M001

SYNTHESIS AND BIOLOGICAL ACTIVITY OF GRAMICIDIN S ANALOGUES CONTAINING CONSTRAINED PHENYLALANINES

C. Solanas[1], B.G. De la Torre[2], L. Rivas[3], A.I. Jiménez[1], **D. Andreu**[2], C. Cativiela[1]

[1]Department of Organic Chemistry, ICMA, University of Zaragoza-CSIC, Zaragoza [2]Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona [3]Centro de Investigaciones Biológicas-CSIC, Madrid, Spain

Development of new classes of antibiotics to counteract bacterial resistance has been intensely pursued in recent years. The search for new molecules has led to the study of naturally occurring antimicrobial peptides. Since most of these molecules target the cell membrane, development of resistance is unlikely. The cationic antimicrobial peptide gramicidin S (GS), isolated from Bacillus brevis, is active against a wide range of bacteria and fungi. Unfortunately, GS exhibits a high haemolytic activity, limiting its use as an antibiotic for topical applications. This peptide is a C2-symmetric cyclic decamer that adopts a rigid β-structure, in which the Val, Orn and Leu residues align to form the antiparallel β-strands and D-Phe and Pro induce type II' β-turns. There is a wide interest in the generation of new GS analogues in order to dissociate the antimicrobial and haemolytic activities. In addition, gramicidin S provides a suitable model to study the structural preferences of non-proteinogenic amino acids. The current study deals with the modification of the β-turn region of GS by incorporating conformationally restricted amino acids in place of both D-Phe residues. On the other hand, the native sequences D-Phe-Pro in GS were replaced by D-Pro-Phe as a well-known type II' β-turn forming dipeptide, and this Phe residue was further exchanged for different constrained surrogates. All linear peptides were prepared by SPPS using Boc-chemistry, cyclized in solution, purified by HPLC and characterized by mass spectrometry. The biological activity of the GS analogues has been evaluated.

Tu002

BIOTIN-DERIVATIVES WITH ENHANCED SOLUBILITY FOR THE LABELLING OF IGG FC PEPTIDES

A. Bartos[1], K. Uray[1], F. Hudecz[1,2]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, [2]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

The biotin – avidin interaction is the strongest non-covalent interaction known in nature. Several biological assays apply peptides or proteins conjugated with biotin. These compounds can be used with avidin-coated ELISA plates or Biacore microchips as targets in binding assays, or can be utilized as labelled entities for capturing enzyme- or fluorophore-labelled avidin, e.g. in FACS analysis. To avoid the blocking of the active sites of peptides/proteins by avidin, a spacer of 6-amino-hexanoic acid is frequently applied between biotin and the compound to be labelled. In some cases the biologically active peptides are also poorly soluble in dimethylformamide, which makes the conjugation reaction with biotin difficult. The biotinylated peptide might have lower solubility than the unlabelled parent compound, making the purification difficult too. Therefore, we prepared a new set of compounds suitable for introduction of biotin without decreasing solubility and preventing steric hindrance. These reagents contain 3,6,9,-trioxaundecanedioic acid moiety. First, N-terminally protected (Boc/Fmoc) building blocks compatible with step-wise solid phase synthesis were produced with ethylene-diamine. This enabled us to prepare biotinylating compounds with different length and solubility. The applicability of this new family of reagents was tested by their coupling to the N-terminal amino group of peptides derived from a β-sheet region of the IgG Fc fragment via their carboxyl groups on solid phase. In this presentation the synthesis of the reagents, their attachment to peptides with low solubility in solution or on solid phase will be described.

These studies were supported by GVOP-3.1.1.-2004-05-0183/3.0 and by OTKA T037749.

Th003

THE CHANGES OF AMINO ACIDS CONTENT IN CONSEQUENCE OF STERILIZATION IN PROCESSED CHEESE

F. Bunka[1], D. Kramarova[1], O. Rop[1], J. Hrabe[1], I. Hoza[1], S. Kracmar[2]

[1]Department of Food Engineering, Faculty of Technology, Tomas Bata University In Zlín [2]Department of Animal Feeding, Faculty of Agriculture, Mendel University of Agriculture And Forestry In Brno, Brno, Czech Republic

Combat rations are designed for boarding of soldiers and members of rescue system during their operation employment. The food components (including processed cheese) should have the shelf life at least 2 years at ambient temperature. The aim of the work was to determine the effect of sterilization on amino acids content in processed cheese.

After production, the first part of processed cheeses (two groups were analyzed) was cooled in 3 hours to the temperature 10 stC. The second part of samples of both sterilized groups there was sterilized (117 stC for 20 minutes) and cooled to 10 stC. There were evaluated crude protein content (Kjeldahl method), ammonia content (Conway method) and amino acids content (ionic chromatography, ninhydrin detection).

The effect of the sterilization on the crude protein content was insignificant. In both groups there occurred the significant decrease of the content of methionine, aspartic acid, serine, glutamic acid, valine, histidine, lysine and arginine ($P < 0,05$). The typical decreases ranged around 2 %. The largest losses occurred at serine (around 4 %). Ammonia may be one of the products of the degradation of amino acids. The sterilization caused an increase of ammonia content (approximately 76 mg/kg).

The conclusions of previous studies were confirmed. Generally only a small decrease of nutrition value of processed cheeses obtained. On the other side it is balanced by multiply extension of their durability (from months to years).

This work was kindly supported by grant No. MSM 7088352101

M004

NOVEL BICYCLIC TEMPLATES FOR PEPTIDOMIMETICS TARGETING GPCRS

J.P. Cain, B.J. Min, A. Mayorov, M. Cai, H. Wang, S. Wallace, Y.S. Lee,
B. Tan, K. Chandler, D. Trivedi, V.J. Hruby

Department of Chemistry, University of Arizona, Tucson, AZ, USA

Two classes of new bicyclic templates have been developed for the synthesis of novel peptidomimetics. Straightforward synthetic steps, starting from amino acids, allow the facile construction of a wide range of analogues. Those systems were initially designed to target the melanocortin receptors (MCRs), with functional group selections based on a known pharmacophore and guidance from molecular modeling to rationally identify positional isomers and stereochemistries likely to be active. Preliminary biological assays have revealed that all compounds tested to date are antagonists which bind with high affinity. Importantly, many are selective for a particular MCR subtype, including some of the first completely MC5R-selective compounds reported. Goals for future work in this area include an increased understanding of the binding mode for these ligands and the production of selective agonists. In addition, the application of these novel templates in targeting other GPCRs, such as the opioid receptors, is being pursued. [Supported by grants from the U.S. Public Health Service DK 17420 and DA 13449]

M010

SYNTHESIS AND IN VITRO ACTIVITY OF A PEPTIDOMIMETIC MYD88 HOMODIMERIZATION INHIBITOR (ST2825) ITS ENANTIOMER AND ITS DIASTEREOISOMERS

N. Fantò[1], M. Semproni[1], D. Vignola[1], G. Gallo[1], O. Serlupi Crescenzi[1], E. Nucera[1], C. Chiapparino[1], R. De Santis[1], M.O. Tinti[1], F. De Angelis[2]

[1]R&D, Sigma-Tau, Pomezia [2]Department of Chemistry Chemical Engineering and Materials, University of L'Aquila, Coppito, L'Aquila, Italy

Background and aims: Homodimerization of MyD88 adapter protein is essential for NF-kB activation in the inflammatory pathway triggered by IL-1 and TLR [1]. We designed a peptidomimetic of the MyD88 TIR domain consensus peptide Arg-Asp-Val-Leu-Pro-Gly-Thr [2], named ST2825. Here, we report its synthesis and biological activity. We also report the synthesis and biological activity of its enantiomer, ST3511, and its diastereoisomers, ST3489 and ST3558.

Methods: The structure of the MyD88 TIR domain consensus peptide is subdivided into three distinct portions, the most important of which is a b-turn. In the peptidomimetic design we changed the b-turn with a tricyclic spirolactam [3], already known [4]. We synthesized this building block, its enantiomer and two of 8 possible diastereoisomers by "in solution" synthesis. Based on semiempirical calculation of heat of formation [5], we could predict the right stereochemistry of the 4 products selectively obtained in the last cyclization step.

Results: These four compounds were tested for their biological activity by Reporter Gene Assay (RGA). Some coimmunoprecipitation experiments were also carried out and we report their results.

Conclusions: The results show the activity of ST2825 and its isomers on our target, with limited specificity towards their stereostructure.

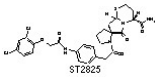
[1] Akira, S. et al. (2004) Nat. Rev. Immunol. 4, 499-511.

[2] Loiarro, M. et al. (2005) J. Biol. Chem.280 (16), 15809-15814.

[3] European Patent requested n° PCT/EP/2005/056847.

[4] Khalil, E.M. et al. (1999) J. Med. Chem.42, 628-637.

[5] Spartan '02 - Wavefunction INC. – Irvine, CA.



ST2825

Tu011

ABOUT EPIMERIZATION OF PEPTIDE ALDEHYDES – A SYSTEMATIC STUDY

J.-A. Fehrentz, A. Moulin, C. Ganneau, L. Demange, J. Martinez

UMR 5810, LAPP, CNRS-UM I-UM II, Faculté de Pharmacie, Montpellier, France

Numerous examples of peptide aldehydes have been found to be potent inhibitors of enzymes. These peptide aldehydes can also be used in a wide range of chemistry including pseudo-peptide bonds or chemical ligation. Various methods for the synthesis of peptide aldehydes have been described in solution and on solid support. However the chosen synthetic route, the obtained peptide aldehydes are known to be very sensitive to epimerization. This phenomenon occurs on the C-terminal carbone and can be explained by the enolisation of the aldehyde function. This epimerization can take place during the synthesis1 or during the purification of peptide aldehydes2. We achieved a systematic study of the epimerisation of di-, tri-, and tetrapeptide aldehyde models. We chose the synthesis in solution in order to purify the Weinreb amide 3 prior to achieve the reduction step.

References:
1. J.-A. Fehrentz, A. Heitz, B. Castro, Int. J. Pept. Protein Res. 1985, 26, 236-241.
2. J.-A. Fehrentz, M. Paris, A. Heitz, J. Velek, F. Winternitz, J. Martinez, J. Org. Chem. 1997, 62, 6792-6796.
3. S. Nahm, S. Weinreb, Tetrahedron Lett. 1971, 22, 3815-3818.

Th012

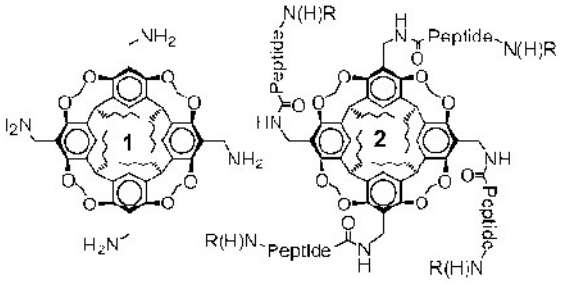
PEPTIDE CAVITANDS

M. Feigel, C. Berghaus, C. Hülsbusch, I. Müller

Department of Chemistry, University of Bochum, Germany

Aminomethyl-substituted bridged resorc[4]arenes (cavitands) 1 are unique rigid anchor points for the attachment of peptides. We prepared several derivatives of the general structure 1(-Aa1-[Aa2-[Aa3]]-N(H)Z)4 (2) to elucidate the influence of the cavitand core on the local conformation of four attached short peptides or amino acids. Dipeptide and tripeptide cavitands having a glycine at position Aa1 build stable inclusion complexes with acetonitrile in solution[1]. NMR data suggest that the peptides form a cyclic array of hydrogen bonds at the upper rim involving the glycine CO- and NH-groups. Chiral amino acids at position Aa1 are not able to develop this type of hydrogen bridging and complex acetonitrile only weakly. However, nmr data suggest, that several amino acids (e.g., (OMe)Glu- and (OMe)Asp) at position Aa1 encapsulate one of their own side chain methyl ester groups within the resorcinarene bowl. The first solid state structure of a peptide–cavitand (1(-Met-N(H)Z)4) is presented and proline cavitands are prepared to explore catalytic properties.

[1] for published results, see: C. Berghaus, M. Feigel, Eur. J. Org. Chem. 2003, 3200.



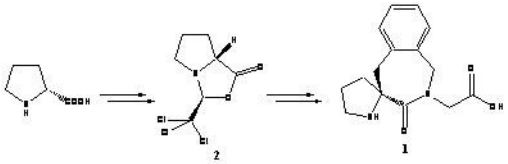
M013

SYNTHESIS OF A SPIROBENZAZEPINONE AS A B-TURN MIMIC IN BRADYKININ

D. Feytens[1], J. Van Betsbrugge[2], S. Ballet[1], R. De Wachter[1], P. Cucchi[3], S. Meini[3], D. Tourwé[1]

[1]Vrije Universiteit Brussel, Department of Organic Chemistry, Brussels, Belgium
[2]Bioquadrant, Montréal, QC, Canada [3]Menarini Ricerce Srl., Florence, Italy

The high affinity of bradykinin (BK) analogues for the B2 receptor has been related to their tendency to adopt a C-terminal beta-turn conformation. We now report the results of the substitution of the Pro-Phe or D-Tic-Oic dipeptide sequence in BK and HOE 140 by spirobenzazepinone 1, which was shown to adopt a beta-turn.
The enantioselective synthesis of 1 was performed starting from D-Pro through the formation of an oxazolidinone 2 and subsequent alkylation with o-cyanobenzylbromide. The enantiomeric excess was determined by means of derivatization with (S)-NIFE and HPLC-analysis. An ee of 99% was obtained. Incorporation into the HOE 140 sequence resulted in an antagonist with good B2 receptor affinity, thus confirming the beta-turn hypothesis.



Tu014

AZIRIDINE-MEDIATED SYNTHESIS OF MULTITOPIC BETA-LACTAM SCAFFOLDS FOR BETA- AND GAMMA-TURN STABILIZATION

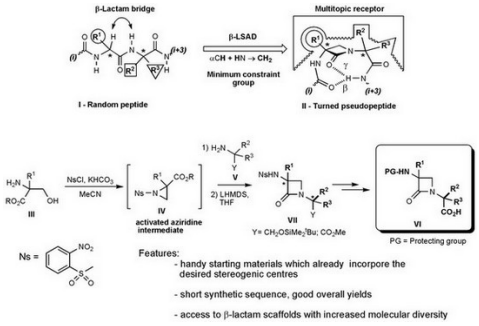
C. Palomo, J.M. Aizpurua, I. Ganboa, A. Benito, I. Loinaz, E. Balentová,
L. Cuerdo, **R.M. Fratila**, A. Jiménez, J.I. Miranda, J. Oyarbide

Universidad del País Vasco, Departamento de Química Orgánica-I, Facultad de Química, San Sebastián, Spain

Introduction of a methylene bridge between the Cα(i+1) and the N(i+2) atoms in an open peptide (I) to mimic simultaneously the CαH(i+1) and HN(i+2) protons (β-Lactam Scaffold Assisted Design - β-LSAD) has proven to be a practical tool for the preparation of monotopic β-turn peptidomimetics (II, R2 = R3 = H), according to the principle of separation of constraint and recognition elements1.

In this work we report a short, general, and stereocontrolled synthesis of multitopic β-lactam scaffolds of type VI. α-Alkyl serinates III are converted into the corresponding enantiopure N-nosyl-aziridines IV which undergo "in situ" ring-opening with amino acids V. Subsequent base-promoted cyclization affords the N-protected α-alkyl-α-amino-β-lactams VII.

Incorporation of the novel scaffolds into linear and cyclic peptides and their conformational features are also presented, most of them showing stabilized β- and γ-turn conformations.



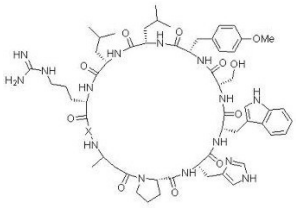
Th015

DESIGN AND SYNTHESIS OF TWO CYCLIC ANALOGUES OF GONADOTROPIN RELEASING HORMONE (GNRH) FOR THE TREATMENT OF CANCER

A. Holeva[1], **I. Friligou**[1], M. Keramida[1,2], T. Tselios[1], J. Matsoukas[1]

[1]Department of Chemistry, University of Patras [2]Department of Fertility, Gynecological Center of Patras, Patras, Greece

Gonadotropin releasing hormone (GnRH) stimulates production of gonadotropin hormones (FSH and LH) through interaction with specific receptors triggering important biological functions. Leuprolide is an agonist of GnRH and it is known in clinical use for cancer treatment and reproductive disorders. In order to improve leuprolide's biological activity and bioavailability, two cyclic peptide analogues were synthesized. Non natural amino-acid azetidine (Aze) at position 9 was used in order to reduce the proteolytic degradation of GnRH peptide analogues. Moreover, substitution of Tyr5 with Tyr(OMe) aimed at avoiding desensitization of GnRH receptors and introduction of D-Leu at position 6 intented to stabilize β-turn between aminoacids 5 to 8 (Tyr5-Gly6-Leu7-Arg8) of GnRH. Also, the usage of Pro and 3-aminobutyric acid at the N and C terminal respectively, simulates the important for activity residues pGlu and ethylamide of leuprolide (Scheme 1). The synthesis was carried out by the Fmoc/tBu methodology utilizing the 2-chlorotrityl chloride resin (CLTR-Cl). The couplings of amino acids were carried out with DIC and HOBt in DMF. The cyclization was achieved by the use of TBTU, HOAt and collidine giving high yield reactions. The purity of the final products was verified by RP-HPLC and their identification was achieved by ESI-MS.



M016

REGULATION OF HELICAL STRUCTURES IN SHORT PEPTIDES BY USING PHOTO- AND ELECTRORESPONSIVE CROSS-LINKING AGENTS

K. Fujimoto, M. Amano, H. Kawai, M. Inouye

Faculty of Pharmaceutical Sciences, University of Toyama, Sugitani, Toyama, Japan

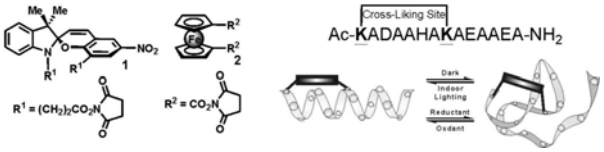
Helical structures in proteins critically relate to molecular recognition events in vivo. In general, particular amino acid sequences that exist as helices in proteins usually adopt the random-coiled structures in their "isolated" short peptide states. Thus, special attention has been paid to external stabilization of helical structures in short peptides. Recently, we have reported the effective stabilization of helical structures in short peptides by using acetylenic cross-linking agents [1]. In addition to such stabilizations, the reversible regulation of helical structures by extrinsic stimuli is a topic of great interest, which may allow the control of biological recognition events.

Photo- and electroresponsive cross-linking agents **1 and 2** were synthesized for the reversible regulation of helical structures in short peptides [2]. The cross-linking agents **1 and 2** possess photochromic spiropyran and redox-active ferrocene, respectively, as a responsive moiety. The helical contents of the peptides cross-linked with 1 were found to be reversibly regulated under ambient indoor lighting and dark conditions at room temperature. In the case of 2, the helical contents could be electrochemically controlled by using suitable oxidant/reductant.

Footnote

[1] K. Fujimoto et al. Chem. Commun. 2004, 1280.

[2] (a) K. Fujimoto et al. Org. Lett. 2006, 8, 285; (b) K. Fujimoto et al. unpublished .



Tu017

PEPTOID-SCAN IN ANTIMICROBIAL PEPTIDES: SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF [NARG]PEPTOID-PEPTIDE HYBRIDS OF APIDAECIN IB

M. Gobbo[1], L. Biondi[1], R. Rocchi[1], V. De Cian[2], E. Reddi[2], G. Bertoloni[3]

[1]Department of Chemical Sciences [2]Department of Biology, [3]Department di Histology, Microbiology And Medical Biotechnology, University of Padova, Italy

Peptoids represent a relative conservative example of oligomeric peptidomimetics in which the side-chains of the amino acid residues present in the sequence have been shifted from the α-carbon to the α-nitrogen, to give N-substituted glycines. Peptoids possess high resistence to proteolytic degradation, can behave as foldamers and have been used in a variety of applications ranging from medicinal chemistry to material science. As valuable tools in drug design the N-substituted glycines can be used to convert a bioactive peptide either into the corresponding peptoid or into a library of peptide-peptoid hybrids containing site-specific substitutions. Following our investigations on the STRUCTURE-ACTIVITY RELATIONSHIP of Pro-Arg rich antimicrobial peptides, we report the preparation of [Narg]peptoid-peptide hybrids of the insect 18-peptide apidaecin Ib in which the arginine residues (positions 4, 12 and 17) have been selectively replaced by the corresponding N-substituted glycine derivatives. The antibacterial action of apidaecin Ib is not membranolytic and probably involves a stereoselective recognition of a chiral cellular target. The straightforward solid-phase synthesis of [Narg]peptide-peptoid hybrids was performed by the sub-monomer method, by using bromoacetic acid and the unprotected 1,3-propanediamine, followed by on resin guanidinylation of the resulting N(3-aminopropyl)glycine residues. The Fmoc-Narg(Pmc)-OH was used for the synthesis of the trisubstituted peptoid-peptide hybrid [Narg4,12,17]apidaecin Ib. According to the circular dichroism measurements the conformational features of the natural peptide and those of the peptoid-peptide hybrids are very similar. Moving the [Narg]residue from the N- to the C-terminal end progressively reduces the antibacterial activity of the [Narg]apidaecin Ib hybrids.

Th018

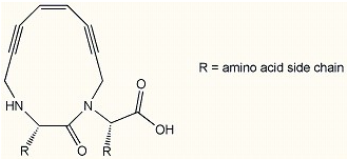
NOVEL PEPTIDE-DERIVED ENEDIYNE STRUCTURES

M. Gredicak, I. Jeric

Division of Organic Chemistry and Biochemistry, Rudjer Boskovic Institute, Zagreb, Croatia

Enediyne anticancer antibiotics (calicheamicins, esperamicins, neocarzinostatin, C-1027, etc.) represent a new class of compounds with complex architecture, high biological activity and the unique reaction mechanism. Apart from differences in their structure, the central position occupies Z-hexa-1,5-diyn-3-en unit (enediyne moiety) embedded within 9- or 10-membered ring. When the enediyne molecule anchors to the minor groove of the DNA, a series of reactions is initiated, leading to a cycloaromatization (Bergman cyclization) of enediyne unit and formation of 1,4-benzenoid diradical. The highly reactive diradical strips hydrogen atoms from sugar phosphate backbone of DNA strands, causing scission of the DNA double helix.

Owing to complex molecular structure and the lack of selectivity of enediyne antibiotics, there is an urge to synthesize more accessible and more selective enediyne-related compounds. Peptides could be good carriers of the enediyne unit, since they allow variations in conformational constrains and acid-base properties by simple amino acid replacement and can also carry certain degree of selectivity towards the certain tissues. The aim of presented research is preparation of cyclic peptide-enediyne molecules suitable for the study of Bergman cyclization triggered by thermal or photo-activation.



M019

USE OF POLYMER-SUPPORTED BASES FOR THE PREPARATION OF HIGHLY PURE PROLINE NCA

O.P. Gulín[1,2], F. Rabanal[1], E. Giralt[1,2]

[1]Department of Organic Chemistry, University of Barcelona [2]Biomedical Research Institute, Barcelona Science Park IRB-PCB, Barcelona, Spain

Poly(amino acids) are emerging as promising therapeutic carriers finding widespread application in the field of drug delivery. In this context, polyproline polymers have been used to solubilize poorly water-soluble proteins, in affinity chromatography for the purification of platelet profilin, and more recently, in the design of dendrimers. Poly(amino acids) are most conveniently synthesized by polymerization of the corresponding amino acid N-carboxyanhydride (NCA). In spite of the interest of polyproline, the preparation of proline N-carboxyanhydride (Pro-NCA) renders poor synthetic yields. In this work a new method for the preparation of Pro-NCA in high yields and purities is described.

Amino acid N-carboxyanhydrides are obtained by the method described by Fuchs. But, in the case of proline, the N-carbamoyl chloride does not cyclise spontaneously as it takes place with other amino acids, and the use of a non-nucleophilic base is required for the cyclisation. A tertiary amine, such as triethylamine, is commonly used but it renders a low conversion of the N-carbamoyl chloride to the expected Pro-NCA, together with the presence of the Pro-Pro diketopiperazine byproduct. In the present work, polymer-supported bases have been used instead of triethylamine. Higher yields of Pro-NCA, and very low percentages of diketopiperazine have been obtained. In addition, no tertiary amine contamination was observed. Polymer-supported bases could also be recycled and Pro-NCA yields were reproducible. In conclusion, we have developed an efficient method for Pro-NCA preparation with polymer-supported bases.

Tu020

SYNTHESIS OF TRIFLUOROMETHYL KETONE-CONTAINING GLUTAMIC ACID AND GLUTAMINE PEPTIDES AS SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 3CL PROTEASE INHIBITORS

M.O. Sydnes[1], **Y. Hayashi**[1], V.K. Sharma[1], T. Hamada[1], U. Bacha[2], J. Barrila[2], E. Freire[2], Y. Kiso[1]

[1]*Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, 21st Century COE Program, Kyoto Pharmaceutical University, Kyoto, Japan*
[2]*Department of Biology, Johns Hopkins University, Baltimore, MD, USA*

Background and aims: The severe acute respiratory syndrome Coronavirus (SARS-CoV) encodes a chymotrypsin-like protease (3CLpro), which has a pivotal role in the replication of the virus. 3CLpro is a cysteine protease that cleaves substrates upon recognizing Gln as the P1 site. An α-CF3 ketone derivative of the amino acids glutamine or glutamate decreases the electron density of the α-carbonyl carbon making it prone to nucleophilic substitution by either hydroxyl or thiol groups in the active site of serine and cysteine proteases, respectively. This results in the formation of a tetrahedral adduct that is believed to imitate the intermediate formed during peptide-bond hydrolysis, thereby inhibiting protease function. Therefore, compounds containing a CF3 ketone moiety may play an important role as SARS-CoV 3CLpro inhibitors. Based on these considerations, we synthesized four tri- or tetra-peptides containing Gln-CF3 or Glu-CF3 at the C-terminus and tested their inhibitory activity against SARS-CoV 3CLpro employing a fluorescence-based inhibition assay.
Methods: CF3-containing β-amino alcohol was synthesized in five steps from Z-Glu-OH. This amino alcohol was then coupled with respective peptide fragments and elaborated to tri- and tetra-peptides with Glu-CF3 or Gln-CF3.
Results: The target peptides were subjected to a fluorescence-based inhibition assay against SARS-CoV 3CLpro that revealed that the compounds moderately inhibited SARS-CoV 3CLpro. 1H and 13C NMR of the target peptides showed that Gln-CF3 exists predominantly in the cyclic form in CDCl3 solution.
Conclusion: A new method of synthesizing Glu-CF3 and Gln-CF3 containing peptides was developed and the peptides obtained exhibited moderate inhibitory activity against SARS-CoV 3CLpro.

Th021

SYNTHETIC STUDY ON DERIVATIVES OF DIMERIC PEPTIDE FROM HUMAN IGG1 HINGE REGION

P. Niederhafner[1], V. Gut[1], H. Hulacova[1], P. Malon[2], J. Slaninova[1], **J. Hlavacek**[1]

[1]Department of Biological Chemistry [2]Department of Organic Structure Analysis, Institute of Organic Chemistry And Biochemistry, Academy of Sciences of The Czech Republic, Prague, Czech Republic

The problem with reproduction of effect in the field of synthetic vaccines could be alleviated using the chemically defined carrier of relatively rigid structure. One of such molecules, the parallel hexadecapeptide (H-Thr-Cys-Pro-Pro-Cys-Pro-Ala-Pro-OH)2 – „hinge peptide"- was found [1] in the central part of protein from the surface region of human IgG1. We developed alternative syntheses of this peptide and its shortened analogs using a solution or soluble (PEG)/insoluble (PS-DVB) polymer supports procedures. In the solution and PEG syntheses, the disulfide bonds were introduced directly to above peptide using bis-Nα-Boc-Cys(Trt)-OPfp. Thus, the parallel arrangement of both the linear sequences was achieved unequivocally [2]. When using PS-DVB support, the Fmoc/tBu,Trt protection and common conditions of SPPS were used. The disulfide bridges closure by the action of pure oxygen under smoothly elevated pressure shortened the time of oxidation in comparison with air oxidation four times and further increased the yield of natural parallel form of the dimer. Dimeric peptide was further coupled on its amino- or/and carboxy- termini with antigenic polypeptides of Epstein-Bar sequence to obtain defined chemical structures for vaccination study.

References: 1.Wűnsch E., Moroder L., Göhring-Romani S., Musiol H.J., Göhring W., Bovermannn G.: Int. J. Peptide Protein Res.1988, 32, 368.
2. Niederhafner P., Safarik M., Sebestik J., Gut V., Malon P., Hlavacek J.: Tetrahedron Lett. 2006, 47, 1023.

Acknowledgment: This work was carried out under research project No.: Z4 055 0506 of the CAS and was supported by grants No.: 203/03/1362 (GA CR) and No.: A400550614 (GA CAS).

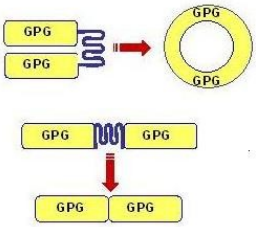
M022

CONTRASTING FIBER MORPHOLOGIES IN PHASED AGGREGATION OF A CYCLIC AND LINEAR HEXAPEPTIDE

K.B. Joshi, S. Verma

Department of Chemistry, IIT-Kanpur, Kanpur, U.P., India

Self-assembly is a widely occurring phenomenon in chemistry and biology, where nanoscopic building blocks are organized to yield well-defined aggregates and supramolecular structures. One such example is ordered protein aggregation which is primarily driven by hydrogen bonding, hydrophobic interactions and other non-covalent stabilizing factors. This report describes differing ultrastructural morphologies of glycine-rich cyclic and linear hexapeptides, possessing same amino acid sequence. Microscopic studies reveal two dissimilar pathways leading to self-assembly: one with spherical pre-fibrillar intermediate, while another one displaying spherulite-like "Maltese-cross" patterns. A remarkable difference in solution phase assembly and phased growth of glycine-rich cyclic and linear peptides suggest dissimilar aggregational pathways via distinct prefibrillar intermediates, thus providing a crucial insight into the interactions controlling occurrence and growth of peptide seeds to persistent length fibers.1



Tu023

THE NEW REARRANGEMENT OF N-ACYLATED 2-HYDROXYMETHYL-2-AMINOACIDS TO 4,5-DIHYDRO-1,3-OXAZOLE-4-CARBOXYLIC ACIDS

J.E. Kaminska[1], S. Olczyk[1], Z.J. Kaminski[2]

[1]Institute of General Food Chemistry [2]Institute of Organic Chemistry, ŁÓdź University of Technology, Poland

It was observed that incorporation of N-acylated 2-hydroxymethyl-2-amino acids into the peptide chain is accompanied by the intensive formation of acidic side-products, isolated and identified as 4,5-dihydro-1,3-oxazole-4-carboxylic acids [1].
Due to the potential application of 4,5-dihydro-1,3-oxazole-4-carboxylic acids as building block very convenient in the synthesis of pharmaceutically interesting natural products such as oxazole (thiazole) antibiotics an efforts were undertaken to modify this reaction conditions in order to prepare this side-product more efficiently. Recently, we found that activated N-acylated 2-hydroxymethyl-2-amino acids after cyclodehydratation to oxazolones at elevated temperature are prone to the rearrangement affording appropriate 4,5-dihydro-1,3-oxazole-4-carboxylic acids with 88-99% yield.

[1] Kamiński; Z.J.; Woszczyna; W.; Kolesińska; B.; Redliński, A., Acta Bioch. Polonica, 48 (4), 1175-78, (2001).

Th024

MONITORING THE OXIDATION OF DECAPEPTIDE(S) Q-E-C-K-D-G(R)-E-C-P-W - DERIVED FROM THE SEQUENCE OF BLOOD COAGULATION FACTOR X - BY REVERSED-PHASE HPLC

L. Kárpáti, I. Komáromi

Clinical Research Center, Medical and Health Science Center, University of Debrecen, Hungary

Blood coagulation factor X (FX) is a zymogen, serine-protease liked glycoprotein. It is activated during the clotting cascade by the concerted action of activated factor VII+tissue factor and/or activated factor IX+activated factor VIII. Prothrombin is transformed into thrombin by the action of activated factor X (FXa). Inherited deficiency of FX is a rare bleeding disorder, but can cause severe bleeding symptoms. A one-year old boy was admitted to the Department of Pediatrics at several occasions because of cerebral bleeding, subdural haematoma and bruising. During the genetic analyzation–made in our departement-a point mutation (guanine?adenine) was found that resulted in a Gly?Arg amino acid exchange at position 204 in FX. The aim of our present study was to identify and purify decapeptide(s) Q-E-C-K-D-G(R)-E-C-P-W - derived from the sequence of blood coagulation FX - by reversed-phase HPLC. We used reversed-phase separation with gradient elution. The obtained peptides were nearly 100% purity. We made different oxidation experiments with the purified peptides and we tried to prove that the Arg residue – found in the mutant sequence – prevents the formation of a disulfide bridge of two Cys residues (Cys201 and Cys206). It causes such conformational changes, which leads to the intracellular degradation of FX by enzymatic cleavage.

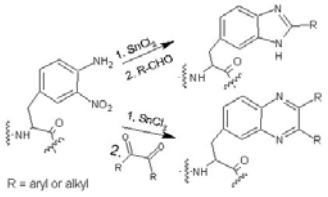
M025

NOVEL HETEROCYCLIC AMINO ACIDS – POST-ASSEMBLY ON-RESIN MODIFICATIONS OF PEPTIDES

A. Kluczyk, A. Staszewska, P. Stefanowicz, H. Bartosz-Bechowski,
M. Koprowska, Z. Szewczuk

Faculty of Chemistry, University of Wroclaw, Poland

The introduction of novel nonproteinaceous heterocyclic amino acids into peptides results in new compounds with interesting structural, physicochemical and biological properties. The transformation of amino acid side chains after the peptide assembly is a convenient method of generating such modified peptides.
Taking into account the biological activity and complexing abilities of nitrogen-containing heterocycles, we investigated the formation of imidazole, benzimidazole and quinoxaline moieties using condensation with various aldehydes and α-dicarbonyl compounds after classical peptide synthesis on solid support. The imidazole synthesis utilizes the N-terminal or side chain amino group of amino acids, whereas a derivative of phenylalanine, β-(4-amino-3-nitrophenyl)alanine, was developed for benzimidazole and quinoxaline synthesis. The modified peptides were purified by preparative HPLC and characterized by ESI-MS, UV and NMR.
In conclusion, we developed a straightforward method of synthesis of peptides with specific ion affinity and spectral characteristic. The broad range of commercially available aromatic aldehydes and dicarbonyl compounds makes possible the synthesis of combinatorial libraries of modified amino acids and peptides.
Part of this work was supported by a grant No. 3T09A 110 28 from the Ministry of Education and Science.



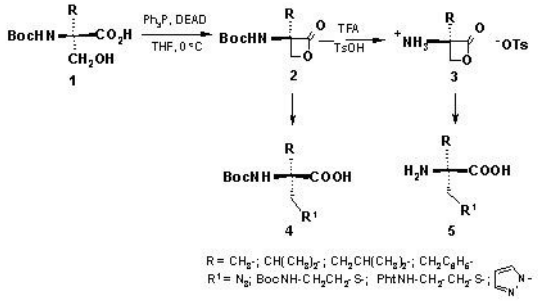
Tu026

EXTENDED ACCESS TO CHIRAL α,α -DISUBSTITUTED α-AMINO ACIDS VIA TRANSFORMATION α-ALKYLSERINE β-LACTONES

A. Kudaj, A. Olma

Institute of Organic Chemistry, Technical University, Łódź, Poland

The biological importance and synthetic utility of noncanonical amino acids continue to stimulate development of new routes to these compounds. In our laboratory, we have been focused on the synthesis of constrained amino acid building blocs and their incorporation into biologically active peptides.
Recently we reported syntheses of optically active N-protected 2 and free 3-amino-3- alkyl-2-oxetanones 3 (β-lactones) from α-alkylserines 1, easy available via procedure developed in our laboratory [1].
In the present study we report the ring opening of β-lactones with various nucleophiles yields protected and free noncanonical amino acids, α-alkyl-β-azidoalanine, α-alkyl-β-pyrazolylalanine and α-alkyl-S-aminoethylcysteine (4, 5).



1. A. Olma, A. Kudaj, *Tetrahedron Lett.*, **2005**, *46*, 6239

Th027

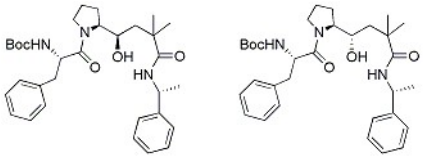
SYNTHESIS AND CONFOMATIONAL ANALYSIS OF THE HYDROXYETHYLENE DIPEPTIDE ISOSTERE OF PRO-AIB

T. Lama, S. Seaman, N. Genest, W.D. Lubell

Département de Chimie, Université de Montréal, QC, Canada

The Pro-Aib sequence is known to adopt beta-turn and beta-bend ribbon secondary structures in peptides (1). Interest in Pro-Aib containing peptides was initially stimulated by its widespread occurrence in microbial polypeptides such as alamethicin and hypelcin (2). More recently, Pro-Aib has served as a key feature in the design of organic catalysts for enantioselective acyl transfer reactions (3). For an efficient synthesis of a hydroxyethylene Pro-Aib isostere, we have now applied a one step synthesis of a key homoallylic ketone intemediate (4), by a reaction that features the conjugate addition of alfa,alfa-dimethyl vinyl Grignard reagent to Boc-Pro-OMe. Studying the influence of the amide bond in the Pro-Aib central residues of a known beta-turn sequence, our report will discuss the synthesis and incorporation of the hydroxyethylene moiety into a tetrapeptide sequence and conformational analysis.

(1) B.V.V. Prasad et al. Biopolymers 1982, 21, 1261-73
(2) R.C. Pandey et al. J. Am. Chem. Soc. 1977, 99, 8469-83
(3) E.R. Jarvo et al. J. Am. Chem. Soc. 1999, 121, 11638-43
(4) K.A. Hansford, J.E. Dettwiler, W.D. Lubell Org. Lett. 2003, 5, 4887-90



M028

SUGAR BUILDING-BLOCKS FOR SOLID-PHASE GLYCOPEPTIDES SYNTHESIS

A. Le Chevalier[1,2], N. Lubin-Germain[3], J. Uziel[3], M. Chelli[1,2], F. Lolli[1,4], P. Rovero[1,5], A.M. Papini[1,2]

[1]Laboratory of Peptide & Protein Chemistry & Biology [2]Department of Organic Chemistry "Ugo Schiff" and CNR-ICCOM, Università di Firenze, Sesto Fiorentino (FI), Italy [3]Organométallique UMR 8123, Université de Cergy-Pontoise, Cergy-Pontoise, France [4]Department of Neurological Sciences [5]Department of Pharmaceutical Sciences, Università di Firenze, Sesto Fiorentino (FI), Italy

The linkage unit to proteins of N-linked carbohydrates in eukaryotic glycoproteins is usually a beta-N-acetylglucosamine coupled to an asparagine. Additional N-glycosyl linkage units have been proven to exist only in the cell surface glycoproteins of bacteria. Among these units, glucose linked to asparagine was found also in the mammalian laminin, an extracellular basement membrane protein [1].
Moreover, an N-glucosyl unit of glucose in alpha-anomeric form linked to Asn (alpha-GlcAsn) has been suggested to occur in eukaryotes [2], but this type of linkage has not been unequivocally characterized. In this context, we demonstrated that the beta-glucosylasparagine (beta-GlcAsn) is fundamental for autoantibody recognition in Multiple Sclerosis (MS) [3]. Therefore, we hypothesize that an aberrant N-glycosylation could trigger pathogenetic autoantibodies in MS.
With the aim of developing a library of CSF114(Glc) mimetic analogues, we synthesized the CSF114 sequence containing different sugars. In particular, we synthesized alpha-glucosylasparagine to obtain anti-(alpha-GlcAsn) antibodies to unequivocally state the anomeric selectivity, specific for MS. Moreover, we synthetised p-glucosylphenylalanine with C-C linkage in beta-configuration, to verify if the increased stability of this bond to chemical and enzymatic cleavage is relevant in autoantibody recognition in MS, and an asparagine modified with a ribose.

[1] R. Schreiner, E. Schnabel and F. Wieland, J. Cell. Biol. 124, 1071-1081 (1994).
[2] S. Shibata, T. Takeda and Y. Natori, J. Biol. Chem. 263, 12483-12485 (1988).
[3] (a) Lolli, F. et al. P.N.A.S. U.S.A. 2005, 102(29), 10273-10278; (b) Lolli, F. et al. J. Neuroimmunology 2005, 167,131-137

Tu029

SUGAR AMINO ACIDS – SYNTHESIS AND CONFORMATIONAL ANALYSIS OF DIMETHYL 3-AMINO-2,3-DIDEOXYHEXOPYRANOSIDURONATES

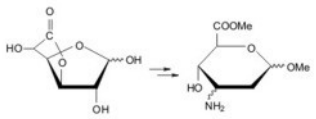
B. Liberek[1], D. Tuwalska[1], A. Konitz[2], A. Sikorski[1], A. Wiśniewski[1]

[1]Department of Chemistry, University of GdańSk [2]Department of Inorganic Chemistry, GdańSk University of Technology, Gdańsk, Poland

Sugar amino acids (SAAs) are sugar moieties containing at least one amino and one carboxyl group.[1] Such the compounds are important class of polyfunctional scaffolds where the carboxyl, amino and hydroxyl termini allow to create structural diversities akin to biologically active molecules.[2] In recent years, sugar amino acids have been used extensively in the area of peptidomimetic studies.[1-3]
Simple and efficient synthesis of dimethyl 3-amino-2,3-dideoxyhexopyranosiduronates, new SAA monomers, from 6,3-lactone of D-glucuronic acid is presented. Conformational analysis of synthesized SAAs, based on the 1H NMR studies, is provided. Influence of the 5-COOMe group on the pyranose ring conformation is discussed. The single-crystal X-ray diffraction data for dimethyl 3-amino-2,3-dideoxy-beta-D-arabino-hexopyranosiduronate is shown.

Acknowledgements: This research was supported by the Ministry of Education and Science under grant
BW/8000-5-0297-6.

1. S.A.W. Gruner, V. Truffault, G. Voll, E. Locardi, M.Stöckle, H. Kessler, Chem. Eur. J.,2002, 8, 4365-4376.
2. T.K. Chakraborty, P. Srinivasu, S. Tapadar, B.K. Mohan, J. Chem. Sci., 2004, 116,187-207.
3. S.A.W. Gruner, E. Locardi, E. Lohof, H. Kessler, Chem. Rev., 2002, 102, 491-514.



Th030

AN NMDA RECEPTOR AGONIST: TETRAZOLYL-GLYCINE AS AN EFFECTIVE COPPER CHELATOR

E. Lodyga-Chruscinska[1], G. Micera[2], D. Sanna[3], P. Gaponik[4], L. Chruscinski[5], J. Olejnik[6], j. Zabrocki[6]

[1]Institute of General Food Chemistry, Technical University of Lodz, Poland
[2]Department of Chemistry, University of Sassari [3]Istituto C.N.R. Chim. Biomolecolare, reg. Baldinca, Sassari, Italy [4]Research Institute for Physical Chemical Problems of the Belarusian State University, Minsk, Belarus [5]Department of Process and Environmental Engineering [6]Institute of Organic Chemistry, Technical University of Lodz, Poland

NMDA receptors belong to the ionotropic group of glutamate receptors. The activity of the receptor can be altered by compounds acting at binding sites. The (R,S)-(Tetrazol-5-yl)glycine (TG) has been shown to be a highly potent NMDA (N-methyl-d-aspartic acid) receptor agonist with exitotoxic effects [1]. The aim of our studies was to investigate the chelating ability of TG towards copper(II) ions. Copper is widely distributed throughout the body with a distinct concentration in the brain. Copper enters cells as complex and seeks out targets requiring it to function. For these reasons it was interesting to evaluate stability and structure of TG – copper(II) complexes. The equilibrium and structural properties of complex species were characterized by pH-metric and spectroscopic (UV-VIS and EPR) methods. In the system, polymeric species are dominant at acidic pH range having { NH2, COO-} coordination with possible Ntetr bridging elements. Monomeric complexes were found at physiological pH. The two TG molecules are bound to copper ion via four nitrogen donors. The formation of two {NH2, Ntetr} donor sets results in very strong metal-ligand interactions and the complex species are very stable over a wide pH region. We have also performed an investigation on similar tetrazole compounds in order to compare the chelating ability of the tetrazole moiety . The targets of our studies were 1,5-diamino-1H-1,2,3,4-tetrazole [2] and tetrazole aspartic acid.

References:

1. D.D. Schoepp, W.H.W. Lunn, C.R. Salhoff, J.W. McDonald, Eur. J. Pharmacol. 270 (1994) 67.
2. A.S. Lyakhov, P.N. Gaponik & S.V. Voitekhovich. Acta Crystall., C57 (2001)185.

M031

SPECTROSCOPIC STUDIES OF CU(II) COMPLEXES WITH INSECT KININ ANALOGS

E. Łodyga-Chruścińska[1], S. Ołdziej[2], D. Sanna[3], G. Micera[4], L. Chruściński[5], K. Kaczmarek[6], R.F. Nachman[7], J. Zabrocki[6], A. Sykuła[1]

[1]Institute of General Food Chemistry, Technical University of ŁÓdź, [2]Faculty of Chemisty, University of GdańSk, Gdańsk, Poland [3]Istituto C.N.R Chim. Biomolecolare, reg. Baldinca [4]Department of Chemistry, University of Sassari, Sassari, Italy [5]Faculty of Process and Environmental Engineering [6]Institute of Organic Chemistry, Technical University of ŁÓdź, Poland [7]Areawide Pest Management Research Unit, Southern Plains Agricultural Research Center, U.S. Department of Agriculture, College Station, TX, USA

Insect kinins share a highly conserved C-terminal pentapeptide sequence Phe-Xaa-Xbb-Trp-Gly-NH2, where Xaa can be Tyr, His, Ser or Asn and Xbb can be Ala but is generally Ser or Pro. The insect kinins preferentially form a cis-Pro, type VI β-turn. An insect kinin analog containing (2S,4S)-4-aminopyroglutamate, a novel cis-peptide bond, type VI β-turn motif demonstrated significant activity in physiological range in a cricket diuretic assay. This is the first instance of a 4-aminopyroglutamate analog of a peptide with a preference for a type VI β-turn that demonstrates significant bioactivity (1).

Many essential metal ions act as important factors influencing the structure of oligopeptides, and as a consequence, they may affect their biological activity. In this work the interaction of copper(II) ions with the insect kinin analogs, Ac-RF[aPy]WG-NH2 and Ac-RF[aPy]WG-NH2, containing (2S, 4R) or (2R, 4R) 4-aminopyroglutamate respectively, was studied. To determine the coordination mode of metal ion in metallopeptide molecules, both spectroscopic (UV-VIS, CD, EPR) and computer modelling optimisation studies, were performed. Complexes with 4N coordination have been found at the 9-10 pH range. The four nitrogens may be derived from Pyr, 2N-amide and NH2Gly. The involvement of these nitrogen atoms has been indicated by spectroscopic parameters found for a similar donor atom system Cu(II)- PyrWG-NH2.

References:

1. Nachman RJ, Kaczmarek K, Williams HJ, Coast GM, Zabrocki J (2004) An active insect kinin analog with 4-aminopyroglutamate, a novel cis-peptide bond, type VI □-turn motif Biopolymers 75 412.

Tu032

B-HOMO-AMINO ACID SCAN OF ANGIOTENSINE IV

A. Lukaszuk[1], H. Demaegdt[2], E. Szemenyei[3], G. Toth[3], D. Tymecka[4], A. Misicka-Kesik[4,5], G. Vauquelin[2], D. Tourwe[1]

[1]Department of Organic Chemistry [2]Department of Molecular and Biochemical Pharmacology, Vrije Universiteit Brussel, Belgium [3]Biological Research Center, Institute of Biochemistry, Szeged, Hungary [4]Faculty of Chemistry, Warsaw University [5]Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Angiotensin IV (Ang IV, H-Val-Tyr-Ile-His-Pro-Phe) was identified as a natural ligand and it exerts significant effects on memory and learning. It was shown that Ang IV binds with high affinity and specificity to novel AT4 receptors, which have been identified as the insulin-regulated aminopeptidase (IRAP or cystinyl aminopeptidase CAP). However, high affinity binding of radiolabelled Ang IV only occurs to the apo-enzyme of CAP in the presence of chelators. Inhibition by Ang IV of the enzymatic activity of CAP takes place with a much lower potency than this high affinity binding. Moreover, Ang IV is rapidly degraded by different proteases. Evidently, there is a need for metabolically stabilised analogues.

The incorporation of beta-amino acids has been successful in creating peptidomimetics that not only have potent biological activity, but are also resistant to proteolysis. In this study the amino acids of Ang IV were respectively replaced by both beta2- and beta3-homo-amino acids. To compare the potencies of these different compounds, we performed enzyme activity measurements, in the presence of different concentrations of compound, of recombinant and endogenous CAP in HEK293 and CHO-K1 cells, respectively. Receptor affinity and metabolic stability were also compared using [125I]Ang IV in CHO-K1 membranes.

Th033

SYNTHESIS AND BIOLOGICAL EVALUATION OF ANALOGUES OF OXYTOCIN, CONTAINING TETRAZOLE ANALOGUES OF AMINO ACIDS

M. Manturewicz[1], L. Borovičkova[2], J. Slaninowá[2], Z. Grzonka[1]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic, Prague, Czech Republic

STRUCTURE-ACTIVITY RELATIONSHIP studies of oxytocin showed that modification of glutamine in position 4 could result in very active analogues. For example one of the most potent agonist of oxytocine for the uterine receptor possess threonine in that position. Continuing work in that field, we synthesized oxytocins containing tetrazole analogues of amino acids. The 5-tetrazolyl group is widely used in medicinal chemistry as an isostere of the carboxyl group. Compounds containing tetrazole ring appear to be metabolically more stable than their carboxylic analogues and have comparable acidity. We synthesized derivatives of aspartic, glutamic, and alpha-aminoadipic acids containing 1H-tetrazole ring in side chains. These derivatives were then used for syntheses of oxytocin analogues substituted in position 4. Apart from above we also obtained two analogues with tetrazole analogue of glycine in position 9. The first one contains 1H-tetrazole ring, the second one has tetrazole ring substituted with methyl group in position 1. Oxytocin analogues possessing amino acids with tetrazole ring in side chains were synthesized on amide resin using Fmoc methodology. In the case of analogues with C-terminal tetrazole ring, fragments 1-7 were synthesized on resin and then coupled with suitable dipeptides in solution. All obtained peptides show no pressor and rather low uteronic activity. However, for some analogues the uterotonic activity when measured in the presence of magnesium ions was several times higher.

Acknowledgement: EFS stipend (ZPORR/2.22/II/2.6/ARP/U/2/05) and grant No. Z40550506 (JS)

M034

SYNTHESIS OF NEW CYCLIC DEPSIPEPTIDES POSSESING ADAMANTANE MOIETY

M. Matkovic, K. Mlinaric-Majerski

Division of Organic Chemistry and Biochemistry, Rudjer Boskovic Institute, Zagreb, Croatia

Depsipeptides, especially cyclodepsipepetides, are very interesting peptidomimetics. These compounds are known to show biological activity which is based on their macrocyclic structure (e.g., valinomycine and closely related molecules, ion-selective antibiotics, etc.).
Incorporation of unnatural amino and hydroxy acids into small peptides and depsipeptides creates new building blocks for synthesis of macrocyclic structures with interesting lipophilic, conformational and electronic features.
Diastereoisomers of 2-(1-adamantyl)-3-hydroxybutyric acid (HyAd) have been synthesized. Ethyl ester of threo HyAd has been incorporated into diastereoisomeric mixture of depsides ethyl 2-(1-adamantyl)-3-O-(Boc-L-Phe)butanoate which were separated. All new compounds were characterized by spectroscopic data and crystallographic analysis.
Threo and erythro 2-(1-adamantyl)-3-hydroxybutyric acid as well as depsid, 2-(1-adamantyl)-3-O-(Boc-L-Phe)butyric acid, are building blocks for synthesis of cyclodepsipeptides of type cyclo(L-Phe-D*L*-HyAd)x.
Cyclodepsipeptides can be prepared by coupling few depsid fragments followed by cyclisation, or by sequentially expanding the starting depsid with D*L*-HyAd and L-PheOH alternatively.

Tu035

N-METHYLATION OF Cα-ALKYLATED LINEAR PEPTIDES: SYNTHETIC ASPECTS AND 3D-STRUCTURAL CONSEQUENCES

A. Moretto[1], M. Crisma[1], B. Kaptein[2], Q.B. Broxterman[2], C. Toniolo[1]

[1]Department of Chemistry, University of Padova, Italy [2]DSM Research, Life Sciences, Advanced Synthesis and Catalysis, Geleen, The Netherlands

Peptides characterized by single or multiple N-methylated, Cα-trisubstituted (e.g., protein) amino acids are of great interest in medicinal chemistry. Several naturally-occurring peptides, remarkably stable to enzymatic attacks, are based on N-methylated residues. The classical conditions (CH3I/Ag2O in DMF, 24 hours, room temperature) for N-methylation of the peptide function(s) is a useful tool for distinguishing solvent exposed from intramolecularly H-bonded –CO–NH– groups in linear and cyclic peptides. In this work we have extended this reaction to terminally blocked linear peptides based exclusively on Cα-tetrasubstituted α-amino acids, e.g. Aib (α-aminoisobutyric acid) or (αMe)Nva (Cα-methyl norvaline) residues.

Under the experimental conditions mentioned, only amide mono-methylation takes place (on the N-terminal, acylated, residue). Methylation of internal peptide groups linking two Cα-tetrasubstituted residues was not observed. Our FT-IR absorption, NMR, and X-ray diffraction investigations support the view that the β-turn and 3-10-helical conformations preferred by the non N-methylated peptides are not significantly perturbed. The tertiary amide bonds are trans. Conversely, the packing mode in the crystals are strongly influenced by the reduction of the number of H-bonding donors. To check the influence of the peptide secondary structure upon N-methylation, we are currently examining as substrates a set of Deg (Cα,α-diethylglycine) homo-peptides, known to form fully-extended (multiple C-5) conformations.

Th036

N-AMIDINO-PROLINE DERIVATIVES AND THEIR APPLICATION IN PEPTIDE SYNTHESIS

Yu. Moskalenko, S. Burov, M. Leko, M. Dorosh, Z. Shkarubskaya, E. Panarin

Institute of Macromolecular Compounds, St. Petersburg, Russia

Unnatural amino acids due to diversity of physico-chemical properties represent valuable tool for the enhancement of peptide activity, bioavailability and stability to enzymatic degradation. Therefore, synthesis of new amino acids and their derivatives with protected functional groups belongs to promising directions of contemporary peptide chemistry.

We selected N-amidino-L-proline [1,2] as hybrid structure, modelling key features of Arg-Pro sequence localised at N-termini of many natural peptide hormones. Its practical application implies synthesis of derivatives to ensure solubility in organic solvents and avoid side reaction at the stage of activation.

Use of PTSA for non-covalent amidino group protection improves solubility of N-amidino-proline in DMF, however it can not prevent side product formation during DIC/HOBt activation. Attempts of TFA-group incorporation using (TFA)2O or TFA-ONP were over with same results. Data of ESI MS, 1H-NMR and 13C-NMR analysis of side product structure were in favour of intramolecular cyclization. Synthesis of Mts-protected N-amidino-L-proline was achieved by L-proline guanidylation by mesitylene-2-sulfonyl-S-methyl-thioisourea. However, HPLC analysis of model dipeptides demonstrates that partial epimerisation of protected derivative made it useless for peptide synthesis. It was shown that amidino group can be successfully modified by the reaction with Mts-Cl. Utility of protected derivative in peptide synthesis was demonstrated for the analogue of fibrinogen □-chain (95-98) fragment, possessed anti-aggregatory properties. Thus, Mts-protected N-amidino-L-proline can be successfully applied both in classical and solid phase peptide synthesis.

References

1. Kaddurah-Daoyk R., Creatine analogues for treatment of obesity: US Patent 5,998,457 (1999).
2. Kim K.S., Qian L., Tetrahedron Lett., 34 (1993) 7677.

M037

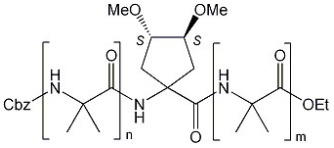
CONTROLLING THE HELICAL SECONDARY STRUCTURE OF HETEROPEPTIDES USING CHIRAL CYCLIC ALPHA, ALPHA-DISUBSTITUTED AMINO ACIDS

M. Nagano[1], M. Tanaka[1], Y. Demizu[1], M. Kurihara[2], M. Doi[3], H. Suemune[1]

[1]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka [2]Division of Organic Chemistry, National Institute of Health Sciences, Tokyo [3]Osaka University of Pharmaceutical Sciences, Osaka, Japan

We have reported that chiral cyclic α,α-disubstituted amino acid (S,S)-Ac(5)c(dOM) could control the helical-screw direction of its homopeptides into the left-handedness. This result means that side-chain chiral centers affect the secondary structure of its homopeptides. Herein, we synthesized heteropeptides containing (S,S)-Ac(5)c(dOM) in Aib sequences, and in proteinogenic amino acid sequences, and studied the conformation. The dominant conformation of Aib peptides containing a chiral cyclic (S,S)-Ac(5)c(dOM) was 3(10)-helix, both in solution and in the solid state. However, the control of helical-screw handedness by one chiral (S,S)-Ac(5)c(dOM) in Aib sequences was difficult. The dominant conformation of the heteropeptides containing a chiral cyclic Ac(5)c(dOM) in normal L-α-amino acid was the right-handed helical structure because the chiral centers of the α-carbon in L-α-amino acid strongly affected the secondary structure.

Ref. M. Tanaka, Y. Demizu, M. Doi, M. Kurihara, H. Suemune, Angew. Chem. Int. Ed. 2004, 43, 5360; M. Tanaka, K. Anan, Y. Demizu, M. Kurihara, M. Doi., H. Suemune, J. Am. Chem. Soc. 2005, 127, 11570.



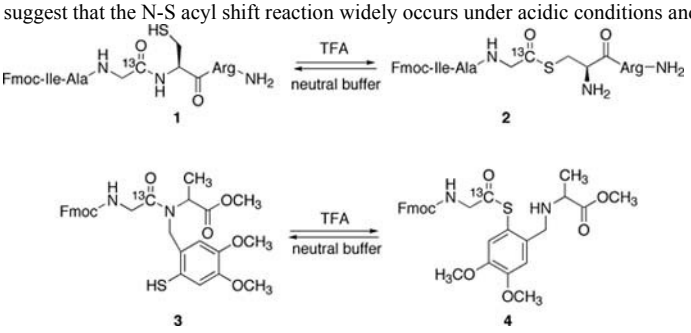
Tu038

N-S ACYL SHIFT REACTION ON PEPTIDE BACKBONE

K. Nakamura, T. Kawakami, S. Aimoto

Institute for Protein Research, Osaka University, Suita, Osaka, Japan

We analyzed products that were generated from thiol-containing peptides by TFA treatment. 13C-NMR spectroscopy suggested that 80% of Fmoc-Ile-Ala-Gly(1-13C)-Cys-Arg-NH2 (1) was transformed into a corresponding peptide thioester 2 in a TFA solution containing 29% CDCl3, after 900 h treatment. An amide bond in a dipeptide, Fmoc-Gly(1-13C)-(4,5-dimethoxy-2-mercaptobenzyl)Ala-OMe (3), was also transformed into thioester 4 to the same extent in a TFA solution containing 14% CDCl3, after 48 h treatment. Addition of sodium 2-mercaptoethanesulfonate to a solution of peptide 4 gave Fmoc-Gly(1-13C)-SCH2CH2SO3H. These results suggest that the N-S acyl shift reaction widely occurs under acidic conditions and that will provide a new route to prepare peptide thioesters.



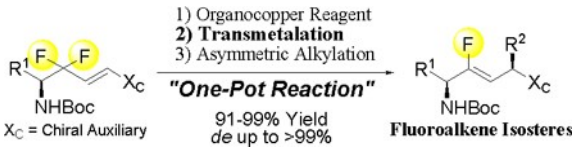
Th039

DEVELOPMENT OF DIASTEREOSELECTIVE SYNTHETIC STRATEGY TOWARD (Z)-FLUOROALKENE DIPEPTIDE ISOSTERES BASED ON ORGANOCOPPER-MEDIATED REDUCTION/DIRECT ALKYLATION VIA TRANSMETALATION

T. Narumi, K. Tomita, A. Niida, S. Oishi, H. Ohno, N. Fujii

Department of Bioorganic Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Backbone replacement of native hydrolyzable peptide bonds with non-hydrolyzable equivalents is a promising approach toward overcoming their major limitations, including rapid proteloysis, in medicinal and biological use of peptides. In particular, (E)- and (Z)-alkene-type dipeptide isosteres have served as potential dipeptide mimetics. We previously reported diastereoselective synthesis of (E)-alkene isosteres, and recently disclosed a new synthetic approach to (Z)-fluoroalkene isosteres utilizing organocopper- or SmI2-mediated reductions via single electron transfer mechanism. Herein, we have succeeded in extending our precedent approaches to the highly diastereoselective synthesis of (Z)-fluoroalkene isosteres with the aid of Oppolzer's sultam as a chiral auxiliary.
To incorporate alpha-sustituents was utilized one-pot organocopper-mediated reduction of substrate followed by direct asymmetric alkylation via transmetalation (91–99% chemical yield, de up to >99%). Applications of fluoroalkene isosteres to bioactive peptides are being examined.



M040

OPTIMIZATION OF THE OXIDATIVE FOLDING REACTION AND DETERMINATION OF THE DISULFIDE STRUCTURE FOR HUMAN ALPHA- AND BETA-DEFENSINS

S. Kubo[1], N. Chino[1], H. Nishio[1], **Y. Nishiuchi**[1], M. Nakazato[2], T. Kimura[1]

[1]Protein Research Foundation, Peptide Institute, Inc., Minoh, Osaka [2]Third Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki, Japan

In humans, two classes of defensins, α-defensin and β-defensin, have been identified on the basis of tissue specificities and structural features including their modes of disulfide pairing. In general, particular combinations with disulfide bonding in cysteine-containing peptides are critical for expressing their intrinsic biological activities. In the case of human α- and β-defensins, however, disulfide isomers without the native pairing were demonstrated to exhibit similar antimicrobial activity to that of the native defensins. Therefore, to assess the biological activities of defensins as well as defensin-based therapeutics, extreme care is required in the chemical synthesis of these peptides to avoid ambiguity in quality. In the present study, we synthesized human α-defensin-1, -2 and -4, and human β-defensin-1, -2, -3 and -4 by employing Boc chemistry, and determined the optimal conditions for folding the respective reduced peptides preferentially into a native conformation. Among the factors affecting the oxidative folding in the presence of reduced and oxidized glutathione, the buffer concentration and reaction temperature were essential. All the synthetic human α- and β-defensins were confirmed to have the respective native disulfide pairing by sequential analyses and mass measurements with cystine segments obtained by enzymatic digestion.

All the human α- and β-defensins could be efficiently oxidized to the α- and β-defensin-type disulfide structure, respectively, under several conditions determined in the present study. These synthetic peptides of high homogeneity were used to accurately assess the antimicrobial activity.

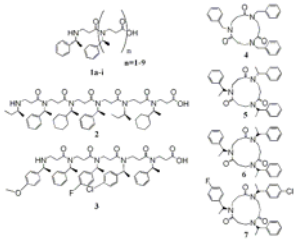
Tu041

SYNTHESIS AND STRUCTURAL INVESTIGATIOND OF LINEAR AND CYCLIC A-CHIRAL N-SUBSTITUTED B-PEPTOIDS.

A.S. Norgren, S. Zhang, P.I. Arvidsson

Organic Chemistry, Department of Biochemistry and Organic Chemistry,
Uppsala, Sweden

New peptidomimetic oligomers are currently a highly interesting field of investigation, due to potential applications in both medicinal chemistry and in material science. Oligomers like these having possibility to adopt well-ordered three-dimensional conformations are known as foldamers. One example of foldamers is the so-called peptoids, i.e. oligo-N-substituted-glycines. These biomimetic oligomers have been widely used both as products of combinatorial syntheses and as foldamers. Peptoids with α-chiral substituents on the nitrogen atom have been shown to fold into helical structures and several interesting biological applications have been reported based on this property.
β-Peptoids, i.e. oligo-N-substituted-β-Alanines were first synthesized in 1998, then with non-chiral substituents on the nitrogen atom. To the best of our knowledge, no investigations have been made concerning synthesis and folding propensities of β-peptoids with α-chiral N-substituents. We have developed a solid-phase synthetic route that enables us to synthesize β-peptoids with α-chiral aromatic N-substituents with up to at least 10 residues, 1a-i. Additionally, pentamers with varying side-chains (2-3) and cyclic trimers (4-7) have also been synthesized. Overall conformation of these β-peptoids has been investigated by circular dichroism (CD) spectroscopy and by NMR spectroscopic studies.



Tu101

DEVELOPMENT OF A DEVICE HAVING 96-CHANNEL COLUMN-LIKE REACTORS FOR SIMULTANEOUS PURIFICATION AND PRODUCTION OF OLIGOPEPTIDES

K. Nokihara[1,2], **T. Ohyama**[1], N. Ono[1], K. Suzuki[1], H. Kawakami[1]

[1]HiPep Laboratories, Kyoto, Japan [2]Nanjing Medical University, Nanjing, China

Recently, we have developed a 96-channel pressurized device which can be mounted up to 96 reactors, which are specially designed column-like form, for high throughput (pre-)purification of labeled peptide libraries such as biotinylated or fluorescent peptides. Additionally, the present device could be used as oligopeptide syntheses. Nowadays peptides and nucleotides are synthesized by the solid-phase synthesis using an automated apparatus. Hence, the complete mixing of acylcomponent with resin is one of the key to avoid formation of deletion peptides. Thus, numerous mechanisms, such as rotation, Vortex mixing, gas bubbling and continuous flow, had been developed and patented. We have found that oligopeptides can be easily obtained by using this device without any agitation or mixing, instead, activated acyl-component has been passed through those columns as natural drop wise. The drop-speed can be controlled by positive pressure and piperdine solution or washing solvents can be rapidly removed from reactors under the reduced pressure. A higher-throughput can be realized by the use of a dispensing system. "Know how" for syntheses of difficult sequences can be adopted such as selection of coupling reagents, solvents and/or coupling at elevated temperature. By the present simple manner various peptide libraries has been constructed in a short time. Deep well microtiter plate (96 format) can be placed inside for recovery and an octachannel pipette can be used for simultaneous addition of solvents/reagents. Cleaved can also be accomplished in the same reactor-columns. The present device was also successfully used for coupling in solution using resin bound reagents.

Th102

FMOC SOLID-PHASE SYNTHESIS OF C-TERMINAL THIOESTERS PEPTIDE USING AN INTRAMOLECULAR N,S-ACYL SHIFT

N. Ollivier[1], J.B. Behr[2], O. El-Mahdi[1], A. Blanpain[1], O. Melnyk[1]

[1]UMR 8161-Service de Chimie des Biomolécules, Institut De Biologie de Lille, Lille [2]Laboratoire de Chimie Bioorganique, UFR-Sciences, Reims, France

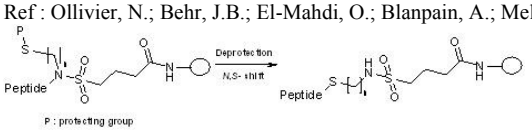
Native chemical ligation is based on the reaction of a peptide bearing a C-terminal thioester group with an N-terminal cysteinyl peptide, leading to the formation of an amide bond at the AA-Cys junction.

The key starting materials for native chemical ligation are unprotected C-terminal thioester peptides. Thioester peptides are often prepared using Boc/benzyl solid-phase peptide chemistry. However, the widespread use of the Fmoc/tert-butyl chemistry for peptide synthesis, over the Boc/benzyl method, has stimulated the development of methods allowing the preparation of thioester peptides that are compatible with the basic treatments used to remove the Fmoc alpha-amino protecting group.

We report here a novel method for thioester peptide synthesis that is based on the use of the sulfonamide safety-catch linker. Once the peptidyl chain is assembled by Fmoc/tert-butyl chemistry, the thioester function is generated on the solid-phase through an intramolecular N,S-acyl shift.

The procedure seems to be insensitive to the bulkiness of the amino acid directly attached to the sulfonamide linker. The thioesters were successfully used for native chemical ligations in solution or on the solid support.

Ref : Ollivier, N.; Behr, J.B.; El-Mahdi, O.; Blanpain, A.; Melnyk, O. Organic Lett. 2005, 7 (13), 2647-2650.



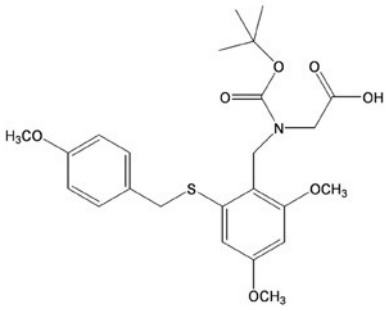
M103

SYNTHESIS OF AN AUXILIARY GROUP FOR CHEMICAL LIGATION AT THE X-GLY SITE.

A. Osler, B. Biondi, A. Calderan, A. Guiotto, P. Ruzza

CNR - Institute of Biomolecular Chemistry, Padua Unit, Padua, Italy

Chemoselective amide forming ligation reactions have facilitated the synthetic access to proteins. In order to generalize this approach, glycine analogues have been developed to promote S to N acyl transfer in manner analogous to native chemical ligation with N-terminal cysteine residues.
Here we report the synthesis of a Gly-derivative starting from 2-mercapto-4,5-dimethoxy benzylammine in which the thiol group is protected as 4-methoxybenzyl (Mob) derivative. Although this group is too TFA-labile for the synthesis of large peptides according to the Boc/Bzl strategy, it has found successful application in many synthesis. The Mob group should be removed by treatment with mercury (II) trifluoroacetate in 80% aqueous acetic acid.
This new auxiliary group, orthogonally protected to C-terminal thioester synthesis condition using Fmoc\tBu chemistry, is tested for the chemoselective peptide ligation involving three or more peptide fragments.



Tu104

THIOAMIDE SOLID-PHASE PEPTIDE SYNTHESIS (T-SPPS): A NOVEL SYNTHESIS METHOD FOR PEPTIDE THIOESTERS

A. Pech[1,2], N. Wehofsky[1,2], D. Wildemann[1], M. Schutkowski[2], F. Bordusa[1,2]

[1]Institute of Biochemistry, Martin-Luther-University Halle-Wittenberg [2]Max-Planck Research Unit for Enzymology of Protein Folding, Halle, Germany

Complete synthesis of proteins still challenges modern chemistry. Since commonly used SPPS protocols can only create peptides of maximal 50 to 60 amino acids in length, combination of peptide fragments, e.g. via native chemical ligation [P.E. Dawson et al., Science, 1994, 226, 776] or protease-catalysis [F. Bordusa, Chemical Reviews, 2002, 102, 4817], is advantageous.

Here a new Fmoc-compatible method for the generation of peptide thioesters as universal precursors for these methods is described. Based on the ability to alkylate the sulphur in the thioamide functionality specifically [D.C. Harrowven, M.C. Lucas, Tetrahedron, 1999, 55, 1187], thioxo amino acids coupled to MBHA resin are generated. After alkylation of the sulphur in the C-terminal thioxo amino acid, the peptide thioimidic acid ester intermediate formed can be hydrolysed to the respective peptide thioester and the free resin (Fig. 1).

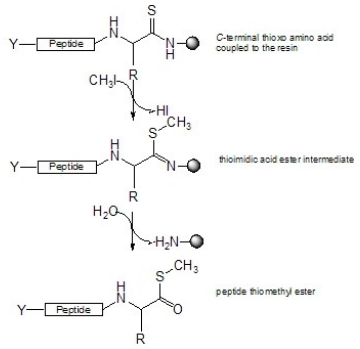


Fig.1: Reaction mechanism for the synthesis of peptide thioesters via alkylation of the thioamide function and hydrolysis

Th105

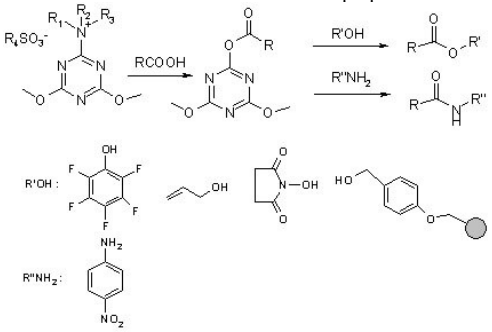
SYNTHESIS OF USEFUL BUILDING BLOCKS FOR PEPTIDE SYNTHESIS BY USING N-TRIAZINYLAMMONIUM SULPHONATES

B. Kolesinska, J. Kolesinska, A. Frymarkiewicz, K.K. Wasikowska,
N. Piatkowska, K.G. Jastrzabek, Z.J. Kaminski

Institute of Organic Chemistry, ŁÓdź University of Technology, ŁÓdź, Poland

Recently, there is an increasing demand for new building blocks necessary for efficient synthesis of complex biomolecules. The most attractive of them are those, prepared from less reactive substrates or formed under reaction conditions too harsh for the final synthetic target.

In this communication, the versatility of N-triazinylammonium sulphonates as coupling reagents have been evaluated for amide or ester bond formation, anchoring Fmoc-amino acids to resins and for the preparation of useful building blocks including esters (allyl, pentafluorophenyl esters) and amides (p-nitroaniline derivatives).



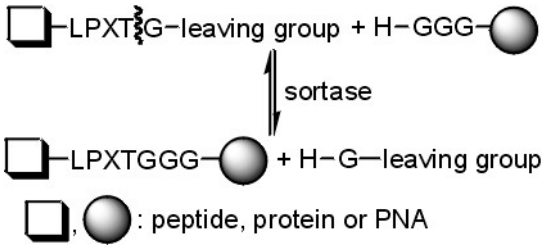
M106

SYNTHESIS OF BIOLOGICALLY ACTIVE PEPTIDE-PNA CONJUGATES AND ARTIFICIAL RECEPTORS BY SORTASE-MEDIATED LIGATION

S. Pritz, Y. Wolf, O. Krätke, J. Klose, M. Bienert, M. Beyermann

Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany

Chemical ligation allows almost selective modification of unprotected peptides or proteins and thereby offers the opportunity for the synthesis of non-natural constructs (Kent et al. Annu. Rev. Biochem. (2000), 923). Even more selective than chemical may be enzymatic ligations.
Sortases are transpeptidases found in Gram-positive bacteria. The Staphylococcus aureus sortase isoform SrtA (sortase A) cleaves proteins at a LPXTG-motif between threonine and glycine, and subsequently transfers the acyl-fragment to a N-terminal oligoglycine. In vivo, this reaction serves for the covalent attachment of proteins to the surface of the bacterium (Mazmanian et al. Science (1999), 760). Recently, sortase-mediated ligation was introduced as a new method for peptide and protein ligation (Mao et al. JACS (2004), 2670).
We optimized the recognition sequence of the substrate and the reaction conditions with respect to the yield. The sortase-mediated ligation was successfully applied to the synthesis of cell-penetrating peptide-PNA conjugates which showed enhanced activity in antisense experiments compared to PNA alone. This ligation strategy was also employed for the coupling of a chemically synthesized construct of the extracellular loops of the CRF-receptor with the corresponding N-terminal receptor domain, which was expressed in E. coli. This 23 kDa protein behaves like an artificial receptor, binding specifically natural ligands.



Tu107

AN EFFICIENT MICROWAVE-ASSISTED SOLID PHASE SYNTHESIS OF GRAMICIDIN A FOR STUDIES IN BILAYER-LIPID MEMBRANE

F. Rizzolo[1,2], G. Sabatino[1,2], I. Paolini[1,2], M.C. Alcaro[1,2], M.R. Moncelli[3], R. Guidelli[3], M. Chelli[1,2], A.M. Papini[1,2], P. Rovero[1,4]

[1]Laboratory of Peptide and Protein Chemistry And Biology [2]Department of Organic Chemistry "Ugo Schiff" and CNR-ICCOM [3]Department of Chemistry, Laboratory of Bioelectrochemistry [4]Department of Pharmaceutical Sciences, University of Florence, Sesto Fiorentino, Italy

Linear gramicidins represent the most investigated family of antibiotic peptides forming ionic channels. Gramicidins produced by Bacillus Brevis are hydrophobic peptides composed of 15 amino-acids with D and L configuration strictly alternate. The presence of D-amino acids in the sequence of gramicidin A (HCO-Val-Gly-Ala-DLeu-Ala-DVal-Val-DVal-Trp-DLeu-Trp-DLeu-Trp-DLeu-Trp-NHCH2CH2OH) should possible make the peptide highly resistant to proteolysis [1].
Striking features like ethanolamine group in C-terminus, the N-terminal N-formylated valine and the high hydrophobicity of the peptide sequence, make the solid-phase synthesis of gramicidin A very tricky.
Therefore, we followed a new synthetic strategy for peptide chain elongation assisted by microwave energy. In fact, microwave energy has been demonstrated to produce high-purity compounds with more rapid reaction times, enhancing coupling rates and efficiency in difficult syntheses [2]. However, microwave-assisted solid phase peptide synthesis (MW-SPPS) has not been yet extensively investigated. In this context, we synthesized gramicidin A by MW-SPPS in high yield and purity, enhancing reaction rate compared to the traditional SPPS. Thermal disruption of peptide aggregation, induced by microwaves, is possible favorable for obtaining this particularly difficult sequence.
Gramicidin A was incorporated in synthetic lipid bilayers, self-assembled on mercury electrodes, characterized by hydrophilic spacers interposed between the metal and the lipid bilayer. We tested the behaviour of gramicidin A in biomimetic membranes using Electrochemical Impedance Spectroscopy (EIS), AC voltammetry and other electrochemical techniques [3].

[1] R.E. Koeppe et al. J. Biol. Chem. 269 (1994) 17: 12567.
[2] C.O. Kappe Angew. Chem. Int. (2004) 43, 6250-6284.
[3] M.R. Moncelli et al. Bioelectrochemistry 63 (2004) 161-167.

Th108

SULFONATES OF N-TIAZINYLAMMONIUM SALTS AS HIGHLY EFFICIENT, INEXPENSIVE AND ENVIRONMENTALLY FRIENDLY COUPLING REAGENTS FOR PEPTIDE SYNTHESIS IN SOLUTION

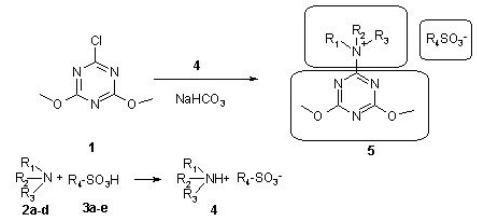
B. Kolesinska, J. Kolesinska, A. Frymarkiewicz, **K.K. Rozniakowski**,
Z.J. Kaminski

Institute of Organic Chemistry, ŁÓdź University of Technology, ŁÓdź, Poland

Tetrafluoroborates of N-triazinylammonium salts were found useful in the peptide synthesis in solution and solid phase (SPPS). We have attempted to expand this family of new generation triazine based coupling reagents by including a new inexpensive and environmentally friendly N-triazinylammonium salts 5. Sulfonates of N-triazinylammonium salts 5 were obtained by treatment 2-chloro-4,6-dimethoxy-1,3,5-triazine with sulfonates of tertiary amine 4 in the presence of sodium bicarbonate [1].

We found all coupling reagents 5 useful for activation of carboxylic components, with the activation rate depended mostly on the structure of tertiary amine constituent 2a-d. The participation of triazine "superactive ester" as intermediate in the condensation has been proved in the model experiments. Utility of reagents 5 was confirmed by peptide synthesis in solution in high yield.

[1] Kamiński, Z. J.; Kolesińska, B.; Kolesińska, J.; Jastrząbek, K. Eur. Pat. Appl. EP1586566 A1 from 19.10.2005, CA 143: 387385 (2006).



2: a) N-methylmorpholine, b) N-methylpiperidine, c) quinuclidine, d) DABCO
3: a) p-toluenosulfonic acid, b) methanosulfonic acid, c) trifluoromethanosulfonic acid, d) camphorosulfonic acid, e) aminosulfonic acid

M109

AUTOMATIC MICROWAVE GLYCOPEPTIDE SYNTHESIS VIA A NEW GENERATION OF TRIAZINE-BASED COUPLING REAGENTS

G. Sabatino[1,2], I. Paolini[1,2], M.C. Pozo-Carrero[1,2], Z.J. Kamiński[3], B. Kolesińska[3], M. Chelli[1,2], P. Rovero[1,4], A.M. Papini[1,2]

[1]Laboratory of Peptide & Protein Chemistry & Biology [2]Dipartimento di Chimica Organica "Ugo Schiff" and CNR-ICCOM, Università di Firenze, Sesto Fiorentino (FI), Italy [3]Institute of Organic Chemistry, ŁÓdź University of Technology, ŁÓdź, Poland [4]Dipartimento di Scienze Farmaceutiche, Università di Firenze, Sesto Fiorentino (FI), Italy

CSF114(Glc) is an N-glucosylated peptide to be produced in large scale by PeptLab because it is the active molecule of the first specific diagnostic/prognostic test for monitoring disease activity and guiding therapeutic treatments of multiple sclerosis patients [1].
In order to develop a synthetic protocol by an automated instrumentation, increasing yield, purity of the crude, and reaction time, a microwave-assisted solid phase peptide synthesis was validated comparing the use of the new generation of Triazine-Based Coupling Reagents (TBCRs) with a series of commonly used ones.
Activation of carboxylic acids by TBCRs is particularly effective because of formation of triazine "superactive esters". The usefulness of TBCRs as coupling reagents has been recently confirmed in the synthesis of Z-, Boc-, and Fmoc-protected dipeptides, sterically hindered amino acids, in the synthesis of esters, in manual and automated SPPS of difficult peptide sequences, and head-to-tail constrained cyclopeptide analogues [2]. Moreover, we also demonstrated TBCRs efficient in a microwave-assisted solution synthesis of the N-glucosylated building block Fmoc-Asn(GlcOAc4)-OH using a manual monomode microwave instrument [3]. This building block was used to obtain CSF114(Glc) comparing the efficacy of a monomode microwave automatic instrument with the traditional solid-phase peptide synthesizers such as the manual and automatic in batch systems, as well as the continuous-flow one.

References
[1] A.M. Papini et al. Proc. Natl. Acad. Sci, U.S.A. 102, 10273-10278 (2005); A.M Papini, Nat. Med. 11, 13 (2005).
[2] Z.J. Kamiski et al J. Am. Chem. Soc. (2005), 127,16912-16920; PCT/EP2005/055793 (2005).
[3] I. Paolini et al Peptides 2005 in press.

CYSTEIN EPROTEASE-CATALYZED PEPTIDE SYNTHESIS

H. Sekizaki[1], T. Fuchise[1], S. Zhou[2], K. Horita[1], Y. Noguchi[1], E. Toyota[1], K. Ohno[1]

[1]Facalty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Hokkaido, Japan [2]Medicinal Department, Medicinal Collage, Qing Dao University, Qing Dao, China

It is known that enzymatic peptide synthesis is more advantageous than chemical synthesis in many aspects; it is highly stereoselective, racemization-free and requires minimal side-chain protection. The method is, however, limited to the use of amino acid derivatives which meet the enzymatic specificity as a coupling component. This problem may be solved using enzymes which have wide specificity of substrate. But in this case, secondary hydrolysis of the resulting peptide may arise from the inherent nature of the protease. In this matter, ficin and ficin-like enzymes were used as cysteine protease to analyze the diminishment of specificity for the substrate.

The cysteine protease-catalyzed peptide coupling reaction has been studied by using synthetic fourteen Boc-amino acid phenyl and naphthyl esters as acyl donor. The reaction conditions were optimized for organic solvent, pH, and concentration of acceptor. The coupling reaction was carried out by incubating an acyl donor (1 mM) with an acyl acceptor (Ala p-nitroanilide, 35 mM) and enzyme (0.1U) in a mixture of GTA buffer (50 mM, pH 9.0) and DMSO (3:2) at 37˚C. The progress of the coupling reaction was monitored by RP-HPLC. The products were obtained in satisfactory yields.

AMADORI-MODIFIED PEPTIDES – A SITE-SPECIFIC STRATEGY FOR SOLID PHASE PEPTID SYNTHESIS

D. Singer, A. Frolov, R. Hoffmann

Bioanalytics, Center for Biotechnology and Biomedicine (BBZ), Faculty of Chemistry and Mineralogy, University of Leipzig, Germany

Non-enzymatic glycosylation, also called glycation, is a common modification in living organisms formed by the reaction of carbohydrates with free amino groups of peptides and proteins. It is a slow chemical reaction yielding Amadori products undergoing further oxidation and degradation reactions finally leading to advanced glycation end-products (AGE). Amadori products are early markers for ageing, diabetes mellitus and Alzheimer's disease. Despite the clinical importance of these Amadori products, universal protocols to synthesize Amadori modified peptides are still missing.
Here we describe a solid phase strategy for the glycation of specific amino groups on partially protected resin bound peptides using a global post synthetic approach. The peptides were synthesized by standard Fmoc/tBu-chemistry using carbodiimide activation. The lysine position to be modified was incorporated with a methyltrityl protected ε-amino group, which can be selectively cleaved after completion of the peptide synthesis with 1% TFA in dichloromethane. The partly deprotected peptide was glycated in methanol using a ten-fold molar excess of 2,3-4,5-di-O-isopropylidene-aldehydo-β-D-arabino-hexos-2-ulo-2,6-pyranose and $NaBH_3CN$ for 18 h at 70°C. After cleavage the overall yields were in the range of 50 – 70 % for the tested octapeptides. All byproducts were well separated by RP-HPLC allowing a simple purification strategy even for medium-sized peptides. Thus the general strategy presented here allows routine synthesis of Amadori peptides at reasonable yields and purities using standard protocols established in most laboratories synthesizing peptides.

M112

A SIMPLE RESIN SWAP PROCEDURE FOR THE EFFICIENT FMOC SYNTHESIS OF LONG C-TERMINAL PROLINE CONTAINING PEPTIDE ACIDS

G.J. Cotton, R. Ramage, K.T. Shaw, **A.S.J. Stewart**

CSS-Albachem, Elvingston Science Centre, Nr. Gladsmuir, East Lothian, UK

2-chlorotrityl chloride resins are recommended for the synthesis of C-terminal proline peptide acids to overcome diketopiperazine formation during chain assembly. However, we have found these (and similar) resins to be unsuitable for the synthesis of peptides greater than 20 residues. For example, the chemokine guinea pig eotaxin, (73 residues C-terminal proline) assembles poorly if not at all on a 2-chlorotrityl resin. We sought to circumvent these problems in the chemical synthesis of peptides and proteins, through the development of a resin-swap procedure. Whereby the initial C-terminal protected tripeptide is assembled on a 2-chlorotrityl resin, liberated from the solid-support, then reattached to a resin that is suited for long chain peptide / protein synthesis. Using this approach, the synthesis of guinea pig eotaxin is reported.

The tripeptide Fmoc-Thr(But)-Lys(Boc)-Pro-OH was assemble on 2-chlorotrityl resin, cleaved with 20% TFE in DCM and attached to Wang resin using standard protocols.
Peptide assembly gave the gp eotaxin in 53% overall yield (as determined by UV monitoring). Fmoc-On cleavage, purification and tag removal followed by folding gave the native chemokine in good yield.

Choice of resin is one of the most critical factors in ensuring a successful peptide synthesis, we have shown the superiority of Wang resin over chlorotrityl resin in the synthesis of medium and long peptides and developed a method for the synthesis of C-terminal proline containing peptides which overcomes the problem of diketopiperazine formation. The technique is being applied to the synthesis of other C-terminal proline peptides e.g. human eotaxin and IP10.

Tu113

A STABLE CONFORMER OF PROLINE TRIPEPTIDES WITH ACE-INHIBITORY ACTIVITY PROVED BY SOLID-STATE IR-LD SPECTROSCOPIC AND THEORETICAL ANALYSIS

T.M. Kolev[1,3], B.B. Ivanova[2], **I.B. Stoineva**[3], E.K. Bratovanova[3], B.P. Tchorbanov[3]

[1]Department of Organic Chemistry, Plovdiv University, Plovdiv [2]Faculty of Chemistry, Sofia University "St. Kl. Ohridsky" [3]Institute of Organic Chemistry With Centre of Phytochemistry Bulgarian Academy of Science, Sofia, Bulgaria

The peptides Val-Pro-Pro and Ile-Pro-Pro possess a high ACE-inhibitory activity and can be applied as food additives with antihypertensive activity. Structural prediction, accompanied by IR-characteristic bands assignment of the tripeptides H-Val-Pro-Pro-OH and H-Ile-Pro-Pro-OH were carried out by means of linear-dichroic infrared (IR-LD) spectroscopy of oriented solid sample as a nematic liquid crystal suspension. These data are supported by theoretical ab initio calculations at the Hartree-Fock level of theory and 6-31++G** basis set. The geometry parameters are compared with known crystallographic ones, indicating a good correlation.

The optimized geometry of the tripeptides studied corresponds to the most stable conformer with ΔE equal to 0.1 kJ/mol obtained by preliminary conformational analysis generated by a minimization of energy varying the dihedral angles. Other data indicate 12 conformers with ΔE within 0.1- 3.2 kJ/mol. The calculations are made on the basis of the assumption supported by 1H-NMR and IR-LD data indicating the stabilization of neutral H-Val-Pro-Pro-OH and H-Ile-Pro-Pro-OH.

The clearance of IR-spectroscopic character of the bands, the IR-data of the tripeptides have been assigned on the basis of IR-LD spectroscopic results in solid-state of amino acids zwitterions L-Valine, L -Isoleucine, and L-Proline, respectively.

In this study we demonstrate the validity of IR-LD spectroscopic conclusions based on theoretical analysis, for investigations of conformation-bioactivity relationships of biogenic peptides.

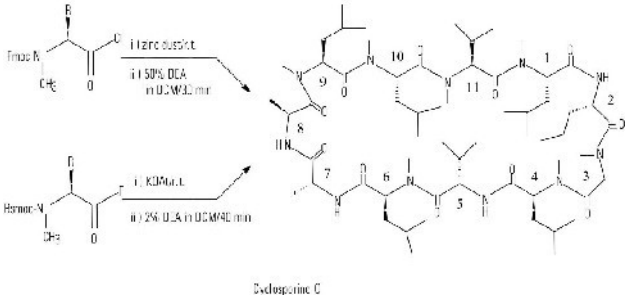
Th114

TOTAL SYNTHESIS OF CYCLOSPORINE O

S.J. Tantry, G. Chennakrishna, **V.V. Suresh Babu**

Department of Chemistry, Central College Campus, Bangalore University, Bangalore, India

Cyclosporines are the family of cyclicundecapeptides. Their most important biological activity is the T-cell specific immunosuppression. Their potential anti-HIV activity has evoked interest for the design of selective cyclosporines active against HIV. Cyclosporine O, devoid of MeBmt at position 1, has marked immunosuppressive activity. The presence of norvaline at position 2 also makes it less nephortoxic. We report the solution phase synthesis of cyclosporine O by the step by step assembly employing Fmoc-amino acid chloride/zinc dust and Bsmoc-amino acid fluoride/KOAt. All the ten intermediate peptides from dipeptide to undecapeptide pertaining to both Fmoc as well as Bsmoc series are isolated as crystalline solids and are characterized by 1H NMR, mass & HPLC techniques. Thus, an efficient and reproducible method for the synthesis of CsO in solution phase by step by step elongation of the peptide chain has been achieved.



Cyclosporine O

M115

N-TERMINAL DIMERIZATION OF BIOLOGICALLY ACTIVE PEPTIDES ON SOLID SUPPORT

Z. Szewczuk[1], M. Biernat[1], P. Stefanowicz[1], H. Bartosz-Bechowski[1], M. Cebrat[1], A. Kluczyk[1], M.M. Cydzik[1], M. Zimecki[2], M. Paduch[3], J. Otlewski[3]

[1]Faculty Of Chemistry, University Of WrocłAw [2]Institute of Biochemistry and Molecular Biology, University of Wroclaw [3]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

Dimerization of cell receptors, involved in antigen presentation, is an essential step in several cellular signal transduction processes, therefore substances that are able to modulate such a process are of potential therapeutic value. Dimeric peptide ligands could represent useful tools to cause dimerization of such receptors. A similar strategy applies dimerization of ligands, interacting with dimeric proteins or proteins with multiple binding sites, to design molecules with enhanced affinity.

Dimeric analogs of the immunosuppressory HLA class II fragments were synthesized using suitably modified, standard Fmoc solid-phase protocols and MBHA-resin. The dimerization was achieved by crosslinking N-terminal amino groups of the peptides with the commercially available mixture of poly(ethyleneglycol)biscarboxylic acid (average MW 600, length range 30-45Å), activated by esterification with pentafluorophenol. The same procedure was applied to synthesize a series of dimeric analogs of C-terminal fragments of plexin-B, consisting of two undecapeptides, linked by the polyethyleneglycol spacers. Other bi- and polyvalent linkers were also investigated.

Our results demonstrated that the amino-terminal dimerizations of the tested HLA-fragments resulted in enhanced immunosuppressive activities, whereas interaction of PDZ dimer with the plexin fragments led to about 20-fold increase in affinity, as compared to their monomeric counterparts.

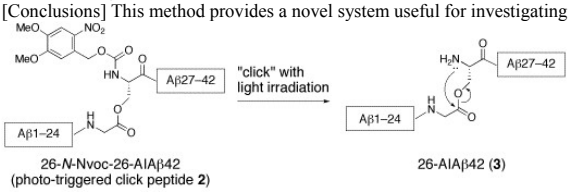
Acknowledgements: This work was supported by the Ministry of Scientific Research and Information Technology Grant No 3T09A08227 (to ZS) and 2P04A 05626 (to JO).

PHOTO-TRIGGERED "CLICK PEPTIDE" BASED ON THE "O-ACYL ISOPEPTIDE METHOD": CONTROLLED PRODUCTION OF AMYLOID ß PEPTIDE (Aß) 1–42

A. Taniguchi[1], Y. Sohma[1], M. Kimura[1], T. Okada[4], K. Ikeda[4], Y. Hayashi[1], T. Kimura[1], S. Hirota[2,3], K. Matsuzaki[4], Y Kiso[1]

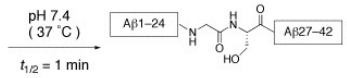
[1]Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, 21st Century COE Program [2]Department of Physical Chemistry, 21st Century COE Program, Kyoto Pharmaceutical University [3]PRESTO, JST [4]Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

[Background and aims] Elucidation of Alzheimer's disease (AD)-related Aß1–42 dynamic events is a difficult issue due to uncontrolled polymerization.
[Methods] Based on the "O-acyl isopeptide method" (Chem. Commun. 2004, 124; J. Am. Chem. Soc. 2006, 128, 696), we have developed a novel photo-triggered "click peptide" of Aß1–42 (1), e.g., "26-N-Nvoc-26-AIAß42 (2)", in which a 6-nitroveratryloxycarbonyl (Nvoc) group was introduced at Ser26 in 26-O-acyl isoAß1–42 (26-AIAß42, 3).
[Results] i) The click peptide 2 did not exhibit the self-assembling nature under physiological conditions due to one single modified ester; ii) photo-irradiation of the click peptide 2 and subsequent O–N intramolecular acyl migration afforded the intact Aß1–42 (1) with a quick and one-way conversion (so-called "click"); and iii) no additional fibril inhibitory auxiliaries were released during conversion to Aß1–42 (1).
[Conclusions] This method provides a novel system useful for investigating the dynamic biological functions of Aß1–42, such as the self-assembly and aggregation processes in AD.



26-*N*-Nvoc-26-AIAβ42
(photo-triggered click peptide **2**)

26-AIAβ42 (**3**)



Aβ1–42 (**1**)

TOTAL CHEMICAL SYNTHESIS OF INSULIN VIA BIOMIMETIC FOLDING

A.P. Tofteng[1,2], K.J. Jensen[1], T. Hoeg-Jensen[2]

[1]Department of Natural Sciences, Royal Veterinary and Agricultural University, Frederiksberg [2]Insulin Chemistry, Novo Nordisk A/S, Bagsværd, Denmark

Several insulin analogues have recently been introduced clinically for improved treatment of diabetes. Industrial productions of such insulins are based on microbial expression systems, which are highly efficient, but generally limited to the 20 proteogenic amino acids. Also, some sequences form inclusion bodies or fail to express. The total chemical synthesis of insulin in research scale was a landmark achievement in peptide science. However, the most commonly used method relies on recombination of A- and B-chains under "random" folding and pairing of the three disulfide bridges. This folding/oxidation step is difficult and low yielding. A general approach using a removable auxiliary which can direct correct formation of disulfide bridges is highly desirable.

In the pancreas as well as in microbial expression systems, insulins are prepared and folded as single chain precursors, with a C-peptide connecting the A and B-chains. The C-peptide helps direct the orientation of A and B-chains in obtaining the correct disulfide pairing and overall peptide folding. Upon folding, the C-peptide is removed enzymatically.

We report here a new method for total chemical synthesis of insulin by use of Fmoc-based step-wise solid-phase synthesis of single-chain precursors followed by C-peptide directed folding and cleavage of C-peptide, thereby allowing total chemical synthesis of novel insulins with unnatural substitutions.

M118

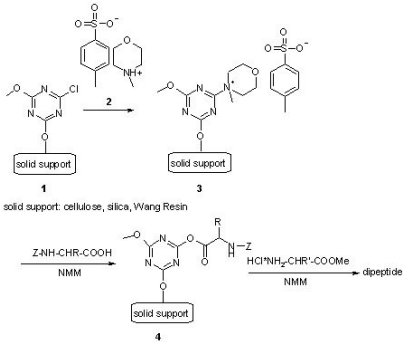
N-TRIAZINYLAMMONIUM SALTS IMMOBILIZED ON SOLID SUPPORT AS COUPLING REAGENTS

Z.J. Kaminski, B. Kolesinska, J. Kolesinska, **K.K. Wasikowska**, N. Piatkowska

Institute of Organic Chemistry, ŁÓdź University of Technology, ŁÓdź, Poland

2-Chloro-4-methoxy-1,3,5-triazines 1a-c anchored on cellulose, silica or Wang resin were prepared by the treatment of 2,4-dichloro-6-methoxy-1,3,5-triazine with appropriate solid support in the presence of a base. Immobilized, environmentally friendly triazine coupling reagents 3a-c were obtained by treatment of 1a-c with N-methylmorpholinium p-toluenesulfonates 2 in the presence of HCl acceptor. The loading of the solid carriers were calculated from N, S contents, determined by microanalysis.

All prepared immobilized N-triazynylammonium toluenosulfonates 3a-c have been found stable at room temperatures. Activation of carboxylic components afforded triazine activate esters 4a-c connected to the support. Treatment of 4a-c with appropriate amino components gave amides or peptides. The final products, chromatographically homogenous amides and peptides, were isolated by filtration or extraction from the solid support.



CRITICAL EVALUATION OF THE USE OF DMB-DIPEPTIDES IN THE SYNTHESIS OF NEUROTOXIC PRION PEPTIDE 106-126

P.D. White[1], J. Beythien[2], P. Schneeberger[2], J. Keyte[3]

[1]Novabiochem, Merck Biosciences Ltd., Beeston, UK [2]Novabiochem, Merck Biosciences AG, Läufelfingen, Switzerland [3]Department of Biochemistry, University of Nottingham, Nottingham, UK

Mutter's pseudoproline dipeptides and Sheppard's Hmb derivatives are powerful tools for enhancing synthetic efficiency in Fmoc SPPS. They work by exploiting the natural propensity of N-alkyl amino acids to disrupt the formation of the secondary structures during peptide assembly. Their use results in better and more predictable acylation and deprotection kinetics, enhanced reaction rates, and improved yields of crude products. However, these approaches have certain limitations: pseudoproline dipeptides can only be used for sequences containing serine or threonine, and the coupling of the amino acid following the Hmb residue can be extremely difficult. To alleviate some of these shortcomings, we have prepared Fmoc-Ala-(Dmb)Gly-OH and Fmoc-Gly-(Dmb)Gly-OH. These Dmb-dipeptides can be incorporated into peptides in place of Ala-Gly and Gly-Gly, resulting in peptides containing structure breaking (Dmb)Gly residues. By introducing the (Dmb)Gly residue as part of a dipeptide unit, the need to acylate the highly hindered secondary amino group of (Dmb)Gly is avoided. On treatment with TFA the Dmb group is cleaved regenerating Gly.

To test the efficacy of our new derivatives in expediting the synthesis of hydrophobic peptides, we undertook the preparation of the challenging neurotoxic prion peptide 106-126 1; this peptide reportedly can not be made using Fmoc SPPS methods. The dipeptides marked in bold were systematically substituted with the appropriate Dmb peptides. The effects of the substitution were evaluated using conductivity monitoring and LC-MS analysis of the crude peptides.

H-Lys-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-Ala-Ala-Gly-Ala-Val-Val-Gly-Gly-Leu-Gly-OH 1

Th120

EFFICIENT DIPEPTIDE PRODUCTION FORM UNPROTECTED L-AMINO ACIDS WITH THE NOVEL ENZYME L-AMINO ACID α-LIGASE.

K. Tabata[2], H. Ikeda[1], **M. Yagasaki**[1], S. Hashimoto[1]

[1]Technical Research Laboratories [2]BioFrontier Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan

Background and aims: Application of α-dipeptides has been limited due to the lack of cost-effective manufacturing methods. The known methods require the protection of amino acid(s) to fix the order of the amino acids (Fig. 1). Furthermore, they usually accompany the formation of longer peptides. To establish the cost-effective manufacturing method, a novel activity which synthesizes α-dipeptides from two unprotected L-amino acids was screened.

Methods and Results: A gene was found in the genome of Bacillus subtilis by in silico screening based on a putative reaction mechanism. The purified protein coded on the gene, i) catalyses α-dipeptide formation from unmodified L-amino acids with a specific order in an ATP-dependent manner, ii) never forms tri- or longer peptides, and iii) takes a wide variety of L-amino acids but no D-amino acids. The enzyme was tentatively named L-amino acid α-ligase (Lal). The whole cell reaction of a recombinant E. coli strain expressing Lal and polyphosphate kinase (Ppk) with two L-amino acids and polyphosphate (polyP) enable the efficient production of many dipeptides with a certain order of the constituent amino acids through the coupling reaction of Lal and Ppk (Fig. 2).

Conclusion: A novel enzyme, Lal, enables to synthesize dipeptides cost-effectively directly from unmodified L-amino acids.

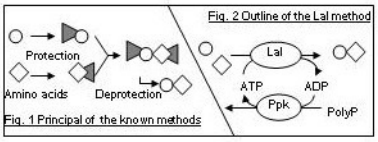


Fig. 1 Principal of the known methods

Fig. 2 Outline of the Lal method

M121

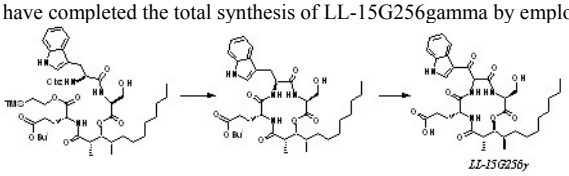
THE FIRST TOTAL SYNTHESIS OF LL-15G256GAMMA□

T. Ye[1], S. Li[2], S. Liang[2], Z.S. Xu[2]

[1]Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong-S.A.R. [2]1Laboratory of Chemical Genomics, The Shenzhen Graduate School of Peking University, Shenzhen, China

Marine organisms continue to provide rich sources of structurally unique and pharmaceutically active compounds. Due to the difficulties in the isolation of significant quantities of these natural products, synthetic chemistry serves an important role in their structural assignment and biological evaluation. Antifungal agents have received considerable attention recently since the spread of HIV has left many people open to fungal infections, and there is a rapidly growing number of drug resistant strains of fungus emerging.

LL-15G256gamma is a cyclodepsipeptide isolated from the marine fungus Hypoxylon oceanicum and structurally assigned in 1998 by Schlingmann. The structure of LL-15G256gamma was determined by a combination of chemical degradation, chiral chromatography and spectroscopic analysis. LL-15G256gamma uniquely combines a beta-ketotryptophan and a polyketide portion within a macrolactone ring. LL-15G256gamma has exhibited potent activity against fungal strains and as such, is an attractive compound to develop as a future therapeutic agent. To date, there have been no reported studies towards the synthesis of LL-15G256gamma. We have completed the total synthesis of LL-15G256gamma by employing the macrolactamization followed by a C-H oxidation as the key step.



APPLICATION OF PEPTOID METHODOLOGY FOR SYNTHESIS OF "DIFFICULT" PEPTIDES FREE OF ASPARTIMIDE AND RELATED PRODUCTS

S . Zahariev[1], C. Guarnaccia[1], C.I. Pongor[1], L. Quaroni[2], M. Čemažar[3], S Pongor[1]

[1]International Centre for Genetic Engineering and Biotechnology [2]ASincrotrone Trieste, Basovizza, Trieste, Italy [3]University of Queensland, Brisbane, QLD, Australia

Aspartimide (aminosuccinimide, Asu) formation is the first step in the degradation of Asp/Asn containing peptides and proteins. The reaction is especially prevalent at Asx-Gly sites and results in a variety of rearranged and racemized products. The bases used in Fmoc-tert-based SPPS promote the formation of Asu and related products.
We recently found that the Dmb backbone protection efficiently prevents secondary structure formation at GG sites and is orthogonal with respect to standard Fmoc SPPS. Here we explore the use of Dmb, Tmb and Nbzl groups (Z) for the synthesis of "difficult"/Asu-prone peptides, in three different schemes: A) Fmoc-Asx-(Z)Gly-OH dipeptide building blocks; B) Fmoc-(Z)Gly-OH monomer building blocks, and C) two steps "submonomeric" approach for synthesis of substituted N-benzyl glycines on the resin. We tested the new methods on two model peptides VKD/NGYI and HA21-20HIV-TAT48-57 (H-G1LFGAIAGFI ENGWEGMIDG20GRKKRRQRRR30-OH) fusion peptide.
The yield and purity of the products reach and even exceed the level in control experiments obtained with Hmb protection and the peptides were found free of Asu/piperidides.
The acid removal of the Dmb protection is ~30% faster than that of Hmb. The submonomeric route (Strategy C) is especially simple, efficient, cost effective and it allows the use of different amines for halogen-displacement. The backbone protecting groups used were in many respects superior to the commercial reagents and applicable for synthesis of both peptide acids and peptide amides. The use of Nbzl-NH2 for halogen displacement represents a new method for preparation of backbone-caged peptides.

TAT-PNA CHIMERIC CONSTRUCTS FOR GENE DOPING DETECTION BY IN VIVO IMAGING TECHNIQUES

B.G. De la Torre[1], Z. Nikolovski[2], N. Andreu[3], V. Gómez[4], J. Llop[4], C. Fillat[3], J. Segura[2], D. Andreu[1]

[1]Department of Experimental And Health Sciences, Pompeu Fabra University [2]Municipal Institute Of Medical Research [3]Center for Genomic Regulation [4]High Technology Institute, Barcelona, Spain

Non-invasive imaging of gene expression has promising applications such as identifying disease-related genes, monitoring gene therapy, or detecting gene doping by athletes. Positron-emission and single- photon emission computerized tomographies (PET, SPECT) are the most sensitive techniques in this regard.
We report preliminary results of a pilot study (IMAGENE) where ectopic gene transfer in mice is detected by image-based techniques. A key tool for this approach are peptide-PNA constructs integrating, in a single molecule, i) a cell-penetrating Tat(48-60) sequence; ii) a PNA sequence complementary to erythropoietin (EPO) RNA, and iii) a Cys residue to which either a fluorophore or an isotopically labeled unit (131I for SPECT, 11C or 18F for PET) can be attached. These chimerae have been built by a combination of Boc/Z (PNA) and Fmoc/tBu (peptide) chemistries, and satisfactorily characterized for purity (HPLC), identity (MALDI-TOF MS) and DNA hybridizing properties (melting curves).
Tat-PNA (fluorescein-labelled, 5 mM) uptake levels nearing 100% were observed by fluorescence microscopy on C2C12 muscle-derived mouse cells transfected with the pCMVmEpo expression vector. Concomitantly, the cells showed inhibition (>50%) of EPO expression at the same PNA concentrations. Once the cell entry of the PNA probe was established, the Tat-PNA constructs, bearing a 131I-label moiety, were injected in mice transfected with the EPO gene. Real-time imaging of the animals by SPECT allowed clear detection of the ectopic gene thus providing proof of principle for our approach. Implications for gene doping analysis and other applications will be discussed.

M124

POLYPROTEIN PROCESSING IN HUMAN PARECHOVIRUSES

F. Ghazi[1], B. Dabirmanesh[2], G. Stanway[3]

[1]Department Microbiology, Iran University of Medical Sciences [2]Department Biochemistry, Azzahra University, Tehran, Iran [3]Department Molecular Virology, Essex University, Colchester, UK

Human parechovirus type1 and 2(HPEV1,2) are common pathogen. They belong to the recently recognized parechovirus genus of picornaviridae. Virus replication involves the synthesis of a large polyprotein, which is cleaved by virus specific proteases to give the final functional proteins. These proteases are important in viral life cycle and are a target for antiviral drugs. One of the earliest steps in picornavirus polyprotein processing occurs at either the N-terminal (as in entero- and rhinoviruses) or C-terminal (as in aphtho – and cardiovirus) boundary of the 2A protein. Activity of this protein differs markedly between these two groups. Recent sequence analysis suggests that HPEV1,2 are distinct from other Picornaviruses and lack the motifs believed to be involved in the protease function of 2A. To study processing directly, contracts containing the HPEV1, 2 2A encoding regions (genomic positions of 2700 – 4200) have been transcribed and translated using an In vitro system. Since no processing of polypeptide containing 2A was observed, and then the 3C region was added to the cDNA constructs to test whether processing has occurred. Expression of this plasmid yielded several small bands in SDS-PAGE, indicating that processing had occurred. Our results showed that the human parechovirus 3C protein seems to be the only virus encoded protease that can catalyze cleavage of all sites in the virus polyprotein.

IMPROVED POLYMERIC REVERSED PHASE RESIN FOR PROCESS SCALE PURIFICATION OF PEPTIDES AND OLIGONUCLEOTIDES

S. Jullien[1], J. Fisher[2], J. Maikner[2], A. Gehris[2], M. Kinzey[2]

[1]Rohm and Haas France S.A.S, Chauny, France [2]Rohm And Haas Co., Spring House, PA, USA

Alkyl bonded silica gels historically have been the standard in reversed phase (RP) purification of biomolecules such as synthetic peptides, small proteins, and oligonucleotides. Silica gels provided the resolving power needed for challenging separations and the mechanical stability required to be operated industrially under high pressure conditions. The chief disadvantage of silica gels is poor chemical stability under alkaline conditions, which limits their capability to withstand rigorous clean and sanitization -in-place (CIP/SIP) protocols. As a result, polymeric media have gained recent market attention because of their excellent chemical stability, which enables full compatibility with modern CIP/SIP protocols. However, first generation polymeric gels lacked both the resolving power and the mechanical stability to be compatible with industrial high pressure dynamic axial compression (DAC) hardware.

Rohm and Haas' Advanced Biosciences division recently introduced a new, monospheric, 10 micron, high performance polymeric RP material. Unlike existing softer polymeric gels, this product has higher mechanical stability which enables it to be used effectively with industrial DAC / HPLC hardware. In addition, this material provides high resolving power for the most challenging industrial separations, because of its unique and selective pore structure, as well as its small monospheric particle size. Finally, because of its excellent chemical stability, the media is not limited in the range of pH that can be used. The combination of mechanical stability for high throughput, chemical stability for long lifetime in use, and high resolution for high yield, together translate to an effective cost-in-use solution for industrial polishing processes.

TRANSPORT OF PYRROLIDINE-BASED OXY-PEPTIDE NUCLEIC ACIDS INTO CYTOPLASM

M. Kitamatsu, A. Takahashi, R. Matsuzaki, M. Sisido

Department of Bioscience and Biotechnology, Okayama University, Okayama, Japan

We have developed new types of peptide nucleic acids with improved water solubility by introducing ether linkages and pyrrolidine rings in the main chain; pyrrolidine-based oxy-PNAs (POPNAs). In this work, cellular uptake and endosomal release of the trans-L-POPNA oligomers, one of stereoisomes of the POPNA, were investigated. The cellular uptake was achieved by combining the POPNA oligomer with an N-terminal 23-mer peptide of an influenza virus hemagglutinin protein (HA2) that is labeled with a rhodamine fluorophore at the N-terminal and covalently linked with a hepta-arginine unit at the C-terminal (Rho-HA2-R7).
The fluorescence images of the CHO cells after incubation with FAM-PO(13) [FAM-O-CAG TTA GGG TTA G-Gly-NH2] in the absence and presence of Rho-HA2-R7 were observed with confocal laser-scanning microscopy. Incubation with FAM-PO(13) alone, no internalization of the oligomer was observed. In the presence of Rho-HA2-R7, however, FAM-PO(13) was successfully internalized into CHO cells and, more importantly, the fluorescence spread over the whole cell. The fluorescence image indicates that the POPNA oligomer in combination with the HA2-R7 peptide was transferred into cytoplasm within 1 h. Since both the red (Rho) and green (FAM) fluorescence spread over the cytoplasm, the POPNA oligomers that were taken up into endosomes together with the Rho-HA2-R7 were released into cytoplasm as the disruption of the endosomes by the HA2 peptide.
In summary, the POPNA oligomers were readily taken up into cytoplasm of CHO cells, when combined with a HA2-R7 peptide.

M127

SPECIFIC RNA BINDING OF IMMOBILIZED PNA'S

T. Ohtsuki, T. Fujimoto, Y. Sugimoto, M. Kitamatsu, M. Sisido

Department of Medical and Bioengineering Science, Okayama University, Okayama, Japan

Most of functional RNAs have post-transcriptional modifications, some of which are quite important for their structure and function. Thus, for studying such RNAs, it is necessary to use purified raw RNAs obtained from living organisms. Isolation of native RNA is necessary also in the case of analyzing the sequence and modifications of mature RNA, which may be different from simple transcript of its gene. Therefore, RNA isolation method is required. Many previous reports demonstrated isolation of RNAs, especially tRNAs.

Most common and traditional purification methods are based on successive column chromatographies. It seems difficult to apply such method to every tRNA because effective combination of columns varies among individual tRNAs. To overcome the difficulty, a sequence-specific selection method using a solid-phase DNA has been devised. In this method, a tRNA can be purified from RNA mixture by a single step. However, this method needs high temperature treatment, which might assist hydrolysis of RNA strand and might impair heat labile modifications.

PNA-RNA hybrid has been known to be much more stable than DNA-RNA hybrid. Thus PNA-based RNA purification method seems to be possible for wider variety of RNAs in lower temperature, in comparison with DNA-based method. In this study, we attempted to purify a single RNA, such as a tRNA and a noncoding RNA, from RNA mixture by using immobilized PNA.

Tu128

PEPTIDE SYNTHESIS OF TROJAN HORSES FOR PLASMID TRANSFER AND TOPICAL ACTIVATION

R. Pipkorn[1], W. Waldeck[2], H. Spring[3], J. Jenne[4], K. Braun[5]

[1]Central Peptide Synthesis Unit,German Cancer Research Center [2]German Cancer Research Center [3]Biomedical Structure Analysis Group [4]Clinical Cooperation Unit Radiation Oncology [5]Division of Molecular Toxicology,German Cancer Research Center, Heidelberg, Germany

Background and aims:
Safe drug delivery technologies are pivotal for genetic interventions, but viral vectors baer the risk of inflammatory reaction. Questions concerning the efficacy of delivery of the genetic substances, the desired topical gene activation and targeting must be answered. Therefore we attempted to develop a membrane non-perturbing delivery system for transport of inactive functional genes into cells and tissues. Genes can be subsequently activated at the target site. Our concept bases on the use of peptide-nucleic-acids (PNAs) resistant against proteases and nucleases, oligonucleotide derivatives, in which the phosphate-backbone has been replaced with ethylen-amin connected alpha-amino-ethyl-glycine-units.
Methods:
Peptides conjugates were composed and synthesized according to the solid phase synthesis and protecting group chemistry strategies. PNA sequences were conjugated covalently, non cleavable, with a capronic acid spacer to the NLS, PKKKRKV.
Complete schematized sequence: [TP -Spacer-Cys-S-S-Cys]n-PKKKRKV-Spacer-[PNAclamp]2.
The hybridization of the 'BioShuttle' modules with the pDNA-EGFP-C3 (Clontech) was accomplished according to first description by Britten & Kohne. Gene transfer into living cells and expression of the DNA-EGFP were determined by CLSM.
Results and implications:
We found a rapid (60 min incubation time) and nearly 100% cellular uptake of pDNA into DU-145 prostate carcinoma cells. By means of GFP-fluorescence, a period of 30 seconds was sufficient for gene activation. After 24 hrs GFP was measured with a 'Quick-Protocol'. Non-activated control cells did not show any fluorescence. 'BioShuttle' carriers proof to be very helpful in gene delivery. We intend to use it for topical, genetic interventions in cells in the differentiation process.

Th129

MODIFICATION OF GUANINE RESIDUES IN PNA-SYNTHESIS BY PYBOP

S. Pritz, Y. Wolf, C. Klemm, M. Bienert

Leibniz-Institute of Molecular Pharmacology, Berlin, Germany

PNAs have gained broad attention in antisense/antigene experiments and as diagnostic tools. In principal, they can be synthesised with several activating reagents known from peptide synthesis. Namely, HATU or PyBOP are often used. Synthesis with HATU is more laborious, because preactivation is needed in order to avoid guadinylation of the N-terminus of the growing PNA-chain. We wanted to use PyBOP, because preactivation should not be needed in this case, which is especially useful in automated synthesis.

Surprisingly, in the PyBOP-mediated syntheses of 18mer PNAs we obtained products showing molecular masses approx. 67 Da above the expected ones. Detailed analysis revealed, that the modification occurred at the only guanine residue in the sequence. In order to further characterise the side reaction, a short PNA fragment was synthesised using HATU and PyBOP activation, respectively, and cleaved from the resin with and without the N-terminal Fmoc-group. While synthesis with HATU gave the desired products, PyBOP partly activates the aromatic carboxy group of the guanine residue, which is substituted by piperidine during subsequent Fmoc cleavage. The modified sequences could be further characterised by MS/MS-fragmentation.

Our results show that care must be taken when synthesising PNAs with PyBOP activation. On the other hand, this reaction possibly opens an opportunity to synthesise guanine derivatives.

M130

SYNTHESIS OF 5-SUBSTITUTED-URACIL PNA MONOMERS BY PD-CATALYZED CROSS-COUPLINGS

F. Sipos, Gy. Sági

Institute of Biomolecular Chemistry, Chemical Research Center of The Hungarian Academy of Sciences, Budapest, Hungary

Peptide nucleic acids (PNA-s) are one of the most promising and powerful DNA mimics due to their strong hybridization ability and complete resistance to nucleases and proteases. In addition, their higher mismatch recognizing ability, compared to the corresponding DNA counterparts, makes them especially suitable for applications in the genetic diagnostics (e.g. PNA microarrays).

By the analogy of higher binding affinity and mismatch discriminating ability of oligodeoxynucleotides containing 5-aryl- or 5-propynyl-pyrimidine bases instead of thymidines and deoxycytidines, we wished to elaborate useful synthetic methods for the synthesis of analogous, new PNA monomers (see Figure). For this purpose, efficiency of different Pd-catalyzed cross-coupling reactions has been investigated and compared. Starting from 5-iodo-uracil (1) number of N-Boc protected 5-aryl- and 5-alkynyl-uracil PNA monomers (2) have been synthesized by the aid of Stille, Suzuki and Sonogashira couplings, respectively. In cases of the two latter methods previous N3-protection of the uracil base was necessary to reach acceptable yields in the coupling reactions.

Incorporation of these modified building units into model homopyrimidine PNA-s, in order to study thermal stabilities of the corresponding PNA:DNA and PNA:RNA complexes, is in process, applying standard solid phase Boc/Z peptide synthesis protocol.

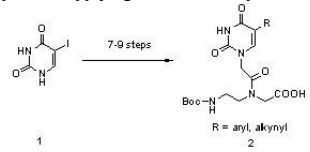


Figure: Synthesis of 5-substituted-uracil PNA monomers

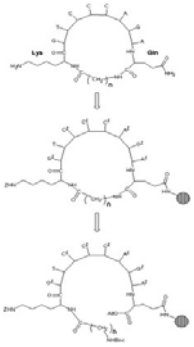
Tu131

CYCLIC PNAS BASED-COMPOUNDS TARGETING THE HIV RNA TAR STRUCTURE

G. Upert, A. Di Giorgio, R. Condom, N. Patino

Laboratoire de Cimie des Molécules Bioactives et des Aromes,University of Nice-Sophia Antipolis, Nice, France

The HIV transcriptional elongation is regulated via the formation of a ternary complex, implying the stem-loop HIV TAR RNA structure, the viral protein Tat an host cellular factors. The formation of this complex is crucial for an efficient viral transcription step and therefore, represents a very attractive target in view of inhibiting HIV replication. Aiming this goal, we have planned to synthesize different cyclic PNAs based-compounds liable to form a kissing-loop complex with the TAR RNA loop. These compounds contain a PNA sequence including the six nucleotides sequence complementary to the TAR loop, flanked by the G and A residues, proned to increase the stability of the duplex. The N and C-extremities of the PNA are linked via spacers of variable length, incorporating a lysine residue for a future vectorisation. The cyclic PNA syntheses were accomplished following an original solid-phase procedure, via on-resin head-to-tail cyclisation of linear PNA derivatives. Their interactions with the HIV TAR RNA fragment were analysed by UV, DC and fluorescence anisotropy studies.



DRUG DEVELOPMENT OF ENDOMORPHIN-1

M.P. Del Borgo[1], Y. Koda[1], J.T. Blanchfield[1], I. Toth[1], Y. Okada[2]

[1]School of Molecular and Microbial Sciences, University of Queensland, St Lucia, QLD, Australia [2]Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Nishi-ku, Japan

The opioid receptor system in the central nervous system (CNS) controls a number of physiological processes including pain, reward, gastrointestinal and cardiovascular functions. As a consequence, most pain modulating compounds currently available cause a variety of side-effects. The endogenous ligands for the opioid receptors are a series of peptides that includes Endomorphin-1. Endomorphin-1 has been shown to elicit potent anti-nociception through the highly selective activation of μ-opioid receptors. It is this receptor that mediates supraspinal analgesia and thus, selectivity for this receptor results in analgesia without affecting other processes. Therefore, endomorphin-1 is considered a promising lead compound for the development of a new, safer pain medication.
We have synthesized a large number of lipid- and carbohydrate-modified endomorphin-1 analogues and screened these compounds for their binding and activation of μ- and δ-opioid receptors in SH-SY5Y cells as well as Caco-2 cell monolayer permeability and plasma stability. Compounds conjugated with either a lipoamino acid or sugar moiety on the C-terminus lost binding affinity by several orders of magnitude, whilst N-terminal conjugations resulted in minimal loss of binding affinity. A number of analogues showed pM binding affinity and high apparent permeability, and of these compounds, one has been selected for assessment in nociceptive and neuropathic pain models. In addition to these pre-clinical studies, internalization and tolerance formation of these compounds has also been measured in an effort to synthesise a non-tolerant opioid agonist. Endomorphin-1 analogues with a high degree of amphiphilicity cause increased receptor internalization and subsequently less tolerance formation.

M133

EN ROUTE TO SYNTHETIC ANTIFREEZE GLYCOPEPTIDES

C. Heggemann, C. Budke, T. Koop, N. Sewald

Department of Chemistry, Bielefeld University, Bielefeld, Germany

Antifreeze glycoproteins (AFGPs) allow organisms to survive at temperatures below the freezing point of physiological solutions. AFGPs usually consist of a varying number of repeating units of (Ala-Ala-Thr)n with minor sequence variations and the disaccharide -D-galactosyl-(1-3)- -N-acetyl-D-galactosamine attached as a glycoside to the hydroxyl oxygen of the threonine residues. The antifreeze activity of these compounds is proven by different experimental observations as thermal hysteresis, change of crystal shape, suppression of recrystallization and ice nucleation.
Until now the mechanism of the shown phenomena is not completely understood due to impure probes from natural sources and problems in synthesis.
A synthetic approach to native antifreeze glycopetides and analogues should lead to a better understanding of molecular mechanisms of antifreeze activity and the relationship between secondary structure motifs and physical properties. The first important point on the way to glycopeptides is a high yield synthetic approach to the disaccharide moiety and in the next step to the glycosylated threonine. This building block is introduced to solid phase peptide synthesis. After conformational analysis with various spectroscopic methods and the microphysical analysis, a deeper understanding of the molecular requirements for antifreeze activity and optimized structures is expected.

MYELIN OLIGODENDROCYTE GLYCOPROTEI(MOG) ROLE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS(EAE) AND MULTIPLE SCLEROSIS

P. Karimi[1], M. Tabatabaai[2]

[1]Medicine Department [2]Neurology Department of Iran University of Medical Sciences, Tehran, Iran

Myelin oligodendrocyte glycoprotein (MOG) is a myelin-specific protein of the central nervous system (CNS). Although MOG is only a minor component of CNS myelin and the contents of this protein in oligodendrogliocyte membrane is very low (approximately 0.1% total proteins) it is considered that MOG is an autoantigen capable to produce Experimental Autoimmune Encephalomyelitis(EAE) as a demyelinating Multiple Sclerosis-like disease. Experimental studies in MOG role in demyelination leads to different results. In rat and marmoset models of MOG-induced EAE demyelination is antibody-dependent and reproduces the immunopathology seen in many cases of MS. In contrast, in mice inflammation in the CNS can result in demyelination in the absence of a MOG-specific B cell response, although if present this will enhance disease severity and demyelination. Clinical studies indicate that autoimmune responses to MOG are enhanced in many CNS diseases and implicate MOG-specific B cell responses in the immunopathogenesis of Multiple Sclerosis. This article provides a summary of our current understanding of MOG as a target autoantigen in EAE and MS, and addresses the crucial question as to how immune tolerance to MOG may be maintained in the healthy individual.

Key Words: MOG, EAE, MS, CNS, demyelination

Th135

OXYTOCIN AND VASOPRESSIN ANALOGUES MODIFIED WITH GLYCOAMINO ACIDS

A. Marcinkowska[1], L. Borovičkova[2], J. Slaninowá[2], Z. Grzonka[1]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic, Prague, Czech Republic

Carbohydrate moieties of glycopeptides and glycoproteins play different decisive roles in various biological phenomena. Conformation and solubility of proteins are influenced by the oligosaccharide chains, which can also inhibit the proteolytic degradation. As a result, the synthesis of glycopeptides is an attractive field that contributes to understanding of mutual interactions between both moieties and for their biological interest.

The synthesis of glycopeptides requires a combination of synthetic methods from both carbohydrate and peptide chemistry. Moreover, this synthesis needs stereoselective formation of the glycosyl bond between a carbohydrate and a peptide (amino acid) part, and also an appropriate protecting group methodology that allows selective deblocking of only one functional group in these polyfunctional molecules.

In the present work we modified the oxytocin and vasopressin structure with glycoamino acids. Transformations of Fmoc-protected serine and threonine derivatives into appropriate O-glycosylated precursors suitable for solid phase peptide synthesis were worked out. The □- and □-O-glycosides were synthesized from Fmoc-serine and Fmoc-threonine allyl esters and appropriate glycosyl bromide using Hanessian's modification of the Koenigs – Knorr reaction. These N-□-Fmoc-protected glycosides were used in synthesis of glycopeptides. Eight analogues of oxytocin modified in position 4 were obtained.

We have also prepared two types of lysin-vasopressin analogues modified with glycoamino acid, in which the glucuronic acid was attached to the ω-amino group of lysine in position 8 through the amide bond.

Glycosylated analogues of oxytocin and vasopressin display an increased stability towards enzymatic degradation, and retain some hormonal activities.

Supported by grants: DS/8350-5-0131-6 (ZG) and Z40550506 (JS)

M136

STUDIES ON THE APOLIPOPROTEIN A-I REGION 104-117 AND ITS LIPOPEPTIDE ANALOGUE (SUBSTITUTION OF MET112 BY AMINOTETRADECANOIC ACID) AS POTENTIAL ANTIATHEROGENIC

K. Harilogis, M. Petraki, A. Tselepis, M. Sakarellos-Daitsiotis, **C. Sakarellos**

Department of Chemistry, University of Ioannina, Greece

Atherosclerosis is a multistep process and the underlying cause of heart disease and stroke in humans. It is considered to be an immune-inflammatory disease, associated with LDL oxidation by numerous studies over the last years. Besides, the antiatherogenic properties of HDL (the “good cholesterol”) are widely accepted. The HDL particles intervene at many different stages of the disease, exhibiting their importance in the defense mechanisms against the disease. Apolipoprotein A-I (apoA-I) is a 243 amino acid secreted amphipathic protein, and the major protein component of HDL. It has been found to play a crucial role in many mechanisms, such as promotion of reverse cholesterol transport, antioxidative and anti-inflammatory functions. As other exchangeable apolipoproteins, it is subjected to great conformational changes during the transition from the lipid-free to the lipid-bound form, resulting in a substantial increase of its helical content. In order to examine the antioxidant role of Met112 of human apoA-I, we have synthesized apoA-I region 104-117 (peptide (1):Ac-FQKKWQEEM112ELYRQ-NH2) and its lipopeptide analogue (Substitution of Met112 by aminotetradecanoic acid), peptide (2). Amphipathic peptides (1) and (2) were tested in their ability to prevent human plasma LDL oxidation in vitro, induced by Cu2+. LDL-associated PAF-AH activity was measured before and after LDL oxidation in order to examine the possible anti-inflammatory action of the peptides (protection against the inactivation of PAF-AH after LDL oxidation). The conformational studies of peptides (1) and (2) were performed using circular dichroism spectroscopy in PBS (pH=7.4), SDS, H2O:TFE 50:50 v/v and TFE 100%.

Tu137

THE STUDIES ON OPTIMIZATION OF THE SOLID PHASE SYNTHESIS OF PEPTIDE-DERIVED AMADORI PRODUCTS

P. Stefanowicz, K. Kapczynska, Z. Szewczuk

Faculty of Chemistry, University of WrocłAw, Poland

According to many authors the formation of Amadori products is a key stage in the glycation process. Glycated proteins may show allergenic properties and potentially initiate autoimmunological processes. They may also serve as the markers of diabetes. To our best knowledge, all procedures concerning the synthesis of peptide-derived Amadori products reported in literature are based on "in solution" approach which makes them tedious and time consuming.

A modified method of the solid phase synthesis of peptide-derived Amadori products based on direct alkylation of the deprotected ε-amino groups with 2,3:4,5-di-O-isopropylidene-β-D-arabino-hexos-2-ulo-2,6-pyranose in the presence of sodium cyanoborohydride was proposed. Isopropylidene groups, protecting the sugar moiety in the obtained conjugate, were removed with trifluoroacetic acid containing 5% water. Studies on optimization of the reaction performed on the model peptide attached to a Wang resin, Fmoc-Lys-Leu-Leu-Phe-(RESIN), showed that the best yield of the product is attained with a two-fold excess of 2,3:4,5-di-O-isopropylidene-β-D-arabino-hexos-2-ulo-2,6-pyranose and a five-fold excess of sodium cyanoborohydride. The identity of the product was confirmed by high resolution MS. The several side products were isolated and their structures will be discussed.

Our results prove that the synthesis of glycated peptides in the solid phase is feasible.

Acknowledgement: This work was supported by a Polish Ministry of Scientific Research and Information Technology Grant No 3T09A02928

SOLID-PHASE SYNTHESIS OF PROLINE TRIPEPTIDES WITH ACE-INHIBITORY ACTIVITY

E.D. Naydenova[1], E.K. Bratovanova[2], M.D. Marinova[2], **I.B. Stoineva**[2], B.P. Tchorbanov[2]

[1]Department of Organic Chemistry, University of Chemical Technology and Metallurgy [2]Institute of Organic Chemistry With Centre of Phytochemistry , Bulgarian Academy of Science, Sofia, Bulgaria

The peptides Val-Pro-Pro and Ile-Pro-Pro have been isolated in 1995 [1] from fermented dairy products and the studies carried out have been summarized by Takano [2]. Proline tripeptides manifesting a high ACE-inhibitory activity can be used as food additives possessing antihypertensive activity.
The aim of the present study is the chemical synthesis of the peptides H-Val-Pro-Pro-OH and H-Ile-Pro-Pro-OH in high yield and analytical purity. The solid-phase Fmoc-strategy was used in this work but the commonly used Wang-resin proved unsuitable as condensation of the second proline resulted in the formation followed by splitting of a diketopiperazine . To avoid this, a scheme for solid-phase peptide synthesis according to the Fmoc-strategy using 2-chlorotrityl resin was developed. The tripeptides were prepared in 45-50 % yield. After deblocking and purification by reversed phase HPLC in gradient mode, H-Ile-Pro-Pro-OH and H-Val-Pro-Pro-OH were obtained as crystals. The proline tripeptides were identified by amino acid analysis and 1H-NMR. Lack of zwitterions form in the peptides structure was observed by the signals of nuclear magnetic resonance and probably it will be important for inhibitory activity.[1] Y. Nakamura, at al., J Dairy Sci ,78:777-783,1995, [2].T. Takano , Antonie van Leeuwenhoek 82: 333–340, 2002.

M139

SYNTHESIS OF HOMOGENEOUS RNASE B GLYCOFORMS BY CHEMICAL AND RECOMBINANT METHODS

C. Unverzagt[1], C. Piontek[1], S. Mezzato[1], D. Varon[1], N Lombana[1], M. Schaffrath[1], C. Poehner[1], M. Püttner[1], P. Ring[1], C. Heinlein[1], A. Martin[2], F.X. Schmid[2]

[1]Bioorganische Chemie Department [2]Biochemie Deprtment, Universität Bayreuth, Bayreuth, Germany

The lack of homogeneous glycoproteins in sufficient quantities is an ongoing challenge in glycobiology. In order to solve this problem researchers have turned to a variety of approaches ranging from mutant eukaryotic strains to the highly demanding total synthesis of glycoproteins.[1] Using RNase B as a model N-glycoprotein[2] we have searched a path to assemble this enzyme employing a combination of chemical and recombinant methods. Native chemical ligation[3] allows the coupling of protein segments of unrestricted size in a chemoselective manner. We have developed solid phase methods to produce the required thioester building blocks 1-25-SR (A) and glycopeptide thioester 26-39-SR (B) containing an N-glycan at Asn34 on a dual linker PEGA resin.[4] The remaining segment 40-124 (C) was expressed in E. coli as a fusion protein and released by intein mediated protein cleavage.[5] Sequential coupling of the three RNase segments requires the use of a protective group at the N-terminus of segment B compatible with the oligosaccharide part. The selective deprotection of Cys 26 in the glycopeptide RNase B 26-124 is the key step towards the synthesis of the full length N-glycoprotein.

[1] Davis B. G., Chem. Rev., 102, 579 (2002).
[2] Liu L. Bennett C. S. and Wong C. H., Chem. Commun., 21 (2006).
[3] Dawson P. E. Muir T. W. Clark-Lewis I. and Kent, S. B., Science, 266, 776 (1994).
[4] Mezzato S. Schaffrath M. and Unverzagt C., Angew. Chem. Int. Ed., 44, 1650 (2005).
[5] Muir T. W., Annu. Rev. Biochem., 72, 249 (2003).

POLYMER ASSISTED SOLUTION PHASE (PASP) SYNTHESIS OF THE M-FACTOR MATING PHEROMONE FROM THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE

H.A. Zebroski

Proteomics Resource Center, Rockefeller University, New York NY, USA

Recent research is demonstrating that post-translational modification of proteins with isoprene residues is an essential feature of several critical signal transduction systems in eukaryotic cells. In general, these modifications occur at the C-terminal cysteines and serve to target the protein to the membrane. Peptide pheromones containing these modifications have been isolated from several species of yeast and other fungi. Many of these peptide pheromones are post-translationally modified by prenylation and/or carboxyl methylesterification, which prove to be highly important for their biological activity. M-factor is a diffusible mating pheromone controlling conjugation in fission yeast, characterized as a nanopeptide YTPKVPYMC in which the C-terminal cysteine is both S-farnesylated and the carboxyl exists as a methyl ester. Chemical synthesis of such peptides has proved to be challenging due to the instability of the farnesyl group to TFA or HF and thus they cannot be directly made using standard SPPS alone. Furthermore, the incorporation of esters into peptides often involves dangerous diazoalkanes. Therefore, a strategy combining SPPS and PASP synthesis was utilized to create this peptide. The method entails the standard Fmoc automated synthesis of the peptide and a post cleavage treatment with polymer bound diazomethane (1). The use of 4-(Methylazoamino) phenoxymethyl polystyrene allowed for the mild introduction of the methyl ester without the hazards normally involved. Farnesyl bromide in DIEA/DMF was used to introduce the prenyl group. The desired peptide was obtained in high purity after final Fmoc deprotection and preparative reversed phase separation.

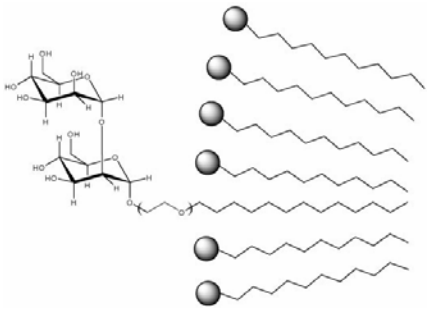
1. Rademann, J., et.al.,(2001) Angew. Chem. Int. Ed., 40,381.

A MODEL FOR CELL SURFACE EXPOSED CARBOHYDRATE UNITS

J. Mares[1], J.U. Müller[2], R.R. Schmidt[2], **O. Zerbe**[1]

[1]Department of Chemistry, University of Zurich [2]Department of Chemistry, University of Konstanz, Switzerland

Cell-cell recognition is an event of prime biological importance in a variety of biological phenomena and interactions with carbohydrate units on the cell surfaces are of prime importance. We have developed a model system for oligosaccharide units presented on cell surfaces. Therein, carbohydrates are linked via a flexible methyleneglycol linker to a lipid chain. The model system was verified by testing its binding capability to cyanovirin N, a cyanobacterial protein that interacts through high-affinity carbohydrate-mediated interactions with the surface-envelope glycoprotein gp120 from HIV and thereby blocks HIV entry We describe methods to characterize glycoconjugates when integrated into the micelles and present data on the polypeptide-carbohydrate interaction.
Moreover, we present data on our studies of the interactions of antimicrobial peptides with LPS, lipid A and model compounds thereof.



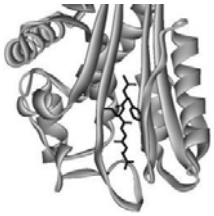
M142

INHIBITION OF ANTITRYPSIN POLYMERIZATION BY COMBINATORIALLY SELECTED 4-MER PEPTIDYL LIGANDS

Y.P. Chang[1], R. Mahadeva[2], W.S. Chang[3], Y.H. Chu[1]

[1]Department of Chemistry and Biochemistry, National Chung Cheng University, Chia-Yi, Taiwan [2]Department of Medicine, University of Cambridge [3]Institute of Cancer Research, National Health Research Institutes, Zhunan, Taiwan

Dysfunctional mutations of antitrypsin can result in a loss of elastase inhibitory activity or allow self-aggregation to occur and cause emphysema and cirrhosis, respectively. Insights of the mechanism of disease provide strategy to cope with the aberrant protein aggregation and may bring potential therapeutic agents. In the present work, we describe our effort to identify effective anti-protein polymerization ligands by the employment of combinatorial technology. Antitrypsin from human plasma was purified by glutathione Sepharose and Mono Q-Sepharose column chromatography. Both Ala-scanning and peptide shortening were carried out systematically to explore the structural requirements necessary for binding. Combinatorial chemistry was then employed to conduct the library screening experiments. Assessment of peptide binding was achieved through an unique gel electrophoresis assay. The structural requirements and the minimal peptide length required for binding were revealed by our systematic approach. This information was critical for the design of combinatorial library and the discovery of antitrypsin binding peptides with much improved affinity and specificity. There is currently no effective cure for Z antitrypsin related cirrhosis and emphysema. The synthesis and screening of combinatorial libraries offer avenues to increase throughput and ultimately lead to the discovery of inhibitory peptides to the polymerization of pathogenic antitrypsin.



Tu143

CHROMOGENIC SUBSTRATES OF SERINE PROTEASES DESIGNERD BY THE COMBINATORIAL CHEMISTRY METHODS

M. Wysocka, B. Kwiatkowska, **A. Lesner**, K. Rolka

Department of Bioorganic Chemistry, Faculty of Chemistry, University of GdańSk Poland

In our previous work [1] we described a simple method for selection of trypsin chromogenic substrates applying combinatorial chemistry methods. Benefit from results obtained we design two libraries of chromogenic substrates. First one was focused on two enzymes: bovine α-chymotrypsin and human leukocyte elastase (HLE). It consists of tetrapeptides containing the 5-amino-2-nitrobenzoic acid (Anb5,2) residue at their C-termini which served as a chromophore. Its general formula was as follow:

P4-P3-P2-P1-Anb5,2-OH

where: P1 =Lys, Arg, Ala, Val, Leu, Nle, Gly, Ser, Phe, Tyr
P2 = all proteinogenic amino acids, except Cys
P3 = Ala, Pro, Val, Phe, Ser, Lys, Arg, Glu
P4 = Phe, Asp, Lys, Val, Ile, Abu, Ala.
Shown below the second library was designed to select substrates of human β tryptase specificity:
P5-P4-P3-Asn-P1-Anb5,2
were P1 and P3 = Arg, Lys, Arg(NO2), Orn, Cit, Hci, Har, p-CN-Phe.
P4 and P5 = all proteinogenic amino acid residues except Cys.
Synthesis of both libraries was performed by the solid-phase method and deconvolution process was carried out in solution by the iterative approach. The selected substrates were resynthesized and their kinetic parameters kcat, KM and Vmax were determined.

1. Zablotna E, Dysasz H, Lesner A, Jaskiewicz A, Kazmierczak K, Miecznikowska H, Rolka K., Biochem Biophys Res Commun. 319, 185(2004).

RAPID COMBINATORIAL DEVELOPMENT OF AFFINITY CHROMATOGRAPHY LIGANDS FOR HUMAN GROWTH HORMONE BY A NOVEL BEAD ENCODING TECHNOLOGY

J.E. Rasmussen[2,1,3], S.F. Christensen[2], L.N. Lauritsen[3], K.J. Jensen[1]

[1]Department of Natural Sciences, The Royal Veterinary And Agricultural University, Frederiksberg [2]Versamatrix A/S, Valby [3]Novo Nordisk A/S, Bagsværd, Denmark

With the rapidly increasing number of biopharmaceuticals in the industrial pipeline the need for efficient and expedient purification procedures is growing ever greater. Affinity chromatography is one of the most promising technologies in this regard, as it offers very high selectivity and can often replace lengthy and expensive traditional chromatographic procedures. The use of combinatorial split-and-mix libraries is a powerful tool for discovering new affinity ligands but the technique has been limited by the laborious spectroscopic and chemical analysis needed to identify the binding ligand. We have previously introduced a novel bead encoding technology based on a 3-dimensional image recognition of patterns made by fluorescent particles randomly distributed inside larger beads.[1] The beads are read prior to each chemical transformation by an instrument featuring three fluorescence microscopes at a rate of 5,000 beads per hour. We here present the development of small peptidomimetic affinity ligands for the human growth hormone (hGH) by the use of this technology. The library was sought enriched prior to synthesis by in silico screening of a virtual combinatorial library using a large number of diverse building blocks. Binding ligands were identified by incubation with fluorescence tagged hGH. [1] Christensen, S.F., Meldal, M., Gen. Eng. News 2005, 25, 58-59

M145

SOLID PHASE SYNTHESIS OF SINAPOYL-PEPTIDE AMIDES

M.G. Spasova[1,2], S.S. Pancheva[2], T.I. Pajpanova[2], T.S. Milkova[1,3], G. Dibó[4], E.B. Dzambazova[5], A.I. Bocheva[5]

[1]South-West University "N.Rilsky", Blagoevgrad [2]Institute of Molecular Biology [3]Institute of Organic Chemistry With Center of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria [4]Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary [5]Department of Pathophysiology, Medical University, Sofia, Bulgaria

The cinnamic acids and their derivatives have been found to possess a variety of biological effects, including antiviral, Antimicrobial, antitumor and antioxidant activity. For example, several hydroxycinnamic acid conjugates with amino acids, isolated from plant sources showed enhanced antioxidant activity. The synthesis of cinnamic acid amides and their opioid activity was also cited in the literature. However the synthesis and pharmacological properties of sinapoyl-peptide amides continues to be virtually unexplored. On the other hand, the synthesis and opioid activity of analogs of Tyr-MIF-1 has been well documented by us. Herein we present a synthesis of a series of sinapoyl - peptide amides where sinapic acid were attached consecutively to both C- and N- end of the Tyr-MIF-1 peptide chain:

SA-Pro-Xaa-Gly-NH2; SA-Tyr-Pro-Xaa-Gly-NH2;

Pro-Leu-Gly-NH(CH2)nNH-SA

SA=sinapic acid; Xaa=Leu, unusual aminoacid; n=2,3

To obtain the sinapoyl-peptide-amides, both Fmoc- and Boc-based SPPS approach were used. Analgesic activity was determined by the Randall-Sellitto paw-pressure test. The antioxidant effects were examined by DPPH test as well. Studies to establish the importance of introducing the sinapoyl moiety in the Tyr-MIF-1 molecule for the antioxidant and opioid activities are underway.

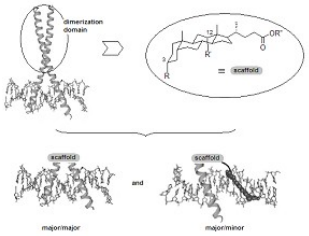
Tu146

DESIGN OF NEW DNA-BINDING STEROIDAL PEPTIDOMIMETICS

D. Verzele, A. Madder

Department of Organic Chemistry, Laboratory for Organic and Biomimetic Chemistry, Ghent University, Ghent, Belgium

Several proteins are involved in the transcription of DNA to mRNA, among which the basic leucine zipper (bZIP) proteins. These transcription factors bind specific DNA sequences by dimerization and inserting short alpha-helices into the DNA major groove.
Because the dimerization domain is only required to obtain the correct geometrical positioning of the alpha-helices, we will replace it by a dipodal steroid scaffold with defined stereochemistry. Due to orthogonal protecting groups, a unique feature of this scaffold is the possibility to design not only homodimers, but also heterodimers. Therefore this strategy allows for the construction of both major/major groove and major/minor groove binding peptides, either mimicking naturally occurring proteins or designing peptides with new binding properties.
Native chemical ligation and Staudinger ligation are both suitable for the construction of these peptide dimers. Moreover, a combination of solution- and solid-phase chemistry allows for the generation of combinatorial libraries.



HIGH-THROUGHPUT SCREENING AND OPTIMIZATION OF CYCLIC ANTIMICROBIAL PEPTIDES

D.F.H. Winkler[1], B.D. Bernard[1], Y. Chen[1], W. Jia[2], W.D. Campbell[1]

[1]PepMetric Technologies Inc. [2]University Of British Columbia, Brain Research Center, Vancouver, BC, Canada

The increasing number of antibiotic-resistant bacteria is a global health problem. Therefore the development of new highly efficient drugs is one of the major tasks of this century.
As an example of peptides, which inhibit the growth of E. coli, we demonstrate an easy and rapid method for finding peptides with optimized antimicrobial properties.
As a first step we built a modular construct. This construct consists of a constant cationic and a variable module. The cationic module was choosen to achieve cell-penetrating properties. The variable module was expected to act as the virtual active part of the peptide. To increase the proteolytic stability of the peptide we synthesized them in cyclic form.
In the first step we used the combinatorial approach to screen approximately 64.000.000 peptide sequences in the variable region in order to find highly active peptides against E. coli.
To optimize the identified sequence, we substituted all amino acids of the sequence with other amino acids and building blocks. Additionally, in order to increase stability we modified the bridging.
In this way we were able to uncover peptides with high antimicrobial activity as well as proteolytic stability and reasonable solubility.

M148

NEW MELANOCORTINS SEQUENCE HFRW MIMETICS

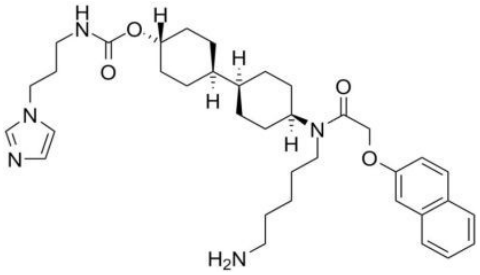
F. Mutulis[1], **S. Yahorava**[1], I. Mutule[1,2], A. Yahorau[1], E. Liepinsh[3], S. Veiksina[1,4], S. Kopanchuk[1,4], J.E.S. Wikberg[1]

[1]Department of Pharmaceutical Biosciences, Division of Pharmacology, Uppsala University, Uppsala, Sweden [2]Department of Organic Chemistry [3]Laboratory of Physical Organic Chemistry, Latvian Institute of Organic Synthesis, Riga, Latvia
[4]Institute of Organic and Bioorganic Chemistry, University of Tartu, Estonia

A series of melanocortin active core tetrapeptide HFRW nonpeptide imitations has been prepared using a combination of solution and solid phase synthesis.

Most of them included residue of 3-(1-imidazolyl) propylamine or histamine as substitutes of histidine. Phenylalanine residue, which is included in melanocortins was replaced by residues of derivatives of 4, 4'-disubstituted isopropylidenedicyclohexane, 4, 4'-disubstituted bicyclohexane, 1,4- disubstituted cyclohexane, 1,5- disubstituted cyclooctane, and 1,2-, 1,3- or 1,4-disubstituted benzenes. Instead of arginine, residues of oligomethylene diamines, 2-butyl-2-ethyl-1,5-pentanediamine, 4,4'-methylene-bis(cyclohexylamine), and 4,4'-diamino-diphenylmethane were introduced. 2-Naphtyloxyacetyl-, (4-1H-indol-3-yl)-butyryl-, 2-phenyl-ethanesulfonyl- and naphthalene-2-sulfonyl- groups served as replacement of tryptophan residue.

Tested on binding assay on melanocortin receptors, active core imitations exhibited a micromolar affinity to them. Isopropylidenedicyclohexane and bicyclohexane derivatives showed about 10 fold higher affinity compared with corresponding derivatives of cyclohexane, cyclooctane or disubstituted benzene.



PEPTIDOME: FORMATION PATTERNS, BIOLOGICAL EFFECTS AND SIGNAL TRANSDUCTION

E.Y. Blishchenko, O.V. Sazonova, A.G. Tolmazova, D.P. Khachin, O.N. Yatskin, A.A. Karelin, V.T. Ivanov

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

Peptidomes are represented in tissues and biological fluids by multiplicity of peptides and are formed by different cell types. Cell cultures were used to model peptidome formation. As composition and content of peptides present intracellularly and released by the cells were different, we considered tissue peptidomes to consist of 2 separate types of sub-peptidomes: intracellular peptide sets and those specifically released by cells into surrounding medium. Alterations of the content and the composition of individual peptide components took place in case of some diseases, i. e., these compounds can be considered as important biomarkers. To study the dependence of peptide release on cell status, we compared the sets of peptides released by intact human erythroid leukemia K562 cells, K562 cells subjected to differentiation and mature erythrocytes. The levels of several peptides released, including hemoglobin fragments, directly depended on cellular metabolism and cell differentiation degree. The endogenous fragments of functional proteins corresponding to different structure/activity groups demonstrated two main patterns of interaction with primary targets: (1) receptors on cell surface; (2) intracellular molecules. The signal transduction patterns of neokyotorphin-related growth stimulatory peptides are given as an example. Neokyotorphin acted intracellularly and increased Ca2+ influx through L-type channel with further involvement of PKA→MAPK/Erk and CaMKII pathways. Both sensitivity of target cells and the effects induced by the peptides depended on the contribution of the PKA-mediated signal transduction system to growth regulation and were most pronounced in the cells subjected to stress conditions, including growth factors deficiency or low cell density.

Th150

DETECTION OF SERUM ANGIOTENSIN CONVERTING ENZYME ACTIVITY, ASCORBIC ACID AND TOTAL ANTIOXIDANTS IN PATIENTS WITH CORONARY ARTERY DISEASE

B. Dabirmanesh[1], A. Shabani[1], M. Firoozray[2], F. Ghazi[2]

[1]Department of Biochemistry, Faculty of Science, Azzahra University [2]Department of Molecular Biochemistry, Iran University of Medical Sciences, Tehran, Iran

Angiotensin – converting enzyme (ACE) is a dipeptidyl carboxypeptidase (EC 3.4.15.1) that catalyzes the conversion of Angiotensin I to the potent vasoconstrictor Angiotensin II . Angiotensin II are responsible for an increase in blood pressure and maintenance of hypertension through the stimulation of oxidative stress . As oxidative stress increases, cardiovascular disease develops because the antioxidant defense systems are over loaded. There is some evidence that antioxidant therapy and ACE inhibitors may beneficial for the prevention of coronary heart disease .

: In this study we investigated the relationship between coronary artery disease (CAD), ACE activity, serum antioxidant status and ascorbic acid (vitamin C).Which may benefit patients with coronary artery disease.

A group of 50 patients with angiographically defind coronary artery disease (CAD) and 50 age and sex-match normal control subjects were studied.

The activity of angiotensin-converting enzyme (ACE) were determined by the reversed-phase high performance liquid chromatography (HPLC) to separate and quantify hippuryl- histidyl-leusin (HHL) and hippuric acid (HA).

We used Ferric Reducing Ability of Plasma (FRAP Assay) as a measure of Antioxidant power.

Serum ascorbic acid were determined photometrically .Ascorbic acid in plasma is oxidized by Cu(II) to form dehyroascorbic acid, which reacts with acidic 2,4-dinitrophenylhydrazine to form a red bis-hydrazone which is measured at A 520 .

Abnormal levels of ACE activity in serum have been related to coronary artery disease.

This study also demonstrated an association of antioxidants with CAD progression.

M151

STRUCTURAL ANALYSIS OF THE NEW OBESITY RELATED PEPTIDE: OBESTATIN

C. Esposito, M. Scrima, P. Campiglia, A.M. D'Ursi

Dipartimento di Scienze Farmaceutiche, Università di Salerno, Fisciano, Italy

Obestatin is a novel endogenous ghrelin-associate peptide, which is involved in the regulation of food intake and weight gain. It was shown to be anorexigenic, able to decrease food intake, gastric emptying and jejunal motility. Although obestatin and ghrelin originate from a common prepropeptide of 117 residues, they are reported to exert opposing physiological roles, by binding distinct receptors belonging to the subgroup of type A GPCRs [1]. Obestatin was found to be the natural ligand of the orphan GPR39 receptor, a GPCR, expressed in jejunum, duodenum, stomach, pituitary, ileum, liver and hypothalamus.
As many other peptides involved in the obesity process, it is a new and interesting drug target for the discovery of new anti-obesity molecules. In particular, the first step for the design of new molecules with potential improved anti-obesity activity, is the elucidation of the obestatin conformational features. Here, we present the synthesis and the conformational analysis by NMR and CD spectroscopies of obestatin and its related 13-mer C-terminal sub-fragment, in aqueous solution and in membrane mimicking environment. The data outline the obestatin C-terminal portion as the region characterized by significant conformational features potentially opened to interesting future developments.

[1] Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake, Science. 2005 Nov 11;310(5750):996-9.

Tu152

SURVEY FOR COMPETITIVE STRAINS OF SINORHIZOBIUM MELILOTI IN IRAN

Sh. Ghorbani

Department of Microbiology, Faculty of Science, Azzahra University, Tehran, Iran

A total of 50 isolates of Rhizobium were collected from root nodules of Medicago sativa and Melilotus officialis plants in different regions of isfahan province .all of isolates on TY medium formed white ,slimy colonies with smooth margins and their inoculation on to roots of young alfalfa plants produced spindly nodules . the nodules developed with some of the isolates were big and pinkish ,although the rest of isolates produced small and white nodules .the speed of nodulation for all the isolates was almost similar and the related nodules were appeared within two weeks . the production of brown pigments on aged colonies of some isolates on TY or TY supplemented with L_tyrosine and copper sulfate revealed that these isolates of S. meliloti are melanin-producing rhizobia.based on the motility and sensitivity to antibiotics tests ,all of the isolates formed a reasonably homogenous group .however a few of them were able to produce an anti-microbial compound which was found to inhibit a number of isolates of S. meliloti .the compound did not suppress the growth of other bacteria . partial purification and spectrophotometery of the compound suggested that it likely belong to the antimicrobial polypeptides .considering on their physiological and biochemical properties ,none of the isolates were selected as a superior and competitive strain ,although based on nodulation efficiency , melanin and antimicrobial compounds production capability the isolate S. meliloti SM2 and SA23 were nominated to investigate in details.

A NOVEL SUITE OF CYCLOTIDES FROM V. ODORATA: SEQUENCE VARIATION AND THE IMPLICATIONS FOR STRUCTURE, FUNCTION AND STABILITY

D.C. Ireland[1,2], **M.L. Colgrave**[1], D.J. Craik[1]

[1]*Institute for Molecular Bioscience, Australian Research Council Special Research Centre for Functional and Applied Genomics and The University of Queensland*
[2]*School of Business, University of Queensland, Brisbane, Australia*

Cyclotides are a fascinating family of plant-derived peptides characterized by their head-to-tail cyclized backbone and knotted arrangement of three disulfide bonds. This conserved structural architecture, termed the cyclic cystine knot, is responsible for their exceptional resistance to thermal, chemical and enzymatic degradation. Cyclotides have a variety of biological activities but their insecticidal activities suggest that their primary function is in plant defense. In this study we determined the cyclotide content of the sweet violet Viola odorata, a member of the Violaceae family. We identified 30 cyclotides from the aerial parts and roots of this plant, 13 of which are novel sequences. The new sequences provide information about the natural diversity of cyclotides and the role of particular residues in defining structure and function. As many of the biological activities of cyclotides appear to be associated with membrane interactions, we used hemolytic activity as a marker of bioactivity for a selection of the new cyclotides. The new cyclotides were tested for their ability to resist proteolysis by a range of enzymes and, in common with other cyclotides, were completely resistant to trypsin, pepsin and thermolysin. The results show that while biological activity varies with the sequence the proteolytic stability of the framework does not, and appears to be an inherent feature of the cyclotide framework. The structure of one of the new cyclotides, cycloviolacin O14, was determined and shown to contain the cyclic cystine knot motif. This study confirms that cyclotides may be regarded as a natural combinatorial template that displays a variety of peptide epitopes most likely targeted to a range of plant pests and pathogens. Furthermore, the inherent stability of the framework makes it an excellent scaffold for protein engineering applications.

M154

INVESTIGATING THE BIOCHEMICAL REASONS FOR SENSITIVITY OF SOME PATIENSTS TO ANTICOAGULANT WARFARIN

Z. Jomeh Farsangi[1], P. Ghadam[1], A. Shabani[1], R.A. Sharifian[2]

[1]Department of Biochemistry, Faculty of Science, Azzahra University [2]3 Faculty of Medicine, Tehran University, Tehran, Iran

Warfarin is the most widely prescribed anticoagulant drug for the prevention and treatment of arterial and venous thromboembolic disorders.Because of large interpatient variability in the dose-anticoagulant effect relationship and a narrow therapeutic index careful dosage adjustment based on INR is essential. Warfarin is available as a racemic mixture of two enantiomers,(S)-and (R)-warfarin. In contrast to (R)-warfarin, which is metabolized by multiple cytochrome P450s(CYPs), Including CYP1A2 and CYP3A4,(S)-warfarin, is predominantly metabolized to 7-hydroxywarfarin by polymorphic CYP2C9. Since the potency of (S)-warfarin is much higher than that of (R)-warfarin, about 3-to 5-fold,any change in the activity of CYP2C9 gene is likely to have a significant influence on the anticoagulant response. Previous in vitro findings revealed that certain variants in the CYP2C9 gene are associated with large interindividual differences in the pharmacokinetic and pharmacodynamic outcomes of warfarin therapy. Three major alleles have been found to date in humans:Arg144/Ile359, and Cys144/Ile359 and Arg144/leu359, and Arg144/Leu359, which have been designated CYP2C9*1 (wild-type), CYP2C9*2, and CYP2C9*3, respectively. We have investigated this polymorphism in Iranians that has not been described previously. Genomic DNA was isolated from whole blood. For detection of CYP2C9*2, and CYP2C9*3 variants, a protocol based on PCR technique and Endonuclease digestion with KpnI, Ava II was used. In this research work, we have studied a group of 56 patients, in which warfarin therapy was initiated.

Keywords: Warfarin, Polymorphism,CYP2C9,Anticoagulant

Tu155

SYNTHESIS OF ARENICIN-1 – AN ANTIMICROBIAL PEPTIDE FROM COELOMOCYTES OF MARINE POLYCHAETA ARENICOLA MARINA

N.I. Kolodkin[1], I.V. Afonina[1], A.D. Krasnodembskaya[2], M.P. Smirnova[1], V.M. Shpen[1]

[1]Laboratory of Peptide Chemistry, State Research Institute of Highly Pure Biopreparations [2]Faculty of Biology and Soil Sciences, Biochemistry Department, Saint-Petersburg State University, Saint-Petersburg, Russia

Recently new 21-residue antimicrobial peptides - arenicins were isolated from coelomocytes of marine polychaeta Arenicola marina and their sequences were determined [1]. There are two isoforms of arenicins which differ only with single amino acid. These peptides have no structure similarity to any previously identified antimicrobial peptides. We have synthesized and estimated the antibacterial properties of arenicin-1: RWCVYAYVRVRGVLVRYRRCW. The linear peptide was prepared by solid phase method using Boc-technology without any problem. However the cyclization caused the appreciable difficulties. The following methods of oxidation were used: oxygen of air, K3Fe(CN)6 and hydrogen peroxide in aqueous or organic media. The best results were obtained by using hydrogen peroxide in methanol, but and in this case the yield of the aim peptide did not exceed 5%. Synthetic arenicin had the same HPLC profile and MALDI-TOF spectra as a natural molecule. The peptide showed an antimicrobial activity against Gram-positive bacteria: Listeria monocytogenes EGD, Staphylococcus aureus MR ATCC 33591, Staphylococcus aureus SG 511, Staphylococcus aureus ATCC 25293, Gram-negative bacteria: Escherichia coli ML-35p, Escherichia coli M-17, Escherichia coli ATCC 25922, Pseudomonas aeruginosa and fungi Candida albicans 820. Minimal inhibitory concentrations of synthetic arenicin-1 against all tested strains were less than 1 mkmol and similar to those of natural arenicin-1.

[1] Ovchinnikova T.V. et al. FEBS Lett. 577, 209 (2004)

SYNTHETIC PEPTIDE IMMUNOCORTIN STIMULATES THE PRODUCTION OF 11-OXYCORTICOSTEROIDS BY RAT ADRENAL CORTEX THROUGH CORTICOTROPIN RECEPTOR

V.M. Lipkin[1], V.I. Vanina[1], A.A. Kolobov[2], E.A. Kampe-Nemm[2], E.V. Navolotskaya[1]

[1]Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Pushchino, Moscow Region [2]State Research Center for Institute of Highly Pure Biopreparations, Ministry of Health of The Russian Federation, St. Petersburg, Russia

We have previously synthesized the decapeptide VKKPGSSVKV corresponding to the sequence 11-20 of human IgG1 heavy chain (referred to as immunocortin, IMC). The study of receptor specificity and biological activity of IMC revealed its high affinity binding to corticotropin receptors on synaptic membranes in the mouse brain and on various immunocompetent cells in human and mouse, as well as the activation of adenylate cyclase in the target cells. In this study we investigated the effects of IMC on the level of 11-oxycorticosteroids (CS) in rat adrenal glands and plasma in vivo, and the binding of [3H]IMC to rat adrenal cortex membranes in vitro. Intramuscular injection of IMC at doses of 10–100 microg/kg was found to stimulate the secretion of CS from the adrenals to the bloodstream. Assuming that the effects of IMC on adrenal cortex cells are mediated by corticotropin receptors, we have prepared [3H]IMC and investigated its receptor binding characteristics. Receptor binding studies revealed that [3H]IMC bound with high affinity and specificity to corticotropin receptor on rat adrenal cortex membranes (Kd = 2.1 nM) and these receptors may mediate the effects of IMC on the CS secretion. IMC at concentrations of 10 – 1000 nM was found to increase the adenylate cyclase activity in adrenal cortex membranes. Thus, IMC interaction with immunocompetent cells, brain synaptic membranes and adrenal cortex membranes includes the following main steps: binding to the corticotropin receptor, activation of adenylate cyclase, and elevation of the intracellular cAMP content.

M157

FUNCTIONAL CRYPTIC PEPTIDES AND THE PHYSIOLOGICAL ROLES OF THEM

H. Mukai[1], N. Ueki[1], K. Someya[1,2], M. Kawanami[1], M. Kiyama[1], K. Wakamatsu[3]

[1]Laboratory of Peptide Biosignal Engineering, Mitsubishi Kagaku Institute of Life Sciences, Machida, Tokyo [2]Department Od Applied Biochemistry, Faculty of Engineering, Tokai University, Hiratsuka, Kanagawa [3]Department of Biochemical and Chemical Engineering, Faculty of Engineering, Gunma University, Kiryu, Gunma, Japan

Peptidergic hormones and neurotransmitters are known to be produced by the specific cleavage of their precursor proteins that per se have no biological functions. The neutrophil-activating peptides we recently identified, however, are the peptides cleaved from mitochondrial proteins by proteolysis. Therefore, we named them "functional cryptic peptides" because they are hidden in protein sequences. Some of these peptides activate Gi type of G proteins directly, and neutrophils are suggested to be stimulated by the direct (i.e., not via GPCRs) activation of G proteins. These peptides had features, in common, in their distributions of charged and hydrophobic amino acid residues, but homologies in their primary structures were not apparent. In the present study, we predicted functional cryptic peptides that activate G proteins, based on the distribution of charged and hydrophobic residues. Receptors for these peptides were also investigated by the direct cross-linking experiments between peptides and their targeted proteins. The finding of functional cryptic peptides is expected to lead to the identification of novel signaling mechanisms where such peptides are involved in the regulation of bio-functions.

Tu158

SYNTHETIC CORTICOTROPIN-LIKE PEPTIDE GKVLKKRR, CORRESPONDING TO THE FRAGMENT 81-88 OF THE PRECURSOR OF HUMAN INTERLEUKIN-1ALPHA, IS AN ANTAGONIST OF CORTICOTROPIN RECEPTOR

E.V. Navolotskaya[1], V.I. Vanina[1], A.A. Kolobov[2], E.A. Kampe-Nemm[2], V.M. Lipkin[1]

[1]Branch of Shemyakin and Ovchinnikov Institute Of Bioorganic Chemistry, Pushchino [2]State Research Center for Institute of Highly Pure Biopreparations, St. Petersburg, Russia

The fragment 81-88 of the precursor of human interleukin-1alpha (pIL-1α) (GK-VLKKRR) appeared to have more than 80% homology with corticotropin fragment 10-18 (GKPVGKKRR). We have previously synthesized the octapeptide GKVLKKRR (referred to as leucocorticotropin, LCT) and found its high affinity binding to corticotropin receptors on various immunocompetent cells in human and mouse. In this study we investigated the interaction of LCT with rat adrenal cortex membranes and the effects of LCT on the level of 11-oxycorticosteroids (CS) in rat adrenal glands and plasma in vivo. LCT was labeled with tritium by the high-temperature solid-state catalytic isotope exchange reaction to specific activity of 22 Ci/mmol. Receptor binding studies revealed that tritium-labeled LCT bound with high affinity and specificity to corticotropin receptor on rat adrenal cortex membranes (Kd = 2.2 nM). LCT at concentrations of 0.1 – 1000 nM was found to have no influence on the adenylate cyclase activity in adrenal cortex membranes, while intranasal injection of LCT to rats at doses of 10 - 50 microg/kg was found to inhibit the secretion of CS from the adrenals to the bloodstream. Thus, LCT is an antagonist of corticotropin receptor.

Th159

BIOCHEMICAL ANALYSIS OF VKORC1 POLYMORPHISMS IN SOME WARFARIN RESISTANCE IRANIAN PATIENTS USING PCR TECHNIQUE

F. Sadeghian[1], P. Ghadam[1], S. Sadrai[2], R. Sharifian[3]

[1]Department of Biochemistry, Azahra University [2]Department of Pharmacology
[3]Department of Medicine, Tehran University, Tehran, Iran

Comarin derivatives such as warfarin are prescribed widely for treatment and prevention of thrombosis. Warfarin is the widespread oral anticoagulant drug employed, but its required dose is highly variable both inter-individually and inter-ethnically. So it is desirable to develop strategies to predict the warfarin dose response in patients before initiation of anticoagulation.

The vitamin K- dependent γ-carboxilation system, Consists of the vitamin K- dependent γ-carboxylase, which requires the reduced hydroquinone form of vitamin K1 as a cofactor and the warfarin sensitive enzyme vitamin K1 2,3-epoxide reductase (VKOR), which produces the cofactor.

Warfarin exerts its anticoagulant effect by inhibiting the vitamin K epoxid reductase enzyme complex (VKOR) that recycles vitamin K1 2, 3-epoxide to vitamin K1 hydroquinone.

A component of the VKOR termed VKORC1, has now been identified as a therapeutic target site of warfarin. Point mutations were identified within the gene encoding VKORC1 in individuals who required large doses of wafarin to maintain therapeutic anticoagulation. However the relationship between the primary structure of VKORC1 and the mechanism of action of warfarin is poorly understood.

In this study we want to show if there is any point mutation in Iranian warfarin resistance patients. So we identified 6 patients who require daily maintenance doses of warfarin > 15 mg. PT and INR of the patients were all be measured.

M160

HOMEOSTATIC POTENTIAL OF FRAGMENTS OF FUNCTIONAL PROTEINS

O.V. Sazonova, A.G. Tolmazova, D.P. Khachin, E.Y. Blishchenko, A.A. Karelin, V.T. Ivanov

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

In previous works we have shown that naturally occurring functional protein fragments affect cell proliferation [1]. Their mechanisms of action involve receptors of "classical" regulatory peptides, or are non-receptoric [1]. Among protein kinases involved are PKA, CaMKII, MAPK [2]. In organism bioactive functional protein fragments could participate in maintenance of tissue homeostasis. In present work, homeostatic potential of functional protein fragments was studied in compare with classical regulatory peptides. The panel of test substances was formed, including signal transduction modulators (PKA, PKC, Ca2+-channel activators), classical regulatory peptides (bradykinin, somatostatin, Met-enkephalin, endothelin, neurotensin) and fragments of functional proteins (β-actin fragments from (75-90) and (69-77) segments; valorphin (β-globin (33-39)); neokyotorphin (α-globin (137-141); short acidic peptides from multiple precursors). Their activity was tested in cultures derived from similar sources but differing in transformation degree (e.g., mouse embryonic vs. tumor fibroblasts) and/or culturing conditions. The factors most affecting cell sensitivity to the test substances were (in order of the importance decrease): (1) cell type; (2) transformed vs. normal phenotype; (3) cell density; (4) serum supply. Activity of fragments of functional proteins, showing general correlation with other test substances, was more influenced by the culturing conditions (i.e., cell population status). Thus, fragments of functional proteins could be regarded as partners of "classical" homeostasis regulators, playing role of finer tuners of tissue proliferative status.

The study was supported by RAS Presidium programme "Molecular and cell biology"

1. Ivanov VT, et al., J. Pept. Sci., 2003, 553.
2. Sazonova OV, et al. in: Peptides 2005, in press.

Tu161

CLONING OF STREPTOKINASE MUTANT GENE

A. Shabani[1], B. Kazemi[2], F. Fallahian[1], **Z. Afraz**[3], M. Asgari Mehr[3], N. Seyed[2]

[1]Department Of Biochemistry, Science Faculty, Azzahra University [2]Cellular and Molecular Biology Research Center, Medical University of Shaheed Beheshti
[3]Medical Faculty, University of Tehran, Iran

Streptokinase (SK) is a streptococcal protein specially used for the treatment of thrombolytic disease and acute myocardial infarction (AMI). This protein is a potent activator of the fibrinolytic enzyme system in humans. It is proved that the presence of anti-SK antibodies in patients due to previous streptococcal infections or thrombolytic therapy reduces the efficiency of thrombolytic therapy and may cause a range of allergic reactions . In this study C-terminal immunodominant epitope of native molecule was eliminated and then the mutant gene was cloned into expression vector , pGEMEX-1 , to produce an engineered variant of SK being functional and less antigenic than the native molecule .
Genomic DNA from streptococci group A was isolated from 5ml bacterial cultures grown overnight at 37˚c . The SK mutant genewas amplified by PCR using two specific primers. The PCR-amplified mutant gene was cloned into SacI and BamHI sites of pGEMEX-1 expression vector .Then this recombinant vector was transferred into DH5α cells as host.

Key words : Streptokinase, Epitope , Immunodominant.

Th162

ASSAYING OF THE PRESENCE OF H_NS PROTEIN IN A NATIVE STRAIN OF HALOMONAS SPECIES

Sh. Shahriari, P. Ghadam, A. Shabani, M.R. Soudi

Department of Biochemistry, Faculty of Science, Azzahra University, Tehran, Iran

In prokaryotic cells, the organization and/or the function of their chromosomal DNA require the involvement of proteins, called histone-like proteins. One of the most abundant of these proteins especially in gram negative bacteria is H-NS. H-NS involved in structurally organizing the nucleoid of bacterial cells. It is also involved in the regulation of many pathways, most of which are related to the response of the cell to environmental changes.
In this study, the presence of H-NS protein in a novel gram negative bacterium was examined. This bacterium belongs to a native strain of Halomonas species which was recently isolated from surface saline soil of the Karaj region, Iran. To do so, perchloric acid 5% was used for extraction procedure. This method was used for extraction of histone proteins from eukaryotic cells previously. Because histone and histone-like proteins are similar to each other, this procedure was chosen. Since H-NS is purified and characterized in E.coli, this bacterium was used as the control. The results of running the protein extracts on SDS-PAGE demonstrated a band around 15 KDa which was seen in protein extracts of control bacteria. The final result of the research supported the hypothesis of the presence of H-NS protein in the studied bacteria.

Keywords: H-NS, gram negative bacteria, histone-like proteins, protein extraction.

M163

ASSAY THE PRESENCE OF SOME HISTONE-LIKE PROTEINS (FOR EXAMPLE HU) IN HALOBACILLUS KARAJIENSIS

N. Shariatian[1], P. Ghadam[1], A. Rabbani[2], M.A. Amoozegar[3]

[1]Department of Biochemistry, Azzahra University [2]Institute of Biochemistry and Biophysics [3]Faculty of Science, University of Tehran, Iran

Histone-like proteins in bacteria are small basic proteins that contribute to the control of gene expression, recombination and DNA replication. They are also an important factor in compressing the bacterial DNA in the nucleoid. Among the HLPs, HU protein is attracted to DNA containing structural aberrations such as four way junctions or single stranded lesions. This protein plays an important role in binding as a dimer and bending DNA. It also contributes to the beginning of the DNA replication. In this study we showed that a 10-KDa protein, probably HU exists in Halobacillus karajiensis which is a novel gram positive moderate halophile bacteria that was recently isolated from surface saline soil of the Karaj Region, Iran. Since HU is purified and characterized in E.coli we used this bacteria as the control in this study. The 10-KDa protein extraction was carried out by using PCA 5% which is normally used for extracting Histones from eukaryotic cells. The results of running the protein extracts on SDS-PAGE demonstrated a band around 10-KDa which was seen in protein extracts of this protocol. These results supported the hypothesis of the existence of a 10-KDa protein in Halobacillus karajiensis.

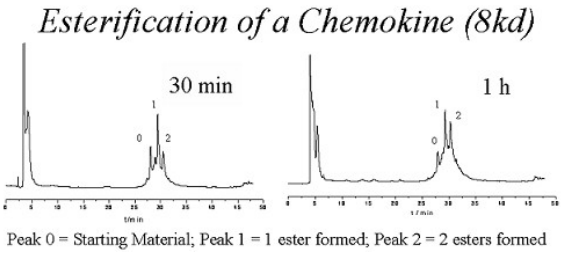
Tu164

NEW STRATEGIES TO ENHANCE (POLY)PEPTIDE AND PROTEIN THERAPEUTICS

J. Vizzavona, **P. Botti**

DBSB Department, CMU, University of Geneva, Switzerland

Coupling of therapeutic peptides and proteins to various polymers (e.g. pegylation) has been employed both to reduce their immunogenic response, to increase resistance to enzymatic degradation and to prolong their circulatory half-life. The chemistry employed to derivatize peptides and proteins with polymers usually produces permanent linkages and in some cases the therapeutic molecule cannot sustain multiple functionalizations without losing activity. Here we present a novel, robust and mild methodology that enables polymers, lipids, and other conjugates and macromolecules to be attached to peptides and proteins in a controlled manner. Conjugation is via ester linkages, which are cleavable in vivo either by (a) uncatalysed hydrolysis or (b) enzymatic cleavage by esterases.



Peak 0 = Starting Material; Peak 1 = 1 ester formed; Peak 2 = 2 esters formed

Th165

IDENTIFICATION OF PEPTIDES HOMING SPECIFICALLY TO CO-OPTED TUMOR VASCULATURE

J.K. Enbäck[1], H. Song[2], A. Närvänen[3], G. Bergers[2], P. Laakkonen[1]

[1]Molecular, Cancer Biology Research Program, Biomedicum Helsinki, University of Helsinki, Finland [2]Department of Neurological Surgery and BTRC, Comprehensive Cancer Center, UCSF, HSE722, San Francisco, CA, USA [3]Department of Chemistry, University of Kuopio, Finland

Sufficient oxygen and nutrient delivery is a necessity for tumors. When oxygen supply decreases, tumors initiate growth of new blood vessels. Low grade astrocytomas, a class of malignant brain tumors, grow along the existing vessels in a process called co-option. Hypoxia is induced in the progression from grade III to grade IV astrocytomas (glioblastoma multiforme, GBM) which in turn triggers the formation of a new and distinct tumor vasculature. The new vessels formed by tumor-triggered angiogenesis differ by molecular composition from their normal vascular counterparts.

We are utilizing phage displayed peptide libraries to identify peptides that specifically home to either co-opted or angiogenic brain tumor vessels. Furthermore, we aim at characterizing differentially expressed endothelial markers (receptor molecules) to get a better understanding of the molecular changes in the vasculature.

Several rounds of in vivo biopanning was performed in mouse models of astrocytomas to isolate a phage pool that has up to a hundred-fold homing to low grade tumor lesions. Out of the selected pool we discovered peptides capable of homing and accumulating to the tumor islets and co-opted vasculature. The homing potential of our newly identified peptides has shown to be highly specific for clusters formed by the tumor cells and co-opted "early" vessels within these palisades.

These homing peptides represent promising candidates to selectively target co-opted vessels and tumor lesions in the brain and act as lead compounds in identification of surface molecules (receptors) differentially expressed by co-opted tumor vessels.

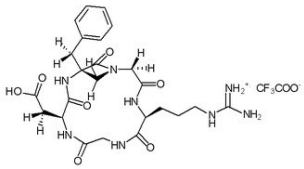
M166

ANGIOGENESIS INHIBITION ACTIVITY OF A CYCLIC RGD-BETA-LACTAM PENTAPEPTIDE

C. Palomo[1], J.M. Aizpurua[1], J.I. Ganboa[1], I. Loinaz[1], **X. Fernandez**[1],
J.L. Castrillo[2], A. Garcia[3]

[1]UPV/EHU,Departamento de Química Orgánica I, Facultad de Química, Paseo Manuel de Lardizabal S/n, San Sebastian [2]CBM "Severo Ochoa" Universidad Autónoma de Madrid, Cantoblanco, Madrid [3]PROTEOMIKA S.A., Parque Tecnologico de Bizkaia, Ed. 801 B, Derio, Spain

The αvβ3 integrin receptors play an important role in human tumor metastasis and growth. The inhibition of these receptors by antibodies or by cyclic peptides containing the Arg-Gly-Asp(RGD) sequence may be used as selectively treatment to suppress the disease.
Our research group has previously described that the formal introduction of a single carbon atom to bridge the Cα (i) and N(i+1) contiguous residues of a linear or cyclic peptide leads to α-amino-β-lactam peptidomimetics containing predictably placed β-turn and γ-turn motifs, respectively. The combination of these results with the well-known capacity of RGD tripeptide for inhibition of the biological answer in integrin led us to the design of the following cyclic peptide.
The adhesion and cell-growth "in vitro" assays using human umbilical vein endothelial cells (HUVEC), as well as "in vivo" assays with xenograph mice revealed that the RGD peptidomimetic was active to micromolar concentrations, slightly better than the reference compound in this field: Cilengitide®.



Tu167

THE STABILITY OF SYNTHETIC STATHERIN IN EXTRACTS OF HUMAN PAROTID, SUBMANDIBULAR AND SUBLINGUAL GLANDS

E. Kamysz[1], D. Jackiewicz-Barańska[1], J. Ochocińska[2], L. Łobocki[1],
B. Kochańska[2], Z. Maćkiewicz[1]

[1]Faculty of Chemistry [2]Department of Conservative Dentistry, Medical University of GdańSk, Poland

Whole saliva is composed of secretions from parotid, submandibular and sublingual glands, and smaller ones from saliva of minor salivary glands (e.g. palatal and labial). Saliva contains a variety of proteins and polypeptides. One of them is statherin, a multifunctional 43-amino acid residue, phosphominiprotein. This peptide is present in human parotid and submandibular saliva .

The aim of our study was to investigate the stability of statherin in extracts of the major salivary glands.

Submandibular, sublingual and parotid gland tissues were obtained at autopsy 12 h after death. Samples of the gland tissues were homogenized, centrifugated (30,000 g, 30 min., 4□C) and the supernatants were frozen and stored at –70 □C prior to analysis. Synthetic statherin was added to the supernatants before analysis (45 microgram/ml). The samples were analysed for the presence of the peptide by the matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS}technique and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE).

Statherin has been found to be decomposed in extracts of parotid and submandibular glands and also in the extract of sublingual glands.

This work was supported by the University of Gdańsk (Grant No. DS 8362-4-0135-6) and Medical University of Gdańsk (Grant No. ST-31) .

Th168

DENDRIMERIC PEPTIDE INHIBITORS OF ANTHRAX LETHAL AND EDEMA FACTORS

L. Lozzi[1], A. Pini[1], C. Falciani[1], B. Lelli[1], J. Brunetti[1], S. Pileri[1], M. Fabbrini[1], A. Bernini[1], F. Tonello[2], F. Dal Molin[3], N. Niccolai[1], L. Bracci[1]

[1]Department of Molecular Biology, University of Siena, Siena [2]Neuroscience Institute, CNR [3]Department of Biomedical Sciences, University of Padova, Italy

The dramatic increase in research for new anthrax therapeutic approach was prompted by potential use of the causative agent of anthrax Bacillus anthracis as a biological weapon. Anthrax toxin consists of three proteins, the protective antigen (PA) and the two enzymes lethal factor (LF) and edema factor (EF) that are carried through the membrane of the target cell upon binding to specific site on the membrane receptor-bound PA. Lethal factor and edema factor were found to cooperate to promote immune evasion of the bacterium. Here we describe the production of peptide inhibitors of PA-LF binding, obtained by selecting PA-binding peptide by a competitive panning of a phage peptide library, using recombinant LF. We selected several 12 mer peptides, which were synthesized in tetra-branched Multiple Antigen Peptide (MAP) form, inducing resistance to proteolytic degradation (1) and maintaining biological activity of phage peptides. Lead tetra-branched peptides were systematically modified by progressive shortening and residue randomization, to obtain an increase of peptide affinity and inhibitory efficiency. Affinity maturation of lead sequences enabled selection of a peptide which has an IC50 at least one log lower that any other lethal-toxin-inhibiting peptide described so far and is effective for in vivo neutralization of anthrax toxin activity (2). The same peptide can also efficiently inhibit the binding of EF to PA and EF-induced cAMP increase in different cell lines.

1) Bracci L. et al., (2003), J. Biol. Chem., 278, 46590-46595;
2) Pini A. et al., Biochem. J., in press

M169

EFFECT OF ARSENIC TRIOXIDE ON SHEEP BRAIN MICROTUBULE POLYMERIZATION IN VITRO

R. Mahinpour[1], G.H. Riazi[1], M.N. Sarbolouky[1], A.A. Moosavi-Movahedi[1],
S.Z. Moosavi-Nejad[2], H. Akhani[3]

[1]Institute of Biochemistry and Biophysics, University of Tehran [2]Department of Biology, Faculty of Science, Alzahra University [3]Department of Botany, School of Biology, College of Science, University of Tehran, Iran

Microtubules are dynamic polymers that have important roles in eukaryotic cellular processes such as signal transduction, cell polarity, vesicular transport and chromosomal movement. The dynamic behavior of microtubules has been studied both in vivo and in vitro. The effect of arsenic trioxide on microtubule polymerization has been studied under in vivo experimentation shown that it inhibits formation of mitotic bundles. We studied the mechanism of arsenic trioxide effect on polymerization of microtubule protein purified from sheep brain in vitro.

Microtubule polymerization has been conducted by adding 1mM GTP to purified tubuline in PEM buffer at 37oC for 30 minutes and simultaneously followed by measuring turbidity (350 nm). The results shown that lag time of polymerization (nucleation step) is affected by increasing concentrations of arsenic trioxide from 0-5 micromolar. Moreover the rate of elongation step was decreased exponentially by increasing arsenic trioxide concentration. Electron micrographs also showed microtubules length decrement due to arsenic trioxide. The results have shown the inhibitory effect of arsenic trioxide on microtubule polymerization via its effect on nucleation step as well as elongation rate.

Tu170

A NOVEL APPROACH TO ALZHEIMER'S DISEASE THERAPY: INHIBITION OF Aß42 OLIGOMERIZATION BY C-TERMINAL Aß42 FRAGMENTS

B.H. Monien[1], E.A. Fradinger[1], S.M. Spring[1], S.L. Bernstein[2], M.T. Bowers[2], G. Bitan[1]

[1]Department of Neurology, David Geffen School of Medicine, UC Los Angeles, Los Angeles, CA [2]Department of Chemistry and Biochemistry, UC Santa Barbara, Santa Barbara, CA, USA

Background and Aims: Alzheimer's disease (AD) is the major cause of dementia among the elderly. The increase in life expectancy worldwide demands new therapies for AD urgently. Self-association of the amyloid ß-protein (Aß) into neurotoxic assemblies, a seminal event in the etiology of AD, is considered to follow interactions of the C-terminus of the 42-residue form of Aß (Aß42). We hypothesized that molecules with high affinity for the C-terminus of Aß42 will disrupt Aß42 oligomerization. A series of C-terminal fragments (CTFs) of Aß42, Aß(x-42) with x = 28-39, has been prepared to study their potential to inhibit Aß42 oligomerization and neurotoxicity.
Methods: Attenuation of Aß42 assembly by CTFs was studied by quantitative analysis of oligomer size distributions using a photo-cross-linking assay followed by SDS-PAGE. Biological activity of CTFs themselves and as inhibitors of Aß42-induced neurotoxicity was assessed by MTT reduction assay using differentiated PC-12 cells. The structure of the CTFs was studied by circular dichroism (CD) spectroscopy and ion mobility spectrometry-mass spectrometry (IMS-MS) coupled with molecular dynamics (MD) simulations.
Results: CTFs were found to inhibit Aß42 oligomerization in a length dependent manner with minimal or no toxicity of the CTFs themselves. Certain CTFs were found to inhibit Aß42-induced neurotoxicity. CD spectra indicate that increasing peptide length results in growing ß-sheet content. Structures based on experimentally determined cross-sections support the existence of a previously proposed turn around residues Gly37-Gly38. The data suggest that Aß42 CTFs can serve as lead compounds for development of peptidomimetic drugs for treatment and prevention of AD.

DIFFERENCES IN PLASMA STABILITY BETWEEN LINEAR AND CYCLIC FORMS OF HK2 PEPTIDE INHIBITOR

M. Pakkala[1], C. Hekim[2], H. Koistinen[2], U.-H. Stenman[2], J. Vepsäläinen[1],
A. Närvänen[1]

[1]Department of Chemistry, University of Kuopio [2]Department of Clinical Chemistry, Biomedicum, University of Helsinki, Finland

Background and aims: Human Kallikrein hK2 is a prostate specific serine protease, which expression level is elevated in aggressive human prostate cancer suggesting a possible role in a tumour growth and spreading. Since hK2 protease is highly prostate specific, inhibition of its activity is a possible method to prevent tumour growth without interfering the function of the other proteases. We have identified hK2 specific linear peptide inhibitor by using phage display techniques. In order to design peptide for in vivo studies we tested the protease stability of the linear and the cyclic forms of the peptide.
Methods: The prerequisite of the binding was studied by using conventional Ala-replacement method and the most optimal sequence was selected for further studies. The stability of the original linear form, acetylated form, peptide with cystein bridge and head-to-tail cyclic peptide was tested with modified trypsin (sequencing grade) and with human plasma.
Results: Both linear versions and peptide with cystein bridge were unstable and were degraded during the first 30 minutes in both stability tests. Head-to-tail form of the peptide was stable in both tests during the first 180 minutes.
Conclusions: Since our peptide contains arginine there was a possibility that our peptide is sensitive to trypsin and other serum proteases. Indeed both linear and one cyclic from degraded in our tests. Only head-to-tail peptide was stable during the first 3 hours suggesting protease resistant folding.

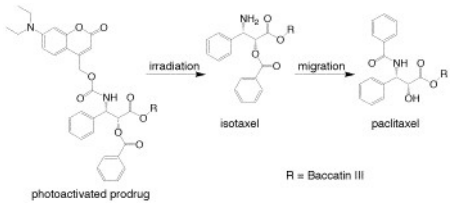
M172

DEVELOPMENT OF FIRST PHOTOACTIVATED PRODRUG OF PACLITAXEL VIA MODIFICATION OF PHENYLISOSERINE MOIETY

M. Skwarczynski[1], **M. Noguchi**[1], S. Hirota[2,3], Y. Sohma[1], T. Kimura[1], Y. Hayashi[1], Y. Kiso[1]

[1]Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science [2]Department of Physical Chemistry , 21st Century COE Program, Kyoto Pharmaceutical University, Kyoto [3]Presto, Jst, Kawaguchi, Saitama, Japan

Background and aims: A large number of anticancer agents has been developed in recent years. However, these agents have very little or no specificity which leads to systemic toxicity. Among them paclitaxel is considered to be one of the most important drugs in cancer chemotherapy; however, this agent has also lack of selectivity to the tumor tissue. Therefore, development of tumor-targeting prodrug is highly promising.
Methods: To activate cytotoxic agent specifically at the tumor tissue, we developed a new prodrug strategy based on O–N intramolecular acyl migration, which is a well-known reaction in peptide chemistry, and photodynamic therapy.
Results: We synthesized a prodrug which has a coumarin derivative conjugated to the amino acid moiety of isotaxel (O-acyl isoform of paclitaxel). The prodrug was selectively activated by visible light irradiation (430 nm) leading to cleavage of coumarin. Finally, paclitaxel was released by subsequent O–N intramolecular acyl migration.
Conclusion: We synthesized and evaluated a novel type of paclitaxel prodrug. This prodrug showed promising kinetic data. Therefore, we believe that photoactivation can be promising novel strategy for design of tumor-targeting prodrugs.



Tu173

ENZYMATIC DEGRADATION OF ENDOTHIOPEPTIDES

J. Nowak[1], R. Andruszkiewicz[1], J.W. payne[2]

[1]Department of Pharmaceutical Technology and Biochemistry, GdańSk University of Technology, Gdańsk, Poland [2]School of Biological Sciences, University of Wales Bangor, UK

It is generally accepted that peptides can be used as vehicles for drug uptake [1,2]. The concept of utilizing peptide transport system for delivery of toxic amino acids into the microbial cells is characterized by authors as „illicit transport" [2]. The peptide transporters are best described at a molecular and biochemical level in bacteria cells, exemplified by the three archetypal permeases for di-, tri- and higher oligopeptides (Dpp, Tpp, Opp) [3].
Peptides are not stable in physiological fluids. That is the reason for their modification in order to improve their stability and bioavailability. Analogs with modified amide backbone are more resistant towards enzymatic degradation. It has been shown that enzymatic hydrolysis of endothiopeptides is often significantly slower than natural peptides [4].
In our studies on antimicrobial peptides containing inhibitors of glucosamine-6-phosphate synthase, a potential target for antimicrobial chemotherapy, three endothiopeptides: Ala-Ψ[CSNH]-Ala, Ala-Ψ[CSNH]-Leu, Ala-Ψ[CSNH]-Phe have been synthesised. Transport and endothiopeptides cleavage studies were performed using five different E. coli strains, purified Carboxypeptidase A and broken cell crude extract of E. coli. Results were also compared to those obtained for adequate natural peptides.

1] J. W. Payne, C. Gilvarg, Adv. Enzymol. Relat. Areas Molec. Biol. 35, 187, 1971
[2] B. N. Ames, G. F. L., Ames, J. D. Young, D. Isuchiya, J. Lecocq, PNAS, 70, 456, 1973
[3] M. W. Smith, D. R. Tyreman, G. M., Payne, N. J. Marshall, J. W. Payne, Microbiology, 145, 2891, 1999
[4] P. A. Bartlett, K. L.Spear, N. E. Jacobsen, Biochemistry, 21, 1608, 1982

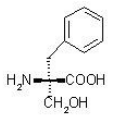
Th174

SYNTHESIS AND BIOLOGICAL PROPERTIES ANALOGUES OF CYCLOLINOPEPTIDE A CONTAINING ALPHA-HYDROXYPHENYLALANINE OR HOMOPHENYLALANINE

I. Derlatka[1], P. DrygałA[1], J. Olejnik[1], **A. Olma**[1], M. Zimecki[2], J. Zabrocki[1]

[1]Institute of Organic Chemistry, Technical University, Łódź [2]Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wrocław, Poland

The search of new immunosupressants, exhibiting the mechanism of action characteristic for cyclosporine A (CsA) and FK-506 is an important challenge for medicinal chemistry. Cyclolinopeptide A (CLA) natural cyclic nonapeptide [cyclo(Leu-Ile-Ile-Leu-Val-Pro-Pro-Phe-Phe)] possesses a strong immunosuppressive activity comparable with that of CsA in low doses. The possibility of practical application of CLA as a therapeutic agent is limited due to its high hydrophobicity. It has been suggested that the tetrapeptide sequence Pro(6)-Pro(7)-Phe(8)-Phe(9) is responsible for the interaction of the CLA molecule with the proper cellular receptor. In order to evaluate the role of this tetrapeptide unit for biological activity of native peptide, we decided to modified this fragment.
In this communication we present linear and cyclic CLA analogues in which phenylalanine residues in position 8 and/or 9 have been replaced with amphiphilic; alpha-hydroxmethylphenylalnine 1 or homophenylalanine 2. The synthetic strategy and biological activity will be evaluated.



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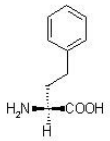

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This work was supported by the Ministry of Education and Science, Grant No 2 505F 035 28

M175

SYNERGY BETWEEN ANTIMICROBIAL PEPTIDES AND ANTIBIOTICS

L. Otvos[1], V.O. Inacio[2], J.D. Wade[3], P. Cudic[2]

[1]Sbarro Institute, Temple University, Philadelphia, PA [2]Department of Biochemistry, Florida Atlantic University, Boca Raton, FL, USA [3]Howard Florey Institute, Melbourne, VIC., Australia

Resistance to currently used small molecule antibiotics develops at an alarming rate. While resistance to β-lactams in clinical isolates is primarily due to hydrolysis of the ring by β-lactamases, when bacteria develop resistance to fluoroquinolones or aminoglycosides, the sequences of the target biopolymers are altered. Earlier we developed a family of antibacterial peptide derivatives that kill bacteria by inhibiting protein folding and are active in animal models of infection. In the current study we examined the synergy between antibiotics acting by different modes of action.

Inhibition of properly folded active resistance enzymes was completely efficacious to recover the activity of amoxicillin, a β-lactam antibiotic against strains that were originally resistant to this molecule. Some activity of ciprofloxacin was also recovered by reducing the load of the induced self-defense DnaK protein, but the synergy between the antibacterial peptide and the fluoroquinolone did not yield full bacterial killing. The mode of action of the synergy is indeed inhibition of protein folding because no such effect could be observed with kanamycin where resistance involves changes in the target protein sequence.

As opposed to current β-lactamase inhibitors and combination therapies that work against only a limited number of strains, inhibition of all protein folding in bacteria is a universally applicable treatment option. Elimination of resistance to β-lactams by proline-rich peptide derivatives may represent a viable avenue to give second life to these antibiotics for which large stockpiles are available for pharmaceutical companies in both patented and generic forms.

Tu176

PEPTIDE ANALOGUES DERIVED FROM THE CYTOPLASMIC DOMAIN OF αIIBβ3 INTEGRIN RECEPTOR INHIBIT PLATELET AGGREGATION

V. Koloka, P. Stathopoulos, **E. Panou-Pomonis**, S. Vaxevanellis, A. Dimitriou,
L. Tsironis, D. Tsoukatos, A. Tselepis, M. Sakarellos-Daitsiotis, C. Sakarellos,
V. Tsikaris

Department of Chemistry, University of Ioannina, Greece

The integrin αIIbβ3 is the major integrin-adhesion receptor on platelets. In unstimulated platelets αIIbβ3 is present in a resting conformation state. Upon platelet activation by agonists, αIIbβ3 receives intracellular signals (inside-out signaling) that allow its rapid conversion to a high-affinity state capable of binding soluble ligands, resulting in platelet aggregation. The intracellular signals include proteins that bind to the cytoplasmic tails of the two subunits α and β of the integrin, or integrin-associated membrane proteins. In vivo charge swapping mutation studies suggested that αIIb and β3 tails have a direct site of interaction between αIIb (R995) and β3 (D723). Peptides derived from the cytoplasmic tail sequences can specifically induce or block αIIbβ3 activation in platelets. The aim of this study is to develop peptide analogues based on the cytoplasmic tail sequences of both αIIb and β3 subunits that could inhibit platelet thrombus formation by specifically disrupting the inside-out signaling pathway. Peptide analogues of the αIIb and β3 subunits spanning the sequences αIIb-989-1008, αIIb -997-1003, αIIb -997-1008, αIIb-1000-1008 and β3-743-750, β3-743-756, β3-749-756 were synthesized in their free state, palmitoylated and/or tagged with the Tat fragment 48-60 and carboxyfluorescein-labeled, in order to investigate their membrane permeability, as well as their inhibition of the platelet aggregation.

Acknowledgements to the GSRT (EPAN YB/88) and E.U. for the financial support.

Th177

DESIGN AND SYNTHESIS OF TRPV1 RECEPTOR ANTAGONISTS WITH ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

M. Quintanar-Audelo[1], L. García de Diego[2], W. Van Den Nest[3], C. Carreño[3], A. Ferrer-Montiel[3], F. Albericio[1]

[1]Institut de Recerca Biomèdica, Parc Científic de Barcelona, Universitat de Barcelona [2]Departamento de Biología Molecular Y Celular, Universidad Miguel Hernández, Elche, Alicante [3]DiverDrugs, SL, Gavá, Spain

Inflammatory pain begins when noxious stimuli (thermal, chemical or mechanical) excite sensorial neurons called nociceptors. The activation of nociceptors leads to the opening of some ionic channels and depolarization of the cell membrane. One of these channels is TRPV1, which is directly implied in thermal hyperalgesia associated to inflammation. In previous work it has been found that peptoid H-Arg-15-15C (Fig. 1) inhibits the activation of TRPV1 by blocking the pore entrance. However, this compound showed toxicity in vivo. The aim of our work is the design and synthesis of new compounds, based on the structure of H-Arg-15-15C, with better therapeutic properties. We synthesized some new non-competitive antagonists of TRPV1 that exhibit notable anti-inflammatory and analgesic activity in vivo.

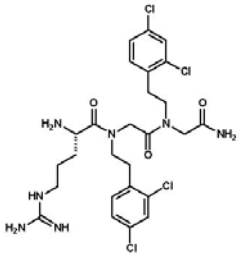


Fig. 1. H-Arg-15-15C

M178

INHIBITORS OF THE RAS/ERK SIGNALING PATHWAY INCORPORATING THE FXF MOTIF FOR THE DEVELOPMENT OF ANTICANCER DRUGS

Th. Skarlas, E. Panou-Pomonis, D. Krikorian, **M. Sakarellos-Daitsiotis**, C. Sakarellos

Department of Chemistry, University of Ioannina, Greece

ERF is a transcriptional repressor with tumor suppressor activity regulated by the RAS/Erk signaling pathway. It has been shown that ERF interacts with, and is phosphorylated by Erks in vitro and in vivo. This phosphorylation determines its subcellular localization and biological function. ERF exhibits a high degree of specificity and sensitivity for Erks. The major objective of our study is to provide proof of principal for a specific anti-cancer approach targeting the RAS-pathway, which is commonly activated in human tumors, via the stimulus of the downstream effector ERF. This will be attained by modeling specific peptide inhibitors that block the ERF phosphorylation and inactivation by the RAS/Erk signaling pathway. We present the design and synthesis of peptide inhibitors incorporating the FSF and FKF motifs, known to play a critical role in the ERF/ERK interaction, in their free forms or conjugated to a carrier.

Acknowledgements to the G.S.R.T. and E.U (bilateral French-Greek cooperation 2003-2006) for financial support

Tu179

PRODUCTION AND PURIFICATION OF ANTIUBIQUITIN ANTIBODIES FOR DETECTION OF DEFECTIVE SPERM IN INFERTILE MALE

A. Shabani[1], M.R. Sadeghi[2], M.M. Akhondi[2], M. Hojet[1], M. Asgari Mehr[3], Z. Afraz[3]

[1]Department of Biochemistry, Faculty of Science, Azzahra University [2]Shaheed Beheshti University [3]Medical Faculty, University of Tehran, Iran

Ubiquitinium is a well known mechanism in protein degredation of Eukaryotic cells ,in which many obsolte and corrupted three dimentional structure protein ,become marked by covalent attachment of ubuquitin through a multi-step enzymatic pathway.Ubiquitin is a small ,8.5 kDa peptide of 76 amino acid residues that targets such substrtes for proteolysis in proteasome .Recnt studies showed that an extra cellular ubiquitination process also taking place in the epididymes of humans and other animals marks protein on the surface of the defective sperm .it appears that structurally and functionally defective sperm become surface ubiquitinated by epididymal epithelial cells. A certain portion of ubiquitin - labeled sperm is phagocytosed and the remaining is ejaculated .Hence ubiquitin on the sperm surface could be a good marker of semen quality control in men. The aim of present study is to purify ubiquitin from packed blood cells , to produce and purify antiubiquitin antibodies,to design an immunofluorescence assy for detection of defective sperm, to compare the percentage of ubiquitinated sperm in Oligoasthenotertozoospermia and Normozoospermia and finally to determine correlations between sperm parameters and sperm ubiquitination.

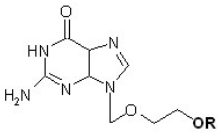
Th180

SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMINO ACID ESTER PRODRUGS OF ACYCLOVIR

I.G. Stankova[1], T.A. Dzimbova[2]

[1]*Department of Chemistry, South-West University "Neofit Rislki" Blagoevgrad*
[2]*Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine (ACV) is an acyclic guanine nucleoside analogue that is widely used clinically as an antiherpetic agent. Its limited absorption in humans after oral administration prompted the search for prodrugs.
The objective of this work has been to design and to synthesize a new amino acid ester (R=Gly,Val) prodrugs containing thiazole, oxazole ring of acyclovir and to evaluate their activity against herpes simplex type I in vitro.
LC/MS was used to characterize the new prodrugs. Both 1H NMR and 13C NMR spectra of the prodrugs of ACV were measured and assigned based on spectral comparison with compounds of similar structures.



acyclovir

M181

SYNTHESIS OF PEPTOID-PEPTIDE ANALOGUES OF SUBSTANCE P FRAGMENTS INCORPORATING D-AMINO ACIDS

P. Vakalopoulou, Ch. Anastasopoulos, G. Stavropoulos, **S. Yiannis**

Department of Chemistry, University of Patras, Greece

C-Terminal analogues of Substance P (SP) have been studied for their ability to prevent tumor growth or the proliferation of several cancer cell lines. The incorporation of D-amino acids into the sequence of SP and N-methylation of peptide bonds have shown to protect SP from the action of plasma and tissue peptidases.

Aiming to design and prepare more potential antagonist of cancer cells proliferation and taking into account that all the metabolites of the C-terminal hexapeptide analogue [Arg6, D-Trp7,9, MePhe8]SP6-11 (antagonist G) possess the N-Me group and D-Trp residue, we proceeded to the synthesis of peptoid-peptide hybrids. They are oligomeric peptido-mimetics containing the residue [-N(Bzl)-CH2-CO-]=(NPhe). The incorporation of N-substituted glycine in peptide chains has been proved to improve their stability against proteases and give biologically active peptides. Thus, the tetrapeptoid-peptide hybrids H-Arg1-D-Trp2-NPhe3-D-Trp4-OH and H-D-Trp1-NPhe2-D-Trp3-Leu4-OH, corresponding to metabolites of antagonist G and also the hexapeptoid-peptide hybrids Glp1-D-Trp2-NPhe3-D-Trp4-Leu5-Glu(OBzl)6-NH2 and Glp1-D-Trp2-NPhe3-D-Trp4-Leu5-Glu(OBzl)6-OH have been synthesized. The latter have incorporated the amino acid residues Glp at the N-terminal and Glu(OBzl) at the C-terminal of the analogue, which have shown to give to the analogues increased resistance and biological activity. All the products were purified (HPLC), identified (ESI-MS) and set about for study their biological properties and activity against cancer cells proliferation.

References

1. J. Cummings, A.J. MacLellan, D.A. Jones, S.P.Langdon, E. Rozengurt, A.A. Ritchie and J.F. Smyth, Annals Oncol., 6 (1995) 595.

2. P. Vakalopoulou, H. Makrodouli, G. Stavropoulos, J. Voutsas and C. Baxevanis, Peptides 2004, p.968

SYNTHESIS AND BIOLOGICAL EVALUATION OF CXCR4 ANTAGONISTS AS PHARMACEUTICAL AGENTS

H. Tamamura[1], H. Tsutsumi[1], H. Masuno[1], T. Ogawa[2], K. Hiramatsu[2], T. Araki[2], S. Ueda[2], S. Oishi[2], A. Esaka[2], Z. Wang[3], S.C. Peiper[3], J.O. Trent[4], N. Fujii[2]

[1]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku [2]Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan [3]Medical College of Georgia, Augusta, GA [4]James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA

A chemokine receptor CXCR4 has multiple critical functions in normal and pathologic physiology. CXCR4 is a GPCR that transduces signals of its endogenous ligand, CXCL12 (stromal cell-derived factor-1, SDF-1). The CXCL12-CXCR4 axis plays an important role in the migration of progenitors during embryologic development of the cardiovascular, hemopoietic, central nervous systems and so on. This axis has recently been proven to be involved in several problematic diseases, including HIV infection, cancer cell metastasis, leukemia cell progression, rheumatoid arthritis (RA) and pulmonary fibrosis. Thus, CXCR4 is a great therapeutic target to overcome the above diseases. Fourteen-mer peptides, T140 and its analogs, were previously found to be specific CXCR4 antagonists that were identified as HIV-entry inhibitors, anti-cancer-metastatic agents, anti-chronic lymphocytic/acute lymphoblastic leukemia agents and anti-RA agents. Cyclic pentapeptides, such as FC131 [cyclo(D-Tyr-Arg-Arg-L-3-(2-naphthyl)alanine-Gly)], were previously found as CXCR4 antagonist leads based on pharmacophores of T140. In this symposium, we would like to report the development of low molecular weight CXCR4 antagonists involving FC131 analogs and other compounds with different scaffolds including leaner-type structures.

[1] Tamamura, H., Fujii, N., et al. (2005) J. Med. Chem., 48, 380-391; (2005) J. Med. Chem., 48, 3280-3289; (2005) Expert Opin. Ther. Targets, 9,1267-1282.

NEW ERYTHROPOIETIN RECEPTOR (EPOR) AGONISTS

O. Vadas, K. Rose

Department of Structural Biology and Bioinformatics, University Medical Center (CMU), Geneva, Switzerland

Erythropoietin (EPO) controls the proliferation and differentiation of red blood cells. It activates EPOR by inducing dimerization and reorientation of two receptor chains. Peptides mimicking the action of EPO, EPO Mimetic Peptides (EMP), have been discovered by phage display, interacting with the receptor on the active site and competing with the hormone [1]. Another peptide, EPOR derived peptide (ERP), was reported to activate the receptor through an alternative site distant from the hormone binding site, and to have synergic action with EPO [2]. We report the design of new synthetic EPO-R agonists by dimerization of active peptides. PEG-based polyamide linkers of precise length were used to link the molecules, using oxime chemistry [3]. These peptides include EMPs that have been homo-dimerized through their N- or C-terminus. A hetero-dimer of one EMP and one ERP peptides was also created. Biological characterization of the molecules is currently under investigation.

[1] Wrighton NC et al, Science, 273, 458-463 (1996)
[2] Naranda T et al, Proc Natl Acad Sci USA, 96, 7569-7574 (1999)
[3] Rose et al, J Am Chem Soc, 121, 7034-7038 (1999)

M184

THE BIOPHYSICAL FOUNDATIONS FOR HIV-1 FUSION INHIBITORS NOT BEING EFFECTIVE AGAINST SARS-COV

A.S. Veiga[1], Y. Yuan[2], X. Li[2], N.C. Santos[3], G. Liu[2], M. Castanho[1]

[1]Centro de Química E Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande C8, Lisbon [2]Chinese Academy of Medical Sciences & Peking Union Medical College, Institute of Materia Medica, Beijing, China [3]Instituto de Biopatologia Química, Faculdade de Medicina de Lisboa and Unidade de Biopatologia Vascular, Instituto de Medicina Molecular, Lisbon, Portugal

The envelope spike (S) glycoprotein of the severe acute respiratory syndrome associated coronavirus (SARS-CoV) mediates the entry of the virus into target cells. Recent studies point out to a cell entry mechanism of this virus similar to other enveloped viruses, such as HIV-1. As it happens with other viruses peptidic fusion inhibitors, SARS-CoV S protein HR2 derived peptides are potential therapeutic drugs against the virus. It is believed that HR2 peptides block the six-helix bundle formation, a key structure in the viral fusion, by interacting with the HR1 region. It is a matter of discussion if the HIV-1 gp41 HR2 derived peptide T20 (enfuvirtide) could be a possible SARS-CoV inhibitor given the similarities between the two viruses. We used fluorescence spectroscopy techniques to test the possibility of interaction between both T20 (HIV-1 gp41 HR2 derived peptide) and T-1249 with S protein HR1 and HR2 derived peptides. Our biophysical data show a significant interaction between a SARS-CoV HR1 derived peptide and T20. However the interaction is only moderate (KB = (1.1±0.3) × 105 M-1). This finding shows that the reasoning behind the hypothesis that T20, already approved for clinical application in AIDS treatment, could inhibit the fusion of SARS-CoV with target cells is correct but the effect may not be strong enough for application.

Tu185

A NEW STRATEGY FOR ACHIEVING ERYTHROPOIETIN MIMETIC PEPTIDES

Y.F. Qi, X.F. Gao, **L.P. Wang**, W. Li

College of Life Science, Jilin University, Changchun, China

Molecular dynamics simulations[1] were used to investigate the structure, dynamics and thermodynamics of the known complex between erythropoietin mimetic peptides (EMP) and erythropoietin receptor (EpoR). With Gromacs 3.2 bioinformatics software, we have obtained from the known EMPs about the key functional amino acids required for effective Epo mimetic action. Then we systematically altered the amino acids in those peptides, and simulated the complex to observe the differences between the altered peptides with the original ones. Based on these results, we designed new EMPs of potential significance. In order to fast identify the mimetic action of these new peptides, we synthesized these peptides and labeled the EpoR binding peptide (EBP) with Quantum dots[2] , to study the binding of these new EMPs to EpoR. Our results illustrate a principle for fast identifying receptor-specific sites importance for receptor internalization, and for enhancing sensitivity to hormones and other agonists.

[1]. Molecular dynamics simulations of biomolecules, Nature Structural Biology 9, 646-652 (01 Sep 2002) Review.

[2]. Hao zhang, Liping Wang, Huanming Xiong, Wei Li, Hydrothermal Synthesis for High-Quality CdTe Nanocrystals. Adv. Mater, 2003, 20:16, 1712-1715

Th186

IDENTIFICATION OF INHIBITORS OF PLGF/ FLT-1 INTERACTION BY THE SCREENING OF PEPTIDE COMPOUNDS LIBRARIES

D. Marasco[1], S. Ponticelli[2], A. Saporito[1], V. Tarallo[2], **E. Benedetti**[1], C. Pedone[1], J.M. Stassen[3], S. De Falco[2], M. Ruvo[1]

[1]Istituto di Biostrutture E Bioimmagini, Sezione Biostrutture, Del CNR [2]Istituto di Genetica E Biofisica Adriano B. Traverso Del CNR, Napoli, Italy [3]Thromb-X N.V., Leuven, Belgium

Blood vessel formation largely contributes to the pathogenesis of numerous diseases, including ischemia and cancer [1-2]. In this regard therapeutic strategies aim to stimulate vascular growth in ischemic tissues and suppress their formation in pathologies like in tumour and diabetic retinopathy. Placental growth factor (PlGF), an homolog of vascular endothelial growth factor (VEGF), (42% amino acid sequence identity), stimulates angiogenesis and collateral growth in ischemic heart and limb. Whereas VEGF exerts it biological function through the binding to both VEGF receptor-1 (VEGFR-1or Flt1) and VEGFR-2 (or KDR) PlGF binds specifically to Flt1. The complex PlGF/Flt1 constitutes a potential candidate for therapeutic modulation of angiogenesis and inflammation [3].

The binding between PlGF and Flt-1 has multipunctual features [4] and potential antagonist must have a sufficient molecular surface to spatially distant contact points. We have used an ELISA-like screening assay to select antagonists of PlGF/Flt-1 complex from a large random library of tetrameric unnatural peptides (complexity: 3^30=27.000 molecules) identifying two active molecules with an about 10 □M IC50. The relative stability of identified peptides were assessed in human serum and their inhibitory properties were tested in a capillary-like tube formation assay performed with Human Umbilical Vein Endothelial Cells (HUVEC).

References

1.Bussolino et al. TIBS, 1997, 22, 251-256.

2.Iyer, S., et al., J. Biol. Chem. 2001, 276(15), 12153-12161

3.Carmeliet P, et al. Nat Med 2001, 7(5):575-83

4.Davis-Smyth T. et al J. Biol. Chem. 1998, 273(6), 3216-3222

M187

NOVEL AND SELECTIVE αVβ3 RECEPTOR PEPTIDE ANTAGONIST: DESIGN, SYNTHESIS AND BIOLOGICAL BEHAVIOUR

A. Del Gatto[1], L. Zaccaro[1], A. Zannetti[2], S. Del Vecchio[2], **E. Benedetti**[1],
M. Salvatore[2], C. Pedone[1], M. Saviano[1]

[1]Institute of Biostructures and Bioimaging, CNR, Department of Biological Sciences, University of Naples "Federico II", And C. I. R. Pe. B [2]Institute of Biostructures and Bioimaging, CNR, Department of Biomorphological and Functional Sciences, University of Naples "Federico II", Naples, Italy

The αvβ3 integrin is a cell adhesion receptor involved in angiogenesis and tumor cell invasion. The tripeptide motif RGD is the αvβ3 minimal recognition sequence and many RGD-containing peptides have been investigated as radiopharmaceuticals for targeting angiogenesis and tumor metastatic phenotype. Since RGD sequence binds also to other integrins, the aim of the present study was to develop and characterize a selective αvβ3 ligand suitable for imaging. A novel peptide containing the RGD loop covalently linked to an echistatin domain (cRGDechi) was designed, synthesized and then tested for selective binding to αvβ3 integrin. A panel of peptides were used for comparison. Adhesion assays showed that the novel peptide was able to inhibit adhesion of αvβ3 overexpressing cells but not αIIbβ3 and αvβ5 overexpressing clones. In conclusion the novel peptide showed a high affinity and specificity for αvβ3 integrin. The design of new molecules, based on the lead compound presented here, is currently ongoing with the aim at developing novel anticancer drugs and/or new class of diagnostic noninvasive tracers as suitable tools for αvβ3 -targeted therapy and imaging.

Tu188

EFFECT OF SHORT PEPTIDES ON Aß(1-42) FIBRILS

Zs. Bozsó[1], B. Penke[1], L. Fülöp[1], T. Martinek[2], A. Lakatos[3], K. Soós[1], I. Laczkó[4], M. Zarándi[1]

[1]Institute of Medical Chemistry [2]Institute of Pharmaceutical Chemistry [3]Department of Inorganic and Analytical Chemistry, University of Szeged [4]Institute of Biophysics, Biological Research Center, Szeged, Hungary

Background and aims: Short peptides like Leu-Pro-Phe-Phe-Asp (LPFFD) and Leu-Pro-Tyr-Phe-Asp-amide (LPYFDa) can influence the structure and aggregation of ß-amyloid peptides. Soto's pentapeptide LPFFD has been published as a ß-sheet breaker (BSB). It is necessary to gain more information about the nature of the interaction of Aß and the pentapeptides mentioned above for the understanding of their action and for the possible development of future therapeutic agents.
Methods: In this study radioligand binding assay, diffusion ordered NMR spectroscopy, dynamic light scattering, circular dichroism and FT-infrared spectroscopy was used.
Results: It was shown by radioligand binding assay and diffusion ordered NMR spectroscopy that both pentapeptides bind to aggregated Aß. Dynamic light scattering, circular dichroism and FT-infrared spectroscopy revealed, that after the treatment of the Aß with the pentapeptides Aß fibrils are still present.
Conclusion: Both peptide can bind to Aß and can cause small conformational changes of Aß, however, they cannot prevent completely the formation of Aß fibrils in 50-100 micromolar concentration using 1:1 molar ratio of Aß and the BSB peptide.

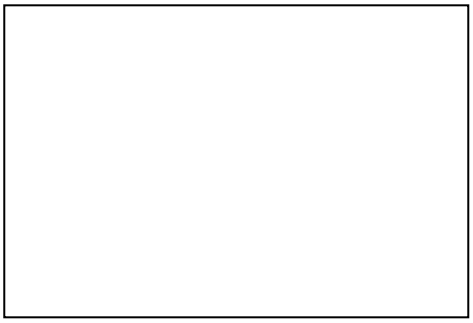
Th189

EPITOPE MAPPING USING A NEW FORMAT OF CELLULOSE SUPPORTED PEPTIDE MICROARRAYS

O. Brandt, H. Gausepohl

INTAVIS Bioanalytical Instruments AG, Koeln, Germany

Peptide arrays are convenient tools for the analysis of antibodies, protein binding domains and to address other biological questions. Here we present a new method to produce identical copies of arrays on microscope slides. The peptides are synthesized on modified cellulose-discs, using a variation of the SPOT-method introduced by Ronald Frank more than 10 years ago [1]. The new array format overcomes several limitations of the SPOT-method, e.g. the low throughput with only one copy of the library and the large sample volumes that are needed for membrane incubations. For the presented arrays modified cellulose discs with covalently bound peptides are dissolved after synthesis. The resulting solutions can be spotted onto glass slides by conventional spotting techniques. Three dimensional layers of cellulose-peptide molecules are formed on the surface of the supports used for spotting. A virtually unlimited number of identical arrays can be printed and assays are performed with a sample volume of 100 µl or less. As application example we show mapping experiments of the streptavidin recognition site with a peptide library containing histidine-proline motives. Because of the much higher peptide loading compared to conventional arrays, the formed 3-dimensional structure might be superior for protein-interaction studies with even low binding constants. [1] Frank, R. Tetrahedron 48, 9217 (1992)



M190

STUDIES ON INTERACTION OF CALMODULIN WITH CALMODULIN-BINDING PEPTIDES M13 AND RS20 IN THE PRESENCE OF ZINC IONS

A. Calderan, P. Ruzza, A. Osler, B. Biondi, C. Rubini, A. Guiotto

CNR Institute of Biomolecular Chemistry, Padova Unit, Padova, Italy

Ca2+-free calmodulin (apoCaM) contains two globular domains connected by a flexible central linker. Each domain contains two well-defined helix-loop-helix EF-motifs that are responsible for Ca2+ binding. Upon binding, the calcium ions organize and stabilize the four-domains structure inducing large conformational changes: in this active form calmodulin (CaM) can bind to its numerous target regulatory proteins. Since most of them are large and multimeric proteins, the CaM-protein complexes are usually simulated with template peptides.

The aim of this study is the set-up of a simple methodology to identify and characterize the CaM altered conformational states induced by ions different from Ca2+. To this purpose we studied, in the presence of Zn2+ ions, the peptide fluorescence and CD spectral changes induced by the formation of the complexes between CaM and two synthetic peptides: M13, corresponding to the sequence 577-602 of skMLCK [1], and RS20, corresponding to the sequence 796-815 of smMLCK [2].

This work was supported by CNR/MIUR - Legge 449/97 - DM 30/10/2000.

[1] Ikura, M., Clore, G.M., Gronenborn, A.M., Zhu, G., Klee, C.B., Bax, A. Science, 256, 632-638 (1992).

[2] Meador, W.E., Means, A.R., Quiocho, F.A. Science, 257, 1251-1255 (1992).

Tu191

IN SITU EIF4E-TEMPLATED CLICK REACTION - EIF4G-DERIVED EIF4E BINDING PEPTIDES AS ANCHORING FRAGMENTS IN SEARCH OF PUTATIVE EXOSITES

S. Cantel[1], A. Natarajan[1], N. Moerke[2], H. Aktas[1], G. Wagner[2], J.A. Halperin[1], M. Chorev[1]

[1]Laboratory for Translational Research, Harvard Medical School, Cambridge, MA [2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

The interaction between the cap binding protein, eukaryotic initiation factor (eIF) 4E, and the scaffolding protein eIF4G is critical for the formation of the heterotrimeric eIF4F translation initiation (TI) complex (eIF4E/eIF4G/eIF4A). Elevated levels of eIF4E and eIF4G found in several human solid tumor cancers and the induction of malignant transformation in animal models by overexpression of eIF4E and the reversal of this phenotype by treatment with anti-sense RNA, suggest the importance of the eIF4E/eIF4G interaction in the excessive translation of oncogenic proteins. eIF4G binds to eIF4E through a conserved eIF4E-binding motif YX4L□ (non-specified (X) and hydrophobic (□) amino acids) that interacts with an hydrophobic hot spot on eIF4E. We report here the identification of a putative eIF4E anionic exosite that is distinct from the hot spot and contributes to the binding of eIF4G-derived ligands.

Our strategy focuses on in situ eIF4E-templated click reaction-mediated assembly of hybrids comprised from an anchoring minimal eIF4G-derived peptide fragment, which binds to the hot spot, and a series of complementing positively charged fragments targeting the anionic exosite.

We synthesized a training set of [1,2,3]triazole-containing hybrid peptides that are potent inhibitors of eIF4E/eIF4G interaction.

Moreover, we achieved in situ eIF4E-templated assembly of these hybrids from the corresponding fragments via click reaction in the absence of Cu(I) catalysis. As such, we demonstrate a proof-of-concept for a new paradigm in the development of inhibitors of protein-protein interaction merging click reaction with fragment-based and in situ target-templated approaches.

Th192

IDENTIFICATION, SYNTHESIS AND CHARACTERIZATION OF AGGREGATION INDUCING PEPTIDES DERIVED FROM GPBP PROTEIN

L. Cascales[1], M.M. Sanchez del Pino[1], D. Serquera[1], J. Saus[2], E. Pérez-Payá[1,3]

[1]Department Of Medicinal Chemistry [2]Department of Autoimmune Pathology, Centro de Investigacion Principe Felipe [3]Instituto de Biomedicina de Valencia, Spain

Goodpasture disease is an autoimmune pathology caused by the accumulation of reactive autoantibodies against the alpha-3 of collagen IV. Goodpasture antigen-binding protein (GPBP) is a Ser/Thr protein kinase that phosphorylates the alpha-3 chain and might be important in human autoimmune pathogenesis [1]. We are carrying out in our laboratories the biophysical and functional characterization of GPBP protein. In the presence of some proteins and at specific experimental conditions, GPBP participates in structurally ordered intra- and inter-protein aggregation processes. Structure prediction programs identify four different domains for GPBP: An N-terminal domain showing pleckstrin homology (PH domain); a central domain with high tendency to form coiled-coils; a domain with WW features; and a C-terminal START domain ('StAR-related lipid-transfer'). Using the TANGO algorithm [2], we have identified several aminoacid sequences in the GPBP START domain of vertebrates with high tendency to participate in protein aggregation. In this work we present the synthesis and structural characterization of a collection of peptides derived from the sequences described above.

REFERENCES

[1].A. Raya, F. Reverte-Ros, P. Martínez-Martínez, S. Navarro, E. Rosello, B. Vieites, F. Granero, J. Forteza, J. Saus, J. Biol. Chem. 2000, 275,40392-399.
[2]. A.M. Fernández-Escamilla, F. Rousseau, J. Schymkowitz, L. Serrano. Nature Biotechnol. 2004, 22,1302.

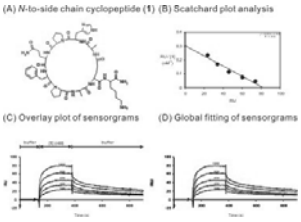
M193

A KINETIC STUDY OF COMBINATORIALLY SELECTED LIGANDS BINDING TO STREPTAVIDIN BY SURFACE PLASMON RESONANCE

Y.P. Chang, Y.H. Chu

Department of Chemistry and Biochemistry, National Chung Cheng University, Chia-Yi, Taiwan-R.O.C.

We recently developed a combinatorial library screening protocol to identify HPQ-containing cyclopeptides that bind streptavidin more tightly than its linear analogues. The relative affinities in IC50 of these structurally constrained ligands and its linear counterparts were measured by a captured enzyme-linked immunosorbent assay. However, their intrinsic binding kinetics remained to be elucidated. In this work, surface plasmon resonance (SPR) was employed to directly determine the kinetics and thermodynamics of the ligands binding to a streptavidin chip. Solid-phase peptide synthesis was carried out using standard Fmoc chemistry. SPR experiments were carried out using BIAcore3000 optical biosensor. Streptavidin was immobilized onto a CM5 sensor chip using the standard amine coupling procedure. The equilibrium dissociation constants and kinetic on/off rates of N-to-side chain and N-to-C cyclopeptides were deduced by Scatchard analysis and computational simulation, respectively. It was found that both cyclopeptides exhibited similar binding kinetics and bound streptavidin far more avidly than its linear form (1000-fold). In addition, the reversed (QPH) linear and cyclic peptidyl ligands were hardly recognized by streptavidin. Not only the binding specificity was distinguished qualitatively, but also the entropic advantage acquired by the pre-organized conformation over its linear analogues was demonstrated quantitatively by SPR in this study.



PEPTIDE APTAMERS AS SYNTHETIC TUMOUR SUPPRESSOR PROTEINS

H.Y. See[1], C. Brown[1], C. Verma[2], D.P. Lane[1], **D.W. Coomber**[1]

[1]Institute of Molecular and Cell Biology [2]Bioinformatics Institute, Singapore, Singapore

The mutation of tumour suppressor genes in the progression of cancer is well characterized. For example, p53 is found to be mutated in approximately 50% of cancers and the loss of this proteins activity has been shown to lead to the deregulation of cell growth and apoptosis. The potential of peptide aptamers to inhibit protein/protein interactions in a highly specific manner makes them very attractive as research reagents or as target validation tools in anti-cancer drug discovery. More interestingly, these molecules have the potentially to inhibit the activity of proteins which are key regulators of cancer cell growth and therefore could act as synthetic tumour suppressor proteins. We used peptides based on known protein/protein interactions, as well as peptides isolated using display technologies, for the design of protein aptamers that were used to analyze pathways critical in controlling cancer cell growth. A range of scaffolds were used to present these peptides in an effort to optimize the peptides activities. Data relating to the activity of these peptide aptamers in vitro as well as in cellular systems will be discussed.

PEPTIDE ANTAGONISTS AND AGONISTS OF UROTENSIN II BIND WITH THE SAME LIGAND GEOMETRY TO THE UROTENSIN RECEPTOR

B.J. Holleran, R. Leduc, **E. Escher**

Département de Pharmacologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, QC, Canada

The cyclic undecapeptide urotensin II (U-II) is the endogenous agonist for the U-II receptor (UT), a Gq coupled GPCR. Current views suggest that binding by agonist, but not antagonist, leads to induction of stabilization of an active receptor conformation. We have previously probed the interactions of urotensin II with rat UT (rUT) using a series of photolabile U-II analogues containing p-benzoyl-L-phenylalanine (Bpa). It was found that positions 1, 2, 3 and 4 of the N-terminus interact with Met288 and position 6 interacts with Met184 or Met185 of rUT. To compare the binding mode of agonist and antagonist ligands to the rUT and gain insight into mechanisms responsible for receptor activation, we applied the same experimental strategy to determine contact points of a peptide antagonist. A peptide was prepared containing the N-terminal portion of the human UII sequence (NH2-Glu-Thr-Pro-Asp-) and the C-terminal heptapeptide of antagonistic urantide (Ala-Pen-Phe-DTrp-Orn-Tyr-Cys-Val): [Pen5,D-trp7,Orn8]U-II . This peptide retained high receptor affinity and had antagonistic properties. Based on this, three photolabelling analogues were synthesized with Bpa in positions 1, 2 and 6, namely [Bpa1,Pen5,D-trp7,Orn8]U-II, [Bpa2,Pen5,D-trp7,Orn8]U-II and [Pen5,Bpa6,D-trp7,Orn8]U-II. All three peptides bound to the rUT with affinities comparable to U-II and were antagonistic in nature. Photolabelling results indicate that the antagonistic peptides interact with the same receptor residues as the corresponding agonists. This approach has allowed us to demonstrate that agonist and antagonist bind UT with a very similar geometry. **Supported by grants from the CIHR and the Quebec chapter of the HSFC.**

M196

INTERACTION OF C-JUN N-TERMINAL KINASE JNK WITH INHIBITORY PEPTIDES

A. Friebe, S. Hanke, B. Heil, C. Kruse, F. Spira, H. Hennemann

Center of Advanced European Studies and Research, Bonn, Germany

The c-jun N-terminal kinases (JNKs) are important mitogen-activated protein kinases. These Ser/Thr protein kinases are activated by various growth factors, cytokines, and cellular stresses. JNKs have been shown to play a key role in phosphorylation of proteins in signal transduction of different diseases including cancer, neurodegenerative, cardiovascular, and inflammatory diseases. Therefore, these enzymes are considered as important therapeutic target proteins.
The interactions of JNK with peptides are of special interest for development of novel specific ATP-noncompetitive inhibitors. Interactions of this kinase and its mutants with various substrates were demonstrated in vivo using yeast Ras-recruitment system. Bioinformatical tools have been developed to predict optimized binding peptides as well as to correlate sequence position and amino acid with binding effiency to extract binding determinants.
Biomolecular interaction analysis have been performed for selected peptide sequences using surface plasmon resonance (SPR) technology. Real time measurements of the binding of peptides to the different isoforms JNK2 and JNK3 resulted in the determination of affinities as well as kinetic constants for association and dissociation. Experimental results and their bioinformatic analysis are discussed with respect to critical features of potential ATP-noncompetitive inhibitors.

Tu197

IDENTIFICATION OF NEW NON NATURAL ANALOGUES OF B DOMAIN OF STAPHYLOCOCCAL PROTEIN A, USING "ONE-BEAD ONE-COMPOUND" COMBINATORIAL LIBRARY

C.G. Granados[1], J.J. Pastor[1], N. Carulla[1], M. Gairí[2], F. Rabanal[3], E. Giralt[1,3]

[1]Institut de Recerca Biomèdica, Parc Científic de Barcelona [2]Servei de Ressonància Magnètica Nuclear [3]Departament de Química Orgànica, Universitat de Barcelona, Barcelona, Spain

The B-domain of is one of the five nearly homologous domains of staphylococcal protein A.
This domain contains three alpha-helices which are assembled in an anti parallel three-helix bundle. The B-domain binds the Fc region of mammalian immunoglobulins through the N-terminal fragment that contains two alpha-helices. The C-terminal helix does not interact with Fc but it is necessary for the correct folding and immunoglobulin recognition of the B-domain. To search for new peptide analogues of the C-terminal helix that bind the N-terminal fragment, a ¨one-bead one-compound¨ library of 300 peptides was designed based on the sequence of the C-terminal helix. Active peptides were obtained after incubation of the library with the N-terminal fragment and rabbit immunoglobulin G labelled with fluorescein. New peptides were found and their sequences identified by MALDI TOF-TOF mass spectrometry. The synthesis of the two most active peptides was carried out and the binding with the N-terminal fragment was confirmed by CD spectroscopy. The N-terminal fragment peptide showed an increase in helicity when the C-terminal wild type peptide or some analogues were present in solution. The complete domains with the C-terminal fragment mutations were synthesized and structurally characterized by CD and NMR spectroscopy. The wild type and the new mutants adopt predominantly an alpha-helical structure. The interaction between rabbit immunoglobulin G and the wild type B-domain and the new analogues was investigated using surface plasmon resonance. Although compared with the wild type, the mutants exhibited different kinetics, they were able to bind the immunoglobulin with high affinity.

CHEMICAL SYNTHESIS AND KINETIC STUDIES OF DIMERIC ANALOGUES OF TRYPSIN INHIBITOR SFTI AND ITS TWO ANALOGUES CONTAINING A CARBONYL BRIDGE

A. Jaśkiewicz, E. Bulak, H. Miecznikowska, K. Rolka

Faculty of Chemistry, University of GdańSk, Poland

SFTI-1, a strong trypsin inhibitor, was isolated in 1999 from seeds of sunflower. It is homodetic 14-amino-acid-residue peptide containing a disulfide bridge. Because of its small size and strong trypsin inhibitory activity, this inhibitor became an interesting model for studying enzyme - inhibitor interactions. SFTI-1 possesses one reactive site located at the Lys5-Thr6 peptide bond and therefore is able to interact with the enzyme in a 1:1 stoichiometry. In this report we describe chemical synthesis and kinetic studies of a series of SFTI-1 analogues containing double sequences of the wild inhibitor. Their structures contain combinations of disulfide bridges and/or head-to-tail cyclization. Each of these analogues contains two trypsin-specific reactive sites. We expect that kinetic studies should answer the question whether such dimeric analogues are able to interact simultaneously with more then one trypsin molecule and how this fact affects their inhibitory potency. In addition, we alsopresenttwo analogues in which we substituted the disulfide bridge with a carbonyl one. Since carbonyl bridge has not been previously introduced into molecules proteinase inhibitors, we decided to check its impact on the activity and proteolytic stability of such modified analogues.

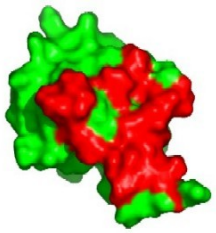
M199

SMALL UBIQUITIN-BINDING PEPTIDES

G. Roth[1], J. Brünjes[1], B. Möhrle[2], K. Wöllner[2], G. Gauglitz[2], S. Freund[3], A. Fersht[3], K.-H. Wiesmüller[4], **G. Jung**[1]

[1]Institute of Organic Chemistry [2]Institute of Physical and Theoretical Chemistry (IPTC), Eberhard-Karls-Universität Tübingen, Germany [3]Cambridge Center for Protein Engineering, MRC Center, Cambridge, UK [4]EMC Microcollections GmbH, Tübingen, Germany

Ubiquitination, the covalent attachment of one or multiple polymerized ubiquitins is a post-translational modification of proteins, which has manifold functions. It mainly determines the protein for degradation, but also activation, deactivation or substrate alteration. Due to its ubiquitinous distribution in all eucarionts no high-affinity antibodies could be originated. High-affinity ligand peptides are of interest to study ubiqutination.
Based on bioinformatical considerations and investigations of ubiquitin-interacting proteins short peptide sequences were selected.
By using a peptide array specific ubiquitin binding was monitored and quantified with label free detection based on Reflectometric Interference Spectroscopy (RIfS). The results from RIfS were confirmed by detection of binding in solution with Fluorescence Correlation Spectroscopy (FCS) using carboxyfluorescein and S0387-labelled peptide amides.
Binding constants were determined by Isothermal Calorimetry (ITC) and RIfS.
Finally 1H,15N-NMR chemical shift analyses of the peptides with the highest affinity were carried out, which allowed the localisation of the interaction site of ubiquitin with the peptide The results from all four methods correlated very good. They showed fast equilibria within 30 s and binding constants down to the low micromolar range. NMR results revealed hints for discrimination possibilities between Lys48 and Lys63 polymerized ubiquitins. (Figure shows interaction site with 15N-ubiquitin with a trideka-peptide in PBS buffer solution as found by 800 MHz NMR)



CHARACTERIZATION OF THE EPITOPE FOR ANTI HUMAN RESPIRATORY SYNCYTIAL VIRUS F PROTEIN MONOCLONAL ANTIBODY (101F) USING SYNTHETIC PEPTIDES

M. Kruszynski[1], E.J. Beil[1], A. Schmidt[1], S.J. Wu[1], P. Tsui[2], A.M. Del Vecchio[3]

[1]Department of Protein Engineering [2]Department of Molecular Discovery Technologies [3]Department of Infectious Diseases, Centocor R&D, Inc., Radnor, PA, USA

101F is a potent neutralizing mAb that binds the human respiratory syncytial virus (HRSV) F protein and is a promising candidate for clinical development. The majority of neutralizing antibodies to HRSV F protein map to two regions of the protein designated site II and site IV,V,VI. To further characterize the 101F epitope, we employed a trypsin digestion of a HRSV F protein-101F mAb complex, followed by mass spectrometry analysis of the resulting recovered mAb bound peptide. One peptide at m/z 3330 was captured by the 101F mAb. Sequence assignment was based upon mass and matched with the database from a virtual digest. This peptide was assigned as residues 420-445 [TKCTASNKNRGIIKTFSNGCDYVSNK] of the HRSV F protein which spans antigenic site IV,V,VI. To further delineate the epitope, the binding of 101F mAb to a series of peptides corresponding to antigenic site IV,V,VI in the HRSV F protein was determined. Based on the peptide ELISA data, the 101F-binding region could be reduced to 422-436 sequence [CTASNKNRGIIKTFS]. As demonstrated by the substitution analysis, R429 and K433 significantly contribute to epitope binding, but another positively charged residue, K427 makes a minor contribution to the binding. Both, the peptide ELISA and proteolytic digestion of the mAb-antigen complex approaches identified the same region of HRSV F protein as being critical for the binding of 101F. Furthermore, these data confirm the results obtained using complementing genetic approaches using a panel of mutations in recombinantly expressed F protein and selection of antibody escape virus mutants (data not presented).

Th201

SYNTHESIS OF URACIL-DNA SPECIFIC ENDONUCLEASE DERIVED PEPTIDES FOR GENERATION OF ENZYME SPECIFIC ANTIBODIES

M. Ligeti[1], A. Békési[1], A. Magyar[1], F. Hudecz[1,2], B.G. Vértessy[3]

[1]Hungarian Academy of Sciences, Research Group of Peptide Chemistry at Eötvös Loránd University of Sciences [2]Department of Organic Chemistry, Eötvös Loránd University [3]Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary

The recently identified uracil-DNA specific nuclease (UDE) is the first representative of a new family of nucleases. The protein sequence has no detectable homology to other proteins except a group of sequences present in genomes of other pupating insects (Vertessy et al, submitted). To analyze the physiological function of this protein, peptide conjugates were prepared to serve as synthetic antigens for the generation of antibodies against isoforms of dUTPase, an enzyme inherently involved in preventing the synthesis of uracil-DNA [1, 2]. We used poly[Lys(Seri-DL-Alam)] (SAK) as a synthetic branched polypeptide [3] or bovine serum albumin (BSA) as a natural macromolecular carrier. Peptides were prepared by solid phase method utilizing Syro2000 (MulltiSynTech GmbH, Germany) peptide synthesizer, using Fmoc chemistry and DIPCI/HOBt-mediated coupling on Rink-amid MBHA resin. A C-terminal Cys(Acm) was added to the native sequences for incorporation of SH group into the peptide. In case of SAK choroacetylated polypeptide was conjugated with SH-peptide to form thioether linkage. The maleimidobenzoyil-N-hydroxyszukcinimid (MBS) derivative of BSA was used to introduce the peptide into the macromolecule.

This work was supported by grant from National Office for Research and Technology, Hungary (GVOP–3.1.1.-2004-05-0412/3.0.)

[1] Bekesi, A., Zagyva, I., Hunyadi-Gulyas, E., Pongracz, V., Kovari, J., Nagy, A.O., Erdei, A.,
Medzihradszky, K.F. and Vertessy, B.G. (2004) J. Biol. Chem., 279, 22362-22370.
[2] Vertessy, B.G. (ed.) (2001) dUTPases. Special Issue. Bentham Science Publishers.
[3] Mező, G., de Oliveira, E., Krikorian, D. (2003) Bioconjugate Chemistry, 14, 1260-1269

M202

ELUCIDATION OF ANTIBODY-PARATOPE PEPTIDES BY A COMBINATION OF AFFINITY PROTEOMICS AND HIGH-RESOLUTION MASS SPECTROMETRY (PAREXPROT)

A. Marquardt, G. Paraschiv, D. Klingler, M. Przybylski

Department of Chemistry, Laboratory of Analytical Chemistry, Konstanz, Germany

Antibodies have been developed as diagnostic tools and therapeutics for many different diseases. However, the isolation and preparation of intact specific antibodies is often very tedious or even unfeasible. Recent studies have shown that single paratope peptides might be well capable to mimic corresponding antigen ligands [1,2], suggesting that paratope peptides from a native antibody might have many advantages, e.g. for molecular vaccine design and targeting. We have developed a new method for identification of paratope-containing peptides by proteolytic affinity-proteome analysis in combination with high resolution FTICR-mass spectrometry (FTICR-MS) [3]. In the present study we used hen eggwhite lysozyme (HEL) and a polyclonal rabbit anti-lysozyme antibody (HELpAb) as a model system. The direct determination of paratope peptides was obtained by selective binding of a DTT-cleavage mixture of the anti-lysozyme antibody to immobilised HEL, followed by proteolytic digestion of the antibody-antigen complex (paratope excision, PAREXPROT). Two specific paratope peptides were identified by MALDI-FTICR-MS, and the corresponding peptide sequences were identified by database search within a 1-2 ppm threshold. Additionally, the identified paratope peptides were synthesised and characterised by affinity mass spectrometry, which ascertained their full binding specificity to lysozyme.

[1] Welling, G. W., van Gorkum, J., Damhof, R. A., Drijfhout, J. W., Bloemhoff, W. & Welling-Wester, S. (1991) J Chromatogr. 548: 235-42.
[2] Marquardt, A., Muyldermans, S., Przybylski, M. (2006) Chemistry 12: 1915-23.
[3] Przybylski, M., E. Amstalden, A. Marquardt, R. Iacob, R. Stefanescu, I. Perdivara and M. Manea (2006) Nature Meth., submitted.

Tu203

PROPEPTIDE INTERACTION WITH CATHEPSIN D: MECHANISM AND REGULATION

M. Máša, L. Marešová, J. Vondrášek, M. Horn, J. Ježek, M. Mareš

Institute of Organic Chemistry and Biochemistry Academy of Sciences of The Czech Republic, Prague, Czech Republic

The propeptide blocks the active site of inactive zymogen of cathepsin D and is cleaved off during maturation. We have designed a set of peptidic fragments derived from the propeptide structure and evaluated their inhibitory potency against mature cathepsin D using kinetic activity assay. The mapping localized two segments in the propeptide involved in the inhibitory interaction with the enzyme core: N-terminus of propeptide plays a major role and the active site anchor plays a minor role according to their respective Ki values. In addition, a fragment derived from the mature N-terminus of cathepsin D displayed inhibition, which supports its proposed regulatory role. The mechanism of interaction of both propeptide segments was characterized by the mode of inhibition and by spatial modeling of propeptide in cathepsin D zymogen. Using fluorescence polarization measurements, Kd in nanomolar range was determined for the N-terminal propeptide segment. The inhibitory potency of the active site anchor segment was modulated by Ala38Val mutation that was reported to be associated with cathepsin D pathology.

3D MODEL FOR THE COMPLEX OF THE TM REGION OF CXCR4 WITH CYCLOPENTAPEPTIDE ANTAGONISTS

J. Våbenø, **G.V. Nikiforovich**, G.R. Marshall

Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, USA

The 3D pharmacophore for binding of anti-retroviral drugs to the chemokine receptor CXCR4 remains elusive, mainly due to conformational flexibility of the identified ligands. We have performed an exhaustive systematic exploration of the conformational space for eleven cyclopentapeptide CXCR4 antagonists of the type c(Gly1-D-Tyr2-L/D-Xaa3-L-Arg4-L/D-Nal5), where Xaa3 = Ala, D-Ala, NMe-Ala, D-NMe-Ala, Pro, D-Pro, Arg, and cis/trans-4-guanidino-Pro, as well as for the retroinverso analogs c(Gly1-D-Nal5-D-Arg4-D-Arg3-Tyr2), c(Gly1-D-Nal5-D-Arg4-Arg3-Tyr2), c(Gly1-Nal5-D-Arg4-Arg3-Tyr2), and c(Gly1-Nal5-D-Arg4-D-Arg3-D-Tyr2). By comparing the resulting low-energy conformations using different sets of atoms, specific conformational features common only to the high/medium affinity compounds were identified. They included the spatial arrangement of the three most important pharmacophoric side chains Tyr2, Arg4, and Nal5 as well as the orientation of the Xaa3-Arg4 amide bond, which together represent a "minimalistic" 3D pharmacophore model for binding of the cyclopentapeptide antagonists to CXCR4. This model rationalizes the data for the cyclopentapeptides as well as for the peptidomimetic CXCR4 antagonist KRH-1636. Automated docking of the pharmacophore model to the 3D structure of the TM region of CXCR4 revealed that the pharmacophoric groups of the cyclopentapeptide ligands were involved in favorable interactions with their counterparts in CXCR4. For instance, the hydroxyl group of Tyr2 formed a hydrogen bond with Lys38, the guanidino group of Arg4 formed a salt bridge with Glu288, and the backbone carbonyl of Xaa3-Arg4 formed a hydrogen bond with Lys282. This finding gives additional support for the suggested 3D pharmacophore model, and also provides opportunities for rational design of CXCR4 mutants to map potential contacts with peptide ligands.

M205

DETECTION OF BIOMOLECULAR INTERACTIONS USING A FLUORESCENT SYSTEM WITH STAUDINGER REACTION

M. Obi, K.T. Tomizaki, H. Mihara

Department of Bioengineering, The COE21 Program, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Japan

With the successful completion of the human genome project, the next challenge is to assimilate enormous amount of genetic information generated and to assign functions to a large number of proteins encoded. Although the DNA chip technology to detect the abundance of mRNAs has been established, it is known that the abundance of mRNAs and proteins does not correlate. Thus, protein detection methods for reproducible and quantitative investigation of protein networks are strongly required. We attempted to establish a novel protein detection system based on a fluorescent measurement that does not require labeling of target molecules and preparation of secondary antibodies.

We focused on a steric hindrance caused by the interaction between a target protein and a specific capture agent. When a target protein interacts with a specific capture agent immobilized on solid surface, we assumed that a steric hindrance in the vicinity of a capture agent increases. In order to detect the differences in the steric hindrance, we utilized a fluorescent system with the Staudinger reaction. This reaction is a chemical ligation between a phosphine and an azide group. These two functionalities are unreactive with protein surfaces under biological conditions. We incorporated an azide group into an immobilized capture agent and investigated the efficiency in the Staudinger reaction between the azide and an external triphenyl phosphine derivative. It was found that a target protein bound to the capture agent immobilized onto the solid support interferes with the efficiency in the Staudinger reaction.

MHC-DERIVED PEPTIDES AS IMMUNOMODULATORS IN AUTOIMMUNE DISEASES

Th. Skarlas[1], A. Kosmopoulou[1], A. Stavrakoudis[1,2], **E. Panou-Pomonis**[1],
M. Zimecki[3], A. Kluczyk[4], Z. Szewczuk[4], M. Sakarellos-Daitsiotis[1], C. Sakarellos[1]

[1]Department of Chemistry [2]Department of Economics, University of Ioannina, Ioannina, Greece [3]Institute of Immunology and Experimental Therapy, PAS [4]Faculty of Chemistry, University of Wroclaw, Poland

The major histocompatibility complex (MHC) has a crucial role to initiate the immune response via the binding of the peptide fragments (epitopes) of foreign antigens and their presentation to the T-cell receptors (TCR). The co-receptor molecule CD4 enhances the binding between TCR and MHC II. Small molecules that mimic surfaces of MHC-II may lead to blockage of the autoimmune response and the development of drugs for immunotherapy. HLA-DQA1*0501/DQB1*0201 (DQ2) and HLA-DQA1*0501/DQB1*0301 (DQ7) are highly correlated to autoimmune diseases as Sjogren Syndrome (SS) and Systemic Lupus Erythematosus (SLE). The non polymorphic β regions of the modelled HLA-DQ7, which are exposed to the solvent and may disrupt the interaction of DQ7 with CD4+ T lymphocytes were determinated using the GETAREA program. It was found that the regions 133-140 (Arg-Asn-Asp-Gln-Glu-Glu-Thr-Thr) and 59-66 (Glu-Tyr-Trp-Asn-Ser-Gln-Lys-Glu) display the highest solvent accessibility. Peptide analogs of these regions were synthesized, by the Fmoc/OtBu solid phase strategy, purified by RP-HPLC and characterized by mass spectrometry ESI-MS. The dimeric analogs of the peptides, designed to mimic the superdimeric nature of the immunosuppressory fragments of HLA class II molecules were also synthesized and investigated. Conformational studies were performed with CD spectroscopy and biological experiments are in progress.

Acknowledgments to the G.S.R.T and E.U. (bilateral Polish-Greek cooperation 2003-2006) for financial support.

LONG-TERM INTERACTION BETWEEN BETA-AMYLOID FIBRILS AND SHORT PEPTIDES

B. Penke[1], L. Fülöp[1], T. Martinek[2], Z. Bozsó[1], I. Laczkó[3], A. Lakatos[4], K. Soós[1], M. Zarándi[1]

[1]Department of Medical Chemistry Institute of Pharmaceutical Chemistry, University of Szeged [3]Institute of Biophysics, Biological Research Center [4]Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary

Background and aims: Aggregates of β-amyloid peptide (Aβ) play central role in the etiopathology of Alzheimer's disease (AD). Short peptides like C. Soto's pentapeptide LPFFD and LPYFD-amide synthesized in our laboratory are neuroprotective agents against Aβ assemblies both in vitro and in vivo. However, the mechanism of their neuroprotective effect has not yet been fully understood.
Methods: transmission electron microscopy (TEM), CD, FT-IR, diffusion ordered NMR spectroscopy, dynamic light scattering, and radioligand binding assays were used.
Results: all the methods applied showed that the pentapeptides mentioned above do not break the fibrillar structure of Aβ, that is these molecules are not real β-sheet breakers (BSB). The pentapeptides bind to Aβ fibrils and cause small structural changes by intercalating into the Aβ assemblies. Fibrils of Aβ survive one week treatment with the pentapeptides using them in 2 to 5-time molar excess.
Conclusion: All the results in our laboratory show that the short peptides have long-term interaction on Aβ-assemblies. In the first step they bind tightly to the Aβ surface and prevent further interaction of Aβ fibrils with the neuronal membranes. After this step the short peptides can be built into the structure of Aβ-assemblies with intercalation causing a less ordered β-conformation. Proteolytic enzymes (neprilisin, IDE) could cleave and hydrolyze Aβ peptides after this structural change, therefore the short peptides are good drug candidates for the treatment of AD.

M208

STRUCTURE-FUNCTION RELATIONSHIP IN THE FBP28 WW-DOMAIN

J. Przezdziak[1], I. Kretzschmar[1], S. Tremmel[2], R. Volkmer[1]

[1]Charite, Institut für Medizinische Immunologie [2]Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany

WW-domains are the smallest naturally occurring, monomeric, triple stranded, anti-parallel beta-sheet domains. Hence, we choose the FBP28 (formin binding protein) WW-domain (1) as a model to investigate the stability of the beta-sheet structure at the amino acid level in the context of its function (ligand binding) (2). The structure-function relationship was investigated by a complete subtitutional analysis of the FBP28 WW-domain synthesized as a cellulose-bound peptide array. Functionality of these FBP28 WW-domain variants was examined by probing the peptid array for ligand binding. In addition, selected domain variants were investigated by CD measurements to determine the stability of the anti-parallel beta-sheet structure. The results of the binding studies not only verify the literature, for example, clearly demonstrating the importance of the hydrophobic cluster (Trp8, Tyr20 and Pro33) for functionality and stability, but also provide new insights into structure-function relationship of the domain. We could show, for instance, that Asn22 and Thr25 are sensitive against replacent and in analogy to the hPIN1 WW-domain (3) we conclude that these residues form a net of stabilization across the beta-strand. Additionally a ligand-induced stucture stabilization effect was clearly revealed by biophysical experiments. (1) M.J. Macias et al., Nat. Struct. Biol., 2000, Vol.7, Num5, 375-379 ; (2) J. Przezdziak et al., ChemBioChem, 2006, in press, "Probing the Ligand Specificity and Analyzing the Folding State of SPOT Synthesized FBP28 WW Domain Variants"; (3) H. Nguyen et al., Proc. Natl. Acad. Sci. USA, 2003, 100, 3948-3953

Tu209

INHIBITION OF INTRACELLULAR PEPTIDE-PROTEIN INTERACTIONS WITH PEPTIDES AND SMALL MOLECULES DERIVED FROM A GENERIC SCREENING LIBRARY

Jörg Rademann

Medicinal Chemistry, Leibniz Institute for Molecular Pharmacology and Institute for Chemistry and Biochemistry, Free University Berlin, Germany

Cellular processes in normal and pathogenic cell states are regulated by external stimuli via complex networks of catalytic and non-catalytic protein-protein interactions.
We have developed methodology for the synthetic variation of peptides and peptidomimetics using polymer reagents including linker reagents enabling polymer-supported C-acylations.[1] In combination with the virtual screening of protein subsites, we have demonstrated the application of the novel synthetic methods to inhibitor optimization for various proteases including plasmepsin II, HIV protease, and SARS coronavirus main protease.[2,3] Moreover, multivalent peptide polymers have been developed for the intracellular targeting of proteins.[4]
This methodology was now extended to the inhibition of PEPTIDE-PROTEIN INTERACTIONS by small molecules. For this purpose, we have composed a library of 20,000 small molecules by algorithmic searching of a database of bioactive molecules with virtually designed substructures (fragments). High throughput assays were developed on the basis of fluorescence and fluorescence polarization detection. Despite the scepticism regarding the inhibition of protein-protein interactions with small molecules, efficient hit molecules have been developed for several intracellular targets and were subjected to synthetic variation and cellular follow-up assays.
Literature:
[1] Angew. Chem. Int. Ed. 2003, 42, 2491-2494. Angew. Chem. Int. Ed. 2001, 40, 4395-4397. [2] ChemMedChem 2006, in press. [3] ChemBioChem 2006, in press. [4] Angew. Chem. Int. Ed. 2005, 44, 1560-1563.

IDENTIFICATION OF VON WILLEBRAND FACTOR BINDING SITE IN COLLAGEN USING TRIPLE HELICAL PEPTIDES

N.F. Raynal[1], T. Lisman[2], E.G. Huizinga[3], P.G. Groot[2], R.W. Farndale[1]

[1]*Department of Biochemistry, University of Cambridge, Cambridge, UK*
[2]*Thrombosis and Haemostasis Laboratory, Department of Haematology, University Medical Centre Utrecht* [3]*Department of Crystal and Structural Chemistry, Utrecht University, Utrecht, The Netherlands*

The essential event in platelet adhesion to the blood vessel wall after injury or in thrombosis is the binding to sub-endothelial collagen of plasma von Willebrand factor (VWF), a protein which interacts transiently with platelet glycoprotein (GP) IbAlpha , slowing circulating platelets to facilitate their firm adhesion through other collagen receptors, e.g. integrin Alpha2beta1 and GPVI. To locate theVWF-binding site in collagen III; we synthesized 57 overlapping triple-helical peptides which comprise the whole native sequence of collagen III . Peptide #23 (GPOGPSGPRGQOGVMGFOGPKGND (O is hydroxyproline)) alone bound VWF, with an affinity comparable to that of native collagen III. Immobilized peptide #23 supported platelet adhesion under static and flow conditions, processes blocked by an antibody which prevents the VWF A3 domain from binding full-length collagen. Truncated and alanine-substituted triple-helical peptides derived from #23 either strongly interacted with both VWF and platelets, or lacked both VWF and platelet binding. Thus, we identified the sequence RGQOGVMGF as the minimal VWF-binding sequence in collagen III. The present work completes our understanding of the collagen-VWF interaction, providing information on crucial sequences in collagen that perfectly complements our existing knowledge of the collagen-binding site in VWF and may assist in targeting the collagen-VWF interaction for therapeutic purposes

M211

POSITIVELY CHARGED PEPTIDES CAN INTERACT WITH EACH OTHER, AS REVEALED BY SOLID PHASE BINDING ASSAYS

J. Rosenbluh[1], A. Kapelnikov[1], D.E. Shalev[2], M. Rusnati[3], A. Bugatti[3], A. Loyter[1]

[1]Department of Biological Chemistry [2]Wolfson Center for Applied Structural Biology, The Hebrew University of Jerusalem, Jerusalem, Israel [3]Department of Biomedical Science and Biotechnology, School of Medicine, University of Brescia, Italy

Solid phase assay systems such as enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), and overlay gels are used to study processes of protein–protein and protein-peptide interactions. The common principle of all these methods is that they monitor the binding between soluble and surface-immobilized molecules.

Following the use of bovine serum albumin (BSA)–peptide conjugates or isolated synthetic peptides and the above-mentioned solid phase assay systems, we were able to demonstrate that positively charged peptides, which would be expected to repulse each other, can interact with each other. Both the ELISA and SPR methods showed that the binding process reached saturation with Kd values ranging between 1 and 14 nM. No interaction was observed between BSA conjugates bearing positively charged peptides and conjugates bearing negatively charged peptides or with pure BSA molecules, strengthening the view that interaction occurs only between positively charged peptides. However, interactions between the same peptides were not observed in solution when was monitored by nuclear magnetic resonance (NMR) or by native gel electrophoresis.

Thus, it appears that for positively charged molecules to interact one of the binding partners must be immobilized to a surface, a process that may lead to the exposure of otherwise masked groups or atoms. The relevance of our findings for the use of solid phase assay systems to study interactions between biomolecules will be discussed.

BINDING STUDIES OF HPK1 PRO-RICH PEPTIDES TO THE CORTACTIN SH3 DOMAIN

P. Ruzza[1], **C. Rubini**[1], C. Cuba[1], A. Calderan[1], A. Guiotto[1], G. Siligardi[2],
R. Hussain[2], L. Cesaro[3], A.M. Brunati[3], A. Donella-Deana[3]

[1]CNR - Institute of Biomolecular Chemistry, Padua Unit, Padua, Italy [2]Diamond Light Source, Diamond House, Didcot, Oxfordshire [3]Department of Biological Chemistry, University of Padua, Padua, Italy

The hematopoietic progenitor kinase 1 (HPK1), a mammalian hematopoiesis-specific Ste20 kinase, contains a cluster of four proline-rich sequences called P1, P2, P3 and P4 located after the kinase domain. These Pro-rich regions play an important role in the interactions of this kinase with different adapter proteins. Previous studies showed that P1, which contains the canonical PxxPxR motif, and P2 and P4 with the canonical PxxPxK motif interact with the C-terminal SH3 domain of hematopoietic lineage cell-specific protein 1 (HS1) even if with different affinity.

HS1 protein shares a high amino acid sequence and structural similarity to cortactin although their functions differ considerably. Here we report the results of our investigation on the interaction between the C-terminal SH3 domain of cortactin and the four proline-rich motifs of HPK1. These interactions were analyzed by non-immobilized ligand interaction assay by circular dichroism (NILIA-CD). Upon peptide addition, the binding was monitored by the CD changes of the Trp side-chains of the conjugate GST-SH3cort. The dissociation constants Kd were determined analyzing the CD data at 294 nm using a non-linear regression method. The results demonstrate that GST-SH3cort displays an affinity binding higher than that found with the corresponding HS1 domain and that the four HPK1 Pro-rich regions are not equivalent. P2 appears to bind with the highest affinity (Kd=0.5 μM), followed by P1 (Kd=10 μM) and P4 (Kd=33 μM), whilst P3 does not interact at all.

Th213

SYNTHETIC PEPTIDES OF 558-565 LOOP OF THE A2 SUBUNIT OF FVIIIA OF BLOOD COAGULATION CASCADE AND THEIR BIOLOGICAL ACTIVITY

Y.M. Sarigiannis[1], G.P. Stavropoulos[1], M.T. Liakopoulou-Kyriakides[2], P.E. Makris[3]

[1]Department of Chemistry, University of Patras [2]Department of Chemical Engineering, Aristotle University of Thessaloniki [3]Haemostasis and Thrombosis Unit, AHEPA Hospital, Thessaloniki, Greece

The generation of a fibrin clot is mediated by the regulated activation of a series of serine proteases and their cofactors. Factor VIII in its activated form, FVIIIa, acts as a cofactor to the serine protease FIXa, in the conversion of the zymogen FX to the active enzyme FXa. Both FVIII and FIX are essential for normal coagulation, deficiencies of either are associated with the bleeding diatheses hemophilia A and B, respectively. The role of FVIIIa is to bind factor IXa, generating the phospholipid-dependent intrinsic factor Xase complex. At least two interactive sites have been identified for the enzyme-cofactor interaction. The Ser558-Gln565 region within the A2 subunit has been shown to be crucial for VIIIa-IXa interaction.

In an attempt to study this interaction, we synthesized a series of peptides of 558-565 loop of the A2 subunit. The syntheses of these peptides were carried out by using SPPS and Fmoc/But methodology. The synthesized compounds were purified by RP-HPLC and lyophilized to give fluffy solid, identified by FT-IR, NMR and ES-MS spectra.

These compounds were tested for inhibitory activity on human platelet aggregation in vitro, by adding common aggregation reagents to citrated platelet rich plasma (PRP). The aggregation was determined using a dual channel electronic aggregometer by recording the light transmission.

M214

SYNTHESIS OF TRITIATED β-AMYLOID PEPTIDES AND THEIR USE IN BIOLOGICAL STUDIES

K. Soós[1], G. Tóth[2], Z. Bozsó[1], I. Rojik[1], Y. Verdier[1], B. Penke[1]

[1]1st Department of Medical Chemistry,University of Szeged [2]Department of Biochemistry, Biological Research Centre of The Hungarian Academy of Sciences, Szeged, Hungary

β-amyloid (Aβ) peptides containing 40 or 42 amino acids form neurotoxic aggregates. The precise mechanism of their neurotoxic action has not yet been discovered. Radiolabeling of the amyloid peptides could help in biological studies as transport, binding and metabolism of Aβ peptides. For this purposes four Aβ peptides were synthesized two of them with tritium labeling. Precursor peptides Aβ 2,5-diiodo-Tyr10(1-40) and Leu34(25-35) were prepared by solid phase peptide synthesis using manual Fmoc strategy. Tritium labeling was carried out by catalytic saturation, yielding Aβ[3H-Tyr10](1-40) and Aβ[3H-Leu34] with specific activities of 35 and 120 Ci/mmol, respectively. Both tritiated Aβ peptides were used in cat brain(in vivo) experiments and it was found that the peptide aggregates enter the neurons within 30 min (electronmicroscopic autoradiography). This transport is most probably an endocytotic pathway. Aβ aggregates could interact also with cytoplasmic proteins such as 3-phosphoglyceraldehyde dehydrogenase etc. We suppose that Aβ assemblies can interact both with membrane receptors (NMDA, AMPA, ACh) and with cytoplasmic proteins triggering neuronal dysfunction and death.

SYNTHESIS, BIOCHEMICAL AND STRUCTURAL ANALYSIS OF PEPTIDES MIMICKING THE BINDING SITE OF HYAP-WW DOMAIN FOR PROLINE-RICH LIGANDS

U. Strijowski, T. Hirsch, J. Eichler

GBF - German Research Centre for Biotechnology, Braunschweig, Germany

Background and Aims: WW domains are the shortest known protein domains and contain a stable three-stranded b-sheet, which presents the binding site for proline-rich ligands. This interaction is mediated by hydrophobic interactions between aromatic and hydrophobic residues of the domain, and the polyproline core of the ligand. As part of our ongoing efforts aimed at synthetically mimicking conformationally defined protein binding sites (1), we have designed and synthesized linear and cyclic peptides covering the binding site of the WW domain of human Yes-associated protein (hYAP-WW), whose structure in complex with a proline-rich ligand had been solved by NMR spectroscopy (2).

Methods: Peptides were synthesized by SPPS, purified by HPLC, and characterized by 2D-NMR spectroscopy, as well as by molecular dynamics calculations. Affinities of the peptides to a hYAP-WW ligand were determined in direct and competitive binding assays.

Results and Conclusions: A cyclic peptide covering the sequence stretch of hYAP-WW that contains its primary contact residues for proline-rich ligands, was found to compete with the domain for binding to a hYAP-WW ligand. Long-ranging NOEs identified in the NMR spectra of this peptide indicate a conformation, in which sequentially distant residues are brought into spatial proximity, likely through formation of a beta-sheet. These result demonstrate the feasibility of functional, as well as structural, mimicry of conformationally defined protein binding sites through synthetic peptides.

Literature:
(1) Eichler, J. Protein Peptide Lett. 11 (2004) 281–290.
(2) Macias, M.J.; Hyvönen, M.; Baraldi, E.; Schultz, J.; Sudol, M.; Saraste, M.; Oschkinat, H. Nature 382 (1996) 646–649.

HIGH-LEVEL EXPRESSION OF GLUCAGON AND GLUCAGON-LIKE PEPTIDE 1 RECEPTORS IN TETRACYCLINE-INDUCIBLE STABLE HEK293S CELLS

C.G. Unson

The Rockefeller University, New York, NY, USA

Despite insights from receptor mutagenesis and structural function studies of peptide ligands the interaction of peptide hormones with family B G protein-coupled receptors (GPCRs) is not fully understood. In family B GPCRs an intact disulfide-bonded amino-terminal domain and extracellular loops linking the seven-helical bundle are believed to contain the peptide binding site. The lack of high-resolution, three-dimensional information is an important factor that limits our understanding of the molecular basis of peptide ligand binding and subsequent development of potential drugs. We have established and characterized a novel tetracycline-inducible system in stable mammalian cell lines for the high-level expression of human glucagon and glucagon-like peptide 1 receptors. This is a strategy frequently used in bacterial expression systems in which membrane proteins are expressed by induction of the desired gene only after the cells have grown to near-maximum density. A gradual loss of high-expressing clones over a given time or severe counter-selection due to the toxicity of the expressed protein in constitutive expression systems are avoided. In addition, these cell lines lack N-acetylglucosaminyltransferase I activity and do not allow formation of heterogeneous N-glycans that may complicate crystallization and NMR analysis. Both receptors contain a 9-mer peptide tag (TETSQVAPA) at the C-terminus for affinity chromatography. Initial receptor yields of 0.81 µg/mg protein for human GLP-1R and 1.27 µg/mg protein GR can be optimized on a bioreactor. Expression of family B GPCRs using this novel system should provide a reliable and sufficient source of receptor protein for structural and biophysical studies.

M217

CYCLIC PEPTIDES AS INHIBITORS OF INTEGRIN-LIGAND INTERACTION

S. Urman[1], K. Gaus[1], U Strijowski[1], Y. Yang[1], N. Sewald[1], S. De Pol[2], O. Reiser[2]

[1]Department of Chemistry, Bielefeld University, Bielefeld [2]Institut of Organic Chemistry, University of Regensburg, Germany

Integrins constitute a family of transmembrane cell surface receptors. They are involved in cell-cell and cell-extracellular matrix interactions. Thus, they participate in many physiological and pathophysiological processes and are of crucial importance for the living organism. Integrins possess two non-covalently bound subunits, α and β, that jointly participate in ligand binding. These dimeric proteins show very high specificity in recognition of natural ligands. For example, α4β1 integrin recognizes VCAM-1 (vascular cell adhesion molecule 1) and fibronectin through binding amino acid motifs TQIDSPLN and LDV, respectively. On the other hand, fibronectin is a classical ligand for α5β1 integrin with the recognition motif RGD.

As shown, identification of the integrin ligands occurs through small recognition amino acid sequences (mostly tripeptides). Thus, small cyclic peptides possessing a recognition motif in the appropriate three-dimensional conformation are able to interfere with the integrin-ligand interactions and act as inhibitors.

The aim of this investigation is the characterisation of small cyclic peptides containing the RGD motif and the determination of the selectivity and specificity of these inhibitors. Two new pentapeptides with 3-amino-cyclopropane-1,2-dicarboxylic acid monomethyl ester ((+/-)ACC) were synthesized and tested. Peptides were characterized in biological assays with living cells (K562 and WM115) and in surface plasmon resonance binding studies. Experiments have shown that cyclic peptide cyclo-(Arg-Gly-Asp-(+)ACC-Val) is a very potent inhibitor (IC50-value in nM range) of interaction between vitronectin and αvβ3 or αvβ5 integrins.

THE IMPORTANCE OF BIOTIN PRESENTATION IN AVIDIN-BASED ASSAYS

P.D. White[1], S. Barthélémy[2], J. Beythien[2], B. Dörner[2], O. Pöschke[3]

[1]Novabiochem, Merck Biosciences Ltd., Beeston, UK [2]Novabiochem, Merck Biosciences AG, Läufelfingen, Switzerland [3]Merck KGaA, Darmstadt, Germany

When preparing biotin-labelled peptides as ligands for avidin-based assays, it is chemically most expedient to locate the biotin label on the N-terminal group of the peptide. This is done without any regard to how this may affect peptide-target interactions, biotin-avidin binding, and the solubility properties of the resultant peptide. In many instances, the products are poorly soluble, have little biological activity, and poor affinity for avidin. Problems can also arise during the synthesis of such N-terminally biotinylated peptides due to the poor solubility and reactivity of many of the reagents used for biotin introduction.

To overcome these limitations, we have developed an extremely simple method for synthesising peptides C-terminally with biotin. Peptides can now be easily prepared by standard solid phase techniques either N- or C-terminally labelled, and screened to determine the optimum presentation for the biotin. In cases studies using protein-protein interaction and kinase assays, peptides C-terminally labelled with biotin gave better sensitivity.

SYNTHESIS OF CYCLIC PEPTIDES AS INHIBITORS OF INVASIN WITH INTEGRIN ALPHA3 BETA1

Y. Yang[1], J. Eble[2], N. Sewald[1]

[1]Department of Chemistry, Bielefeld University, Bielefeld [2]Institute of Physiological Chemistry and Pathological Biochemistry, University of Muenster, Muenster, Germany

Many bacterial pathogens bind and enter eukaryotic cells to establish infection. Invasin is an outer membrane protein required for efficient uptake of Yersinia into M cells. Invasin mediates its entry into eukaryotic cells by binding to members of β1 integrin family that lack insertion domains (I domains), such as α3β1,α4β1,α5β1,α6β1, and αvβ1. This type of peptide-protein interaction is an ideal subject for the rational design of inhibitors.

The integrin binding motif consists of one loop region with a conservative Asp911 residue and two synergistic regions. The aim of this project is to synthesize cyclic peptides based on the invasin binding epitope SDMS. This sequence has to be positioned in a β-turn with Asp in i+1 position for optimal activity of the peptide. Also the Arg883 and Asp811 residue, which are about 27.29Å and 31.54Å respectively away from the Asp911 residue of the SDMS loop in invasin, should be investigated. Peptides that mimic these recognition sites have been synthesized and tested as ligands for the integrin

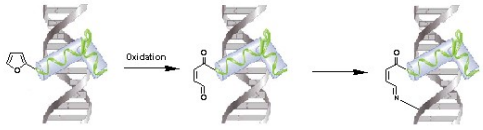
M220

VALIDATION OF A FURAN MOIETY AS A MASKED REACTIVE ENAL FUNCTIONALITY FOR PEPTIDE-DNA CROSS-LINKING PURPOSES

A. Deceuninck, A. Madder

Department of Organic Chemistry, Laboratory for Organic and Biomimetic Chemistry, University of Ghent, Belgium

Peptide-DNA cross-linking is a very powerful tool for studying peptide-DNA complexes. It transforms non-covalent complexes into covalent complexes, which renders characterization of the adduct by classical techniques (mass spectroscopy, NMR,…) much easier. The aim of our research is to develop a new method for peptide-DNA cross-linking involving the incorporation of a furan moiety. The strategy is inspired by the naturally occurring process of oxidative furan ring opening by Cytochrome P450. The resulting cis-butene-1,4-dial has been shown to react with amino- and sulfhydryl groups of macromolecules such as proteins and DNA. In our research, DNA binding peptides are modified with a furan moiety and then chemically oxidized into a reactive enal. This enal can react with DNA to form a covalent peptide-DNA complex. Previous attempts to selectively oxidize furan modified minor groove binding peptides consisting of N-methylpyrrole building blocks failed. We are now applying the same strategy on major groove binding peptides consisting of natural amino acids. Currently the oxidation conditions are being optimized so that the furan moiety undergoes selective oxidation. These optimized conditions will be applied to known DNA binding peptides, in order to obtain a peptide-DNA cross-link.



Transformation of a non-covalent complex into a covalent complex

THE N-TERMINALLY COUPLED ACYL CHAIN LENGTH INFLUENCES THE OLIGONUCLEOTIDE BINDING AND DELIVERING ABILITIES OF SV40

I. Laczkó[1], S. Bottka[2], Gy. Váró[1], Z. Bálint[1], E. Illyés[3], E. Vass[3], J.R. Bertrand[4], C. Malvy[4], M. Hollósi[3]

[1]Institute of Biophysics [2]Institute of Plant Biology, Biological Research Center, Szeged [3]Department of Organic Chemistry, Eötvös Lóránd University, Budapest, Hungary [4]Institute Gustave Roussy, Villejuif, Paris, France

We coupled octanoyl or palmitoyl group to the N-terminus of an analogue of SV40 nuclear localization signal (NLS) peptide, SV126-133(Ser128) to investigate the effect of fatty acid chain length on the conformation of the lipopeptide-antisense oligodeoxynucleotide (ODN) complexes and to establish the optimal peptide/ODN molar ratio (rM) for the effective delivery of ODN into the cells. The ODNs used in this study were targeted towards either the green fluorescent protein (GFP) mRNA and the junction sequence between EWS and Fli1 genes. The conformational change of ODN at different rM values was followed by circular dichroism (CD), attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy and atomic force microscopy (AFM). The SV40 peptide-mediated ODN transfer into NIH/3T3 cells was studied by epifluorescence microscopy.

The strongest binding affinity was observed for palmitoylated-SV peptide as shown by the largest changes in CD and ATR-FTIR spectra. This finding may indicate a better accessibility of the positively charged lysyl and arginyl side chains to the phosphate groups due to the turn structure(s) stabilized by palmitoyl group. In titration of ODN with the peptides, the CD spectral changes show a gradual unstacking of the bases reaching the maximum rate at rM = 10. At rM > 10, restacking of the nucleotide bases is detected accompanied by the dissociation of aggregates to smaller particles where the ODN is completely encapsulated in liposome-like object made up of palmitoylated-SV peptides. Cell translocation experiments revealed a highly efficient cell transport of ODN by palmitoylated-SV peptide at rM > 10.

THE DESIGN, SYNTHESIS AND BINDING STUDIES OF A NOVEL CLASS OF ?-HAIRPIN REV PEPTIDOMIMETICS

K. Patora[1], Z. Athanassiou[2], K. Moehle[1], G. Varani[2], J.A. Robinson[1]

[1]*Institute of Organic Chemistry, University of Zurich, Switzerland*
[2]*Department of Chemistry and Department of Biochemistry, University of Washington, Seattle, WA, USA*

The interaction between the HIV-1 regulatory protein Rev and Rev responsive element (RRE) of HIV-1 mRNA has emerged in the last decade as an important target in antiviral therapy. The Rev-RRE interaction is essential for the replication of HIV. The Rev protein binds to the RRE site located in the env coding region of the full length viral mRNA and facilities the export of the RNA from the nucleus, while protecting it from the cell's splicing machinery.
In the published NMR structure of the RRE/Rev-derived peptide complex, an -helical segment of Rev binding domain recognises a specific region of RRE. An approach is described to design a new class of -hairpin peptidomimetic ligands for HIV-1 Rev protein, which inhibit its binding to the RRE RNA. A model -hairpin peptide served as a scaffold to pre-organise side chains into a geometry similar to that seen in a helical peptide. A library of peptidomimetics was prepared by grafting sequences related to the RNA recognition element in Rev onto a hairpin-inducing D-Pro-L-Pro template. The electrophoretic mobility shift assay (EMSA) revealed that all of the designed peptidomimetics bind to RRE and the best examples show affinities (Kd) in a nanomolar range.
These new ligands show a novel approach to designing Rev peptidomimetics, represent interesting leads for the development of more potent HIV RRE/Rev inhibitors and permit more detailed studies of the mechanism of binding to RNA.

M223

CAPILLARY ELECTROPHORESIS ANALYSIS OF INTERACTION OF NUCLEOBASE-CONTAINING TAT PEPTIDES WITH TAR RNA HIV-1

A. Napiorkowska, A. Sawula, M. Olkowicz, P. Mucha, **P. Rekowski**

Faculty of Chemistry, University of Gdansk, Poland

Tat (trans-activator of transcription) is the protein which controls the early phase of HIV-1 replication cycle. It is a potent viral trans-activator containing from 86 to 101 amino acid residues which binds to TAR RNA. The fundamental role of Tat is promoting effective elongation of viral mRNA (vmRNA). Binding of Tat to TAR is mediated by a 9-amino-acid, highly basic Arg49-Lys-Lys-Arg52-Arg-Gln-Arg-Arg-Arg57 sequence of the ARM (arginine rich motif); the key role in these interactions is played by Arg52.

The goal of our research was to investigate the interaction of 27-nucleotide TAR RNA with synthesized Tat peptide analogues using capillary electrophoresis (CE), a powerful analytical technique of biochemical studies. Changes in electrophoretic mobility of the TAR peak are employed for monitoring TAR-Tat complex formation. CE experiments were performed using LPA-coated capillary and a physical gel containing buffer. Native ARM fragment Tat(49-57)NH2, its analogues Ac-Tat(49-57)NH2 and Ac-[Lys52]Tat(49-57)NH2 and analogues substituted in position 52 with alanine-, homoalanine and lysine-derived amino acids containing nucleobases (adenine, guanine, cytosine, uracil, thymine) and nucleosides (adenosine, guanosine, uridine and cytidine) in the side chain were studied. Specific interactions and complex formation were observed for both the native ARM peptide fragment and selected Tat analogues. The research is aimed at improving our understanding of the molecular mechanism of peptide-nucleic acid interaction, as well as evaluating the usefulness of selected nucleobase-containing amino acids as point probes for investigating peptide-RNA interactions.

This work was supported by grant DS-8291-4-0130-6 from the University of Gdansk.

DNA-PEPTIDE INTERACTION FORCES ON THE SINGLE MOLECULE LEVEL

K. Wollschläger[1], R. Eckel[2], S.D. Wilking[1], M. McIntosh[3], A. Becker[3], R. Ros[2], D. Anselmetti[2], N. Sewald[1]

[1]Department of Chemistry [2]Department of Physics [3]Department of Biology, Bielefeld University, Bielefeld, Germany

Interactions between proteins and DNA are important to all living organisms. The goal is to investigate the molecular recognition between DNA and the transcription factor PhoB of E. coli on the single molecule level and to identify amino acids required for DNA binding.

PhoB is composed of a transactivation domain (amino acids 1-127) and a DNA binding domain (amino acids 123-229) that binds to specific DNA sequences (pho boxes) containing a TGTCA sequence.[1]

Chemical synthesis of peptide epitopes present in the DNA binding domain of PhoB and isolation of the whole DNA binding domain of PhoB was performed. The protein was purified using intein mediated protein purification. An additional cysteine residue was ligated to the protein using intein mediated ligation reactions and will be used for immobilization and labeling.

In single molecule force spectroscopy (AFM) experiments it has been shown that both a peptide with a native PhoB-sequence and the recombinant protein bind to DNA. Competition experiments were performed to prove specific DNA binding.[2] Mutated peptides and proteins where strategic amino acids were replaced by alanine have also been examined to reveal the contributions of single residues to molecular recognition.

The binding contribution of the proteins is determined by surface plasmon resonance, electrophoretic mobility shift assays and fluorescence correlation spectroscopy.

Acknowledgment: This project was supported by DFG (SFB 613).

[1] H. Okamura et al., J. Mol. Biol. 295, 1225-1236 (2001).
[2] R. Eckel et al., Angew. Chem. Int. Ed. 44, 3921-3924 (2005).

Th225

BIOPHYSICAL PROPERTIES OF ALAMETHICIN F50/5 AND SELECTED ANALOGUES INSERTED IN ROD OUTER SEGMENT MEMBRANES

C. Baldini[1], C. Peggion[1], C. Toniolo[1], N. Vedovato[2], G. Rispoli[2]

[1]Department of Chemistry [2]Department of Biology, University of Ferrara, Italy

We investigated the biophysical characteristics and the pore formation dynamics of naturally occurring and synthetic peptides forming membrane-spanning channels by using isolated rod outer segments (OS) of reptilia and amphibia recorded in the whole-cell configuration. Once blocked the two OS endogenous conductances (the cGMP channels by light and the retinal exchanger by removing one of the transported ion species from both sides of the membrane, i.e. K+, Na+ or Ca2+), the OS membrane resistance (Rm) could be >5 GΩ. Therefore, any exogenous current can be studied down to the single channel level.
Macroscopic currents of amplitude of ~300 pA were recorded in symmetric K+ or Na+ (>100 mM) and Ca2+ (1 mM) from the commercially available alamethicin mixture, the synthetic alamethicin F50/5 (a major component of the natural mixture), and selected analogues applied at 1 μM concentration at –20 mV. Once applied and removed the peptide, the current activates and deactivates with a time constant of about 160 ms. The synthetic analogues [Glu(OMe)7,18,19] and [Glu(OMe)18,19] produce a current of about 100 pA at 1 μM concentration, and they show a strong activation by hyperpolarization as alamethicin F50/5 itself. Clear single channel events were observed when the concentration of all of the alamethicin peptides is reduced to <250 nM.
These results indicate that the three Gln residues at positions 7, 18 and 19 of alamethicin F50/5 are not a key factor for pore formation and its conduction properties. In general, the pore assembly and disassembly are very fast and cooperative events.

M226

INTERACTION BETWEEN PENETRATIN AND PHOSPHOLIPIDS BILAYERS

Z. Bánóczi[1], F. Reig[2], F. Hudecz[1,3]

[1]Research Group of Peptide Chemistry, Eötvös L. University; Hungarian Academy of Sciences, Budapest, Hungary [2]Peptide Department, Institute for Chemical and Environmental Research, IIQAB, CSIC, Barcelona, Spain [3]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

The translocation mechanism of penetratin (RQIKIWFQNRRMKWKK) is not clear, but the involvement of cell membrane was supposed. Recent studies with phospholipid model membranes have shown that penetratin interacts only with negatively charged liposomes. We aimed to analyse the effect of penetratin on liposomes composed of different phospholipids (DPPC/DPPG 2:8-8:2) by fluorescence spectroscopy. In the first set of experiments, liposomes labelled with fluorescent markers (DPH, ANS and TMA-DPH) were incubated with penetratin and the fluorescence polarisation was determined as a function of the temperature. In the range of 15-200 mol/mol phospholipid/penetratin ratio, no change in the transition temperature was observed indicating that penetratin has no influence on the membrane structure. Next, we have analysed the interactions between phospholipids and penetratin through changes in the intrinsic fluorescence of the peptide due to the presence of two W residues in its sequence. Comparing the emission spectra corresponding to penetratin in aqueous media or in presence of vesicles one can clearly appreciate a blue shift. This indicates that that tryptophan residues are mainly exposed to a hydrophobic environment. Analysis of the main band shows low values of polarization suggesting a free motion of the peptide chain. On the contrary polarization measured for penetratin mixed with liposomes results in higher values. This indicates that hydrophobic residues, like Trp, are inserted into the bilayer and their motion is restricted. These data suggest the presence of interation sensed strongly by Trp properties.

These studies were supported by Medichem2 (1/A/005/2004) and Spanish-Hungarian Intergovernmental Programme CSIC-CID (12/2003).

Tu227

GRAMICIDIN S EFFECT ON HUMAN BLOOD PLATELETS DEPENDS ON THE MOBILITY OF MEMBRANE LIPIDS

E. Hackl[1], **V. Berest**[2], A. Alamush[2], S. Gatash[2]

[1]Faculty of Life Sciences, University of Manchester, Manchester, UK [2]Department of Biological and Medical Physics, V.Karazin Kharkiv National University, Kharkiv, Ukraine

Cyclopeptide antibiotic gramicidin S (GS) has antimicrobial activity against Gram-positive and Gram-negative bacteria and some fungi. But non-specific action of GS and its high lytic potential limits therapeutic application of GS. We attempted to elucidate in which way GS molecule could be modified to lose its haemolytic side effects. GS molecule interacts directly with membrane phospholipids due to electrostatic and hydrophobic interaction. Naturally, changes in the state of a lipid bilayer cause changes in the GS molecule binding to a bilayer.

We studied the effect of GS on human blood platelets and the effect of platelet membrane state on the GS-induced disaggregation of cells with the help of turbidimetric and microscopy techniques. We modified the membrane state by temperature, osmotic stress, ionizing irradiation, lipid oxidation.

Depending on concentration GS causes platelet shape change and activation. When added to preliminary aggregated (in response to physiological agonists - thrombin, epinephrine, ADP) platelets, GS causes crumble of cells aggregates. The rate and extent of platelet disaggregation under the effect of GS non monotonously depends on temperature (range of 5-40°C) and irradiation dose (up to 200 Gy). Parameters of the GS interaction with membranes are determined by the mobility of membrane lipids. Factors modifying the lipid bilayer change the degree and the speed of the GS interaction with platelet membranes.

Results obtained permit to use GS for testing the state of membrane lipids and on the other hand allow to suppose ways of GS molecule modifications to achieve its tolerance to blood cells.

Th228

INSIGHTS ON THE INTERACTIONS BETWEEN TWO CECROPIN-MELITTIN PEPTIDES AND MODEL MEMBRANES: CALORIMETRIC AND SPECTROSCOPIC RESULTS

G. Bai[1], **P. Gomes**[1], R. Seixas[1], M. Hicks[2], M. Prieto[3], M. Bastos[1]

[1]CIQ (UP), Department of Chemistry of Faculty of Sciences of The University of Porto, Portugal [2]Department of Chemistry, University of Warwick, Coventry, UK [3]Centro de Química-Física Molecular do Instituto Superior Técnico - UTL, Lisbon, Portugal

Eukaryotic antibiotic peptides (EAPs) have been widely studied for the past years as an alternative to conventional antibiotics due to emergence of multi-resistant microbial strains, and significant efforts targeting increasingly potent and specific antimicrobial peptides are being made.

One interesting approach in peptide antibiotics is based on hybrid sequences derived from natural EAPs, with CA(1-8)M(1-18) as one of the most successful examples in this approach. CA(1-8)M(1-18) is derived from Cecropin A and Melittin, being more potent than the former and less hemolytic than the latter parent peptides, respectively. A subsequent approach has been to reduce CA(1-8)M(1-18) size while retaining its antimicrobial activity, which led to CA(1-7)M(2-9). Both CA(1-8)M(1-18) and CA(1-7)M(2-9) have been subject to extensive microbiological and biochemical studies, but not to detailed biophysical studies focused on the molecular mechanisms through which these peptides exert their action. We have been studying the interaction of these hybrids with liposomes by several techniques, namely calorimetry (DSC, ITC), circular dichroism, light scattering, SPR and fluorescence spectroscopy. The peptides were prepared by Fmoc/tBu SPPS, purified by RPLC and characterized by HPLC, AAA and MALDI-TOF MS. LUV's from DMPC, DMPG and their 3:1 mixture were used as model membranes.[1]

We now wish to present our recent results on peptide-membrane interactions, obtained by ITC, Time Resolved Fluorescence Spectroscopy and Linear Dichroism. These techniques provided us information on the energetics, partition and tertiary structure of the peptides (in buffer and in the presence of membranes), as well as on their orientation relative to the liposome surface.

M229

MOLECULAR IMAGING OF MEMBRANE LYSIS BY ANTIMICROBIAL PEPTIDES

K.N. Hall[1], A.I. Mechler[2], L.L. Martin[2], M.I. Aguilar[1]

[1]Department of Biochemistry And Molecular Biology [2]School of Chemistry, Monash University, Melbourne, Vic., Australia

Resistance to conventional antibiotics has stimulated a search for alternative therapeutics for microbial infections, a possible source that has gained much interest in recent years are antimicrobial peptides. Antimicrobial peptides target the cell membrane directly, which is a key feature as evolution has shown bacteria have had difficulty in altering their membrane composition and organization to mount a suitable defence against these peptides. A common theory is that peptides that bind strongly exhibit high biological activity, but our real-time quantitative binding studies via surface plasmon resonance (SPR) have shown that this correlation does not always hold. As more information on the molecular details of membrane disruption is required, we have used Atomic Force Microscopy (AFM) to visualise peptide insertion and changes in membrane morphology by a range of antimicrobial peptides in situ. Interaction studies were performed with a series of phospholipid mixtures that mimic either mammalian cells (high in phosphatidylcholine and cholesterol) or microbial cells (high in phosphatidylethanolamine and phosphatidylglycerol). The present study may assist in the design of new specific antimicrobial peptides with high antimicrobial activity and low host toxicity.

Tu230

OMIGANAN INTERACTION WITH BACTERIAL MEMBRANE MODELS. THE ROLE OF SATURATION AND CORRELATION WITH CLINICAL PROPERTIES

M.N. Melo, M.A.R.B. Castanho

Centro de Química E Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Portugal

The interaction of the dodecapeptide antimicrobial peptide Omiganan pentahydrochloride (ILRWPWWPWRRK-NH.5Cl) with bacterial and mammalian model membranes was characterized by means of UV-Vis absorption and fluorescence emission spectroscopy using large unilamellar vesicles of different proportions of POPC and POPG as models. Very high molar ratio partition constants ((18.9+-1.3)x10^3 and (43.5+-8.7)x10^3) were obtained for the bacterial models (POPG:POPC 4:1 and 2:1, respectively), these being about one order of magnitude greater than the partition constants obtained for the less anionic mammalian model systems ((3.7+-0.4)x10^3 for the 100% POPC system). At low lipid:peptide ratios there were significant deviations from the usual hyperbolic-like partition behavior of peptide vesicle titration curves, especially in the case of the most anionic systems. Membrane saturation was shown to be related to such observations and mathematical models were derived to further characterize the peptide-lipid interaction under these conditions. The calculated peptide-to-lipid saturation proportions, together with the determined partition constants, suggest that the Minimal Inhibitory Concentrations of Omiganan pentahydrochloride could represent the conditions required for bacterial membrane saturation to occur.

Th231

MODEL PEPTIDES MIMIC THE STRUCTURE AND FUNCTION OF THE N-TERMINUS OF THE PORE-FORMING TOXIN STICHOLYSIN II

S. Schreier[1], F.J.F. Oliveira[1], J. Paulino[1], F.C. Souza[2], E.J. Crusca[2], U. Ros[3], M. Tejuca[3], D. Martinez[3], E.M. Cilli[2], M.E. Lanio[3], C. Alvarez[3]

[1]Department of Biochemistry, Institute of Chemistry, University of São Paulo [2]Department of Biochemistry, Institute of Chemistry, State University of São Paulo, Araraquara, Brazil [3]Center for Protein Studies, Faculty of Biology, University of Havana, Cuba

The hemolytic pore-forming toxin sticholysin II (StII) produced by the sea anemone Stichodactyla heliantus belongs to the actinoporin protein family. The N-terminal domain of these proteins is required for interaction with membranes. To investigate the role of StII´s N-terminal domain in membrane binding and in the molecular mechanism of hemolysis, peptides corresponding to residues 1 to 35, or shorter fragments from this region, were synthesized. In some peptides Leu was replaced by Trp. All peptides exhibited hemolytic activity, albeit to a lesser extent than the whole protein. Moreover, peptides lacking the 1-14 hydrophobic stretch were less active. The longer peptides were also able to permeabilize phospholipid vesicles. Conformational studies were performed in aqueous solution and in membrane-mimicking environments. CD spectra showed that, while the shorter, more hydrophilic peptides, displayed a random conformation, the longer peptides underwent aggregation with increasing concentration, pH, and ionic strength. In the presence of trifluoroethanol and upon binding to detergent micelles and phospholipid bilayers, the peptides showed a propensity to acquire □-helical conformation, as expected for the sequence comprising residues 14 to 26. Fluorescence spectra demonstrated that the first residues of StII´s N-terminus penetrate more deeply into the bilayer, whereas residues 14-26 are located more superficially. This is in agreement with the predicted amphipathic nature of the helix formed by these residues and corroborates the existing hypotheses for the role of the N-terminal domain in the process of membrane insertion and pore formation.

Supported by FAPESP, CNPq (Brazil), and Ministry of Science and Education (Cuba).

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TRP-RICH ANTIBACTERIAL PEPTIDES AND THEIR ACTION ON MEMBRANES

M.P. Smirnova[1], V.G. Afonin[1], D.S. Orlov[2], O.V. Shamova[2], V.M. Shpen[1], N.I. Kolodkin[1]

[1]Laboratory of Peptide Chemistry, State Research Institute of Highly Pure Biopreparations [2]Department of General Pathology and Pathophysiology, Institute for Experimental Medicine RAMS, Saint Petersburg, Russia

Among a great number of antibacterial peptides a group of Trp-rich peptides is of special interest. Taking into consideration, that in most of proteins tryptophan is not frequently occurring amino acid, the biological meaning of a high content of tryptophan in structure of these antimicrobial peptides is particularly interesting. In the present study we carried out the investigation of antimicrobial and hemolytic activities of selected Trp-rich peptides and their action on microbial membrane:

ILPWKWPWWPWRR-NH2 - indolicidin (I)
PITWPWKWWKGG-NH2 - 3B3 (II)
PLSWFFPRTWGKR-NH2 - GSP-1a (III)
FPVTWRWWKWWKG-NH2 - puroindoline (IV)
VRRFPWWWPFLRR-NH2 - tritrypticin (V).

All peptides were synthesized by solid phase method using Boc-technology and their antimicrobial activity was estimated in radial diffusion assay. The set of microorganisms used for testing included Gram-negative bacteria Escherichia coli, Gram-positive bacteria Listeria monocytogenes and fungi Candida albicans. All the peptides exhibited potent antimicrobial activity against three listed microorganisms with MICs (minimal inhibitory concentrations) ranged from 1 to10 μg/ml. While the peptides I and V were substantially toxic for human erythrocytes, the peptides II - IV had no hemolytic effect. Influence of the peptides on the permeability of E. coli ML35p outer and inner membranes was evaluated by fluorometric and photometric methods and compared with the effects of well known antimicrobial membranolytic peptides mellitin and protegrin.

PEPTOMERIC ANALOGUES OF THE TRYPSYN INHIBITOR SFTI-1

A. Łęgowska, E. Zabłotna, A. Jaśkiewicz, D. Dębowski, J. Kossakowska, W. Łozińska, K. Rolka

Department of Bioorganic Chemistry, Faculty of Chemistry, University of GdańSk, Poland

Sunflower trypsin inhibitor SFTI-1 is the smallest and the most potent known peptidic trypsin inhibitor from the Bowman-Birk class of proteins [1]. This head-to-tail-cyclized 14-amino-acid peptide contains one disulfide bridge and a lysine residue (Lys5) present in the P1 position, which is responsible for inhibitor specificity.As was reported by us and other groups, SFTI-1 analogues with one cycle only retain trypsin inhibitory activity. Very recently we have shown [2] that introduction of N-substituted glycine residues mimicking Lys and Phe (denoted as Nlys and Nphe) in the P1 position of monocyclic SFTI-1 with disulfide bridge yielded potent trypsin and chymotrypsin inhibitors, respectively. In this novel class of proteinase inhibitors contains completely proteolytic resistant P1-P1' reactive site.
In the present communication we report chemical synthesis and determination of trypsin and chymotrypsin inhibitory activity of a series of ten SFTI-1 analogues modified in the P1 position by these peptoid monomers (Nlys and Nphe). Each of the synthesized peptomeric (peptide-peptoid hybrid polymer) SFTI-1 analogues contains one of the following cycles: head-to-tail, disulfide bridge formed by Cys, by Pen and by Cys/Pen residues. The impact of the different cycles introduced into SFTI-1 structure on proteinase inhibitory activity will be discussed.

1. Luckett S., et al., J. Mol. Biol., 290,535 (1999).
2. Stawikowski M., et al., ChemBioChem., 6, 1 (2005).

PROCESSING OF HUMAN SARS-COV SPIKE PROTEIN AND HEPTAD REPEAT DOMAINS OF CLEAVED C-TERMINAL FRAGMENT IN SARS INFECTION

A. Basak, X. Hao, S. Basak

Department of Hormone, Growth and Development, Ottawa Health Research Institute, Ottawa, ON, Canada

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is the causative agent for the recent outbreak of SARS infection. SARS-CoV spike glycoprotein (S) is considered as one of the prime targets for SARS therapeutics and intervention. Consistent with type I viral fusion protein, the SARS-CoV S-protein contains a proteolytic processing site and two interacting heptad repeat regions denoted as HR-N and HR-C. Following processing of S-protein mediated by host cellular protease/s, the C-terminal S2-fragment fuse with host cell membranes via its HR-N and HR-C domains that form coiled coil 6-helix bundle (trimeric of dimers)-crucial for its receptor-mediated viral fusion. Our objective in this work is to study the proteolytic site using model peptides and also to examine the interaction of HR-N and HR-C domains using fluorescence microscopy and other techniques. Thus we synthesized an intramolecularly quenched fluorogenic peptide containing the proposed cleavage site [Abz-EQDRNTR761□EVFATyx, Abz=2-amino benzoic acid and Tyx=3-nitro tyrosine] and showed by kinetic measurements that this cleavage is mediated most efficiently by furin, followed PC5 and PC7. Other potential substrates were also tested and compared. Above cleavage can be blocked by specific-PC-inhibitors in a dose-dependent manner. In addition using fluorescent-labeled peptides derived from HR-N and HR-C domains, circular dichroism spectra and surface assisted laser desorption mass spectral (SELDI-MS) analysis we confirmed that a short domain within HR-C (1151-1185) can bind to HR-N(890-930). Our studies revealed crucial roles of host PCs and heptad repeat domains in SARS pathogenesis.

M235

PSEUDO AND BRANCH-PEPTIDE INHIBITORS OF SUBTILISIN KEXIN ISOZYME-1: DESIGN, CELLULAR DELIVARY AND BIOCHEMICAL APPLICATIONS

D. Mohottalage, S. Basak, **A. Basak**

Department of Hormone Growth Development, Ottawa Health Research Institute, Ottawa, ON, Canada

Subtilisin Kexin Isozyme-1 (SKI-1)/Site1 Protease (S1P) is a Ca+2-dependent membrane bound mammalian subtilase of pyrolysin subtype. It cleaves peptide bonds at motif Arg-X-Leu/Ile/Val-Leu/Thr/Gly/Lys□ where X=any amino acid other than Cys. SKI-1, a Golgi enzyme is clinically linked to lipid metabolism, cholesterol homeostasis and infections caused by hemorrhagic fever viruses such as Lassa and others. Interest has grown to develop specific and potent inhibitors of this enzyme. Our objectives in this study are to generate soluble recombinant human (h)SKI-1 enzyme, design potent inhibitors and study its 3D-model structure. We have successfully expressed hSKI-1 enzyme lacking its transmembrane domain in HEK-293 cells and purified the enzyme via chromatography. In addition we developed SKI-1 inhibitors by using pseudo- and multi-branch peptide approaches. In first approach we inserted dipeptide isosteres amino oxy acetic acid (Aoaa) or 8-amino 3, 6 dioxa octanoic acid (Adoa) at scissile P1-P1' position ((R175□L) of hSKI-1. A typical example is 167GRYSSRRL(Adoa)AIP179. Other dipeptide isosters were also incorporated at the cleavage site of either SKI-1 prodomain or Lassa virus glycoprotein. In second approach we prepared 2 and 4-branch peptides containing hSKI-1128-137 segment. These peptides inhibit SKI-1 in competitive manners with varying degrees ranging from low □M to high nM IC50. Circular dichroism spectra indicated strong interactions of inhibitors with SKI-1 consistent with observed inhibition profile. A 3D-model structure of catalytic domain of SKI-1 indicated a broad catalytic pocket

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ENZYMATIC SYNTHESIS OF HIGH SPECIFIC SUBSTRATE FOR CYSTEINE PROTEASES ASSAY

A.V. Belyaeva[1], T.A. Semashko[2], E.N. Lysogorskaya[2], V.I. Lozinsky[1], I.Yu. Filippova[2]

[1]A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences [2]Chemistry Department, Lomonosov Moscow State University, Moscow, Russia

Cysteine proteases are of great importance in biochemical processes and these enzymes are used in biotechnology, food industry and agriculture. In this connection synthesis of high selectivity and high specificity substrates for cysteine proteases is of importance. Enzymatic synthesis of peptides is a good tool for obtaining different biologically active peptides. Immobilized serine proteases, subtilisin Carlsberg and α-chymotrypsin immobilized on poly(vinyl alcohol) cryogel (PVA-cryogel), proved to be a convenient biocatalyst for such kind of syntheses.

The high specific chromogenic substrate for cysteine proteases assay Glp-Phe-Ala-pNA was obtained with high product yields (up to 88% in 24 h) using subtilisin and chymotrypsin immobilized on PVA-cryogel. The reaction was carried out according to the following scheme:

Glp-Phe-OMe + Ala-pNA → Glp-Phe- Ala-pNA, where

Glp – the residue of pyroglutamic acid, pNA – p-nitroanilide. The influence of initial concentrations of components, the reaction mixture composition, the biocatalyst content and time on product yield was studied. It was shown that the optimal conditions are: dimethylformamide-acetonitrile mixture 20:80 (v/v), initial concentrations – 85 mM, and enzyme-to-substrate ratio – 1:3900.

This approach was used in order to synthesize analogous substrates, containing different fluorogenic and chromogenic groups as well as other amino acids in P1-position. The obtained substrates were tested for the papain assay.

This work was supported by RFBR grant № 03-03-32847, CRDF grant RUC 2-5027-MO-04 and RSSF grant for PhD Students of Russian Academy of Sciences.

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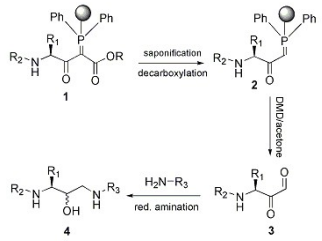
RAPID SYNTHESIS OF PEPTIDE ISOSTERS VIA C-ACYLATION ON LINKER REAGENTS

A. El-Dahshan[1], S. Weik[2], J. Rademann[1,3]

[1]*Leibniz Institute for Molecular Pharmacology (FMP), Berlin, Germany*
[2]*FE Patents, Boehringer Ingelheim, Austria GmbH, Vienna, Austria*
[3]*Free University Berlin, Berlin, Germany*

Peptidyl-a-ketoaldehydes 3 represent attractive lead compounds and intermediates in the development of potent protease inhibitors due to their structural similarity with peptide aldehydes, previously known to be excellent inhibitors of serine- and cysteine protease. Recently, we demonstrated the application of polymer cyano methylene- and carboxylato methylene phosphoranes in the assembly of a-hydroxy-b-amino esters (norstatines), a,b-diketoesters, and a,b-unsaturated ketones. [1,2,3] We now present a further development of our reagent linker 2 approach employing peptidyl-a-ketoaldehydes 3 and diamino propanoles 4. Carboxylato methylen phosphoranes 1 derived from bromo acetic esters which are readly acylated without racemization, play the key role in our synthetic concept. Herein we show the oxidative cleavage to peptidyl-a-ketoaldehydes 3 using dimethyldioxirane (DMD) in acetone as oxidant, after saponification and decarboxylation on the solid support. Diamino propanoles 4 were furnished via the reductive amination of resin-bound peptides.



[1] S.Weik, J. Rademann, *Angew. Chem.* **2003**, 115, 2595-2598; *Angew. Chem. Int. Ed.* **2003**, *42*, 2491-2494.
[2] S. Weik, T. Luksch, A. Evers, J. Böttcher, A. Hasilik, H. Löffler, G. Klebe, J. Rademann, *ChemMedChem*, **2006**, in press.
[3] A. El-Dahshan, S. Weik, J. Rademann, submitted.

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NEW SCREENING METHODOLOGY BY 19F-NMR

S. Frutos[1], E. Giralt[1], M. Pellecchia[2]

[1]*Irb-Pcb, Barcelona, Spain* [2]*Burnham Institute, San Diego, CA, USA*

Over the past few years Nuclear Magnetic Resonance has emerged as a powerful means for lead molecular identification and optimization.On the other hand, the 19F NMR has been used succesfully in several structural studies, protein folding studies and for the identification of active compounds, using a very similar methodology that the one used in the present work.
The methodology required the labeling of the substrate with a CF3 moiety. The enzymatic reaction is performed with the CF3 substrate and quenched, using an enzyme inhibitor. 19F NMR is then used to monitor the evolution of both substrate and product. Only two peaks are observed, the starting substrate and the cleaved substrate. This NMR method has some advantages: Fluorine NMR is very sensitive, 0,83 times that of the proton. There are no spectral interference from protonated solvents, buffers or detergents typically present in the enzymatic reactions.The 19F isotropic chemical shift is extremely sensitive to small structural perturbations resulting in different chemical shift for the signals of the substrate and product. Isotopic labeling of the protein is not required. As a model, Caspase-8, which play a critical role in the initiation of apoptosis process5 and HIV-1 protease were chosen. Two different kind of libraries were screened: One based on natural products from plant and animal extracts used in tradicional Chinese medicine and a second one corresponding of a synthetic library with two sublibraries of 160 and 144 compounds.With this methodology it has been possible to identify some compounds with very promising inhibitory properties.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL HUMAN KALLIKREIN 2 (HK2) INHIBITING PEPTIDES

C. Hekim[1], J. Leinonen[1], H. Koistinen[1], A. Narvanen[2], U.H. Stenman[1]

[1]Department of Clinical Chemistry, University Of Helsinki [2]Department of Chemistry, University of Kuopio, Finland

Background and aims: Human kallikrein 2 (hK2), a prostate specific serine protease, regulates the activity of several factors that may participate in proteolytic cascades promoting tumor growth and metastasis. Thus, inhibition of its enzymatic activity is a potential way of preventing growth and metastasis of prostate cancer. Moreover, specific ligands for hK2 have potential use for targeting and in vivo imaging of prostate cancer and for development of novel assays.
Methods: To find peptide ligands we panned several phage display peptide libraries against active recombinant hK2 captured by a monoclonal antibody exposing the active site of the enzyme. Alanine scanning and amino acid deletion analyses were performed to elucidate the motifs required for hK2 inhibition.
Results: From libraries expressing 10 and 11 amino acid long linear peptides we isolated six different hK2-binding peptides. Three of these peptides are specific inhibitors of the enzymatic activity of hK2. Amino acid substitution and deletion studies indicated that motifs of 6 amino acids are necessary for the inhibitory activity.
Conclusions: We have developed specific hK2 inhibitors by phage display technology. These novel hK2 specific peptides are potentially useful for treatment and targeting of prostate cancer.

THE RECOGNITION MECHANISM OF THE N-TERMINAL REGION OF HISTONE H2 AND H3 BY PEPTIDYLARGININE DEIMINASE IV

M. Watase, H. Matsubayashi, **Y. Hidaka**

Department of Life Science, Kinki University, Higashi-Osaka, Osaka, Japan

Peptidylarginine deiminase IV (PADIV) catalyzes the citrullination of Arg residues in various peptides and proteins, such as histone, resulting in the production of citrullinated proteins in granulocytes [1,2]. The citrullination mechanism of histone subunits and its functional effects in cells are not well known yet in detail. Recently, it has been reported that the protein deimination/citrullination by PAD IV plays a role in rheumatoid arthritis [3]. This implicates that the citrullination of histone may be related to rheumatoid arthritis. In order to further study the citrullination mechanism of histone, we explored the citrullination sites of histone H2A and H3 by PAD IV using a series of synthetic peptides.

Recently, Hagiwara et. al. reported that PAD IV only citrullinates the Arg3 of histone H2A as well as the Arg3 in histone H4 [4,5]. In order to investigate the citrullination mechanism, the N-terminal peptides of histone H2A and H3 were chemically synthesized and examined the citullination by PAD IV. The N-terminal acetylation effect of the N-terminal synthetic peptide was also estimated on the citrullination by PADIV. The velocity of each Arg residues in the N-terminal peptides were estimated in vitro. The results indicated that PADIV recognizes the specific Arg residues in the synthetic peptide, and that the N-terminal acetylation of the histone peptides dramatically affects on the substrate recognition of PADIV. In addition, the CD spectra of the N-terminal peptides were measured to elucidate the structural specificity for the recognition of PAD IV.

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THE EFFECTS OF TWO L-PROLINE MIMETICS ON POTENCY, LIPOPHILICITY AND BINDING KINETICS OF PROLYL OLIGOPEPTIDASE INHIBITORS

E.M. Jarho[1], J.I. Venäläinen[2], J.A. Garcia-Horsman[2], J.A.M. Christiaans[2], M.M. Forsberg[2], P.T. Männistö[2], E.A.A. Wallén[1]

[1]Department of Pharmaceutical Chemistry [2]Department of Pharmacology and Toxicology, University of Kuopio, Finland

Background and aims. Prolyl oligopeptidase (POP) is a serine peptidase that cleaves oligopeptides after prolyl residues. It has been associated with cognitive disorders. POP inhibitors have been shown to enhance cognition in monkeys (1) and to improve performance in verbal memory tests in humans (2). In the present study, the P2 L-prolyl residue of POP inhibitors was replaced by two L-proline mimetics, the 5-t-butyl-L-prolyl group and the (R)-cyclopent-2-enecarbonyl group. The effect of the mimetics on in vitro potency, lipophilicity and binding kinetics were studied.

Methods. The L-proline mimetics were synthesized according to the published procedures (3,4) with minor modifications. The IC50 and Ki values and the binding kinetics were determined for porcine POP. The log P values were determined with the shake-flask method.

Results. The replacement of the P2 L-prolyl residue by the L-proline mimetics gave compounds which were equipotent with their parent structures. Both L-proline mimetics increased lipophilicity but the effect of the 5-t-butyl-L-prolyl group was more pronounced. While the 5-t-butyl-L-prolyl group increased the dissociation half-life of the enzyme-inhibitor complex, the (R)-cyclopent-2-enecarbonyl group decreased it.

Conclusions. Both L-proline mimetics perfectly mimicked L-proline at the P2 position of POP inhibitors. These mimetics can be used to modify the lipophilicity and the binding kinetics of POP inhibitors.

(1) Schneider, J. S. et al. Neuropsychopharmacology 2002, 26, 176-182.
(2) Morain, P. et al. Br. J. Clin. Pharmacol. 2000, 50, 350-359.
(3) Beausoleil, E. et al. J. Org. Chem. 1996, 61, 9447-9454.
(4) Nöteberg, D. et al. J. Med. Chem. 2000, 431, 705-13.

NEW PSEUDOPEPTIDE INHIBITORS FOR THE TYPE-1 METHIONINE AMINOPEPTIDASE FROM ESCHERICHIA COLI

J. Jiracek[1], R. Liboska[1], L. Zakova[1], A.M. Dygas-Holz[2], R.C. Holz[2], M. Zertova[1], M. Budesinsky[1]

[1]Institute of Organic Chemistry and Biochemisty of The Academy of Sciences of The Czech Republic, Praha, Czech Republic [2]Department of Chemistry and Biochemistry, Utah State University, Logan, UT, USA

Methionine aminopeptidases (MetAP) are ubiquitous enzymes found in both eukaryotic and prokaryotic cells. These enzymes are involved in the selective removal of the initiator N-terminal methionine residue from newly synthesized polypeptide chains. Deletion or complete inhibition of MetAPs in E. coli and S. cerevisiae will lead to slower growth or lethality of the organism. Although there are intensive efforts in searching for new MetAP inhibitors, there are only few inhibitors for type-1 MetAPs, eventhough it is an essential enzyme in bacteria. Clearly, there is a need for potent inhibitors of bacterial and fungal MetAPs for antibiotic applications.
We present herein a new class of MetAP inhibitors, pseudopeptides, having two different non-hydrolyzable isostere moieties mimicking the transition-state of peptide hydrolysis by MetAPs. At first, we prepared a series of 20 pseudopeptide inhibitors of a general structure MSta-Xaa, in which each compound contained a statine derivative of methionine (MSta) and a different amino acid (Xaa) attached to the C-terminus of the statine synthon. These compounds were tested for their ability to inhibit the type-1 MetAP from E. coli. This step allowed us to select cysteine and valine as the most potent substitutions in the Xaa position. In a second series of inhibitors, we synthesized phosphinic pseudopeptides Met-ψ[PO2-CH2]-Gly-Val, Met-ψ[PO2-CH2]-Gly-Cys, Met-ψ[PO2-CH2]-Ala-Val and Met-ψ[PO2-CH2]-Ala-Cys. The described approach has allowed us to identify new inhibitors for the type-1 MetAP from E. coli.

NOVEL PEPTIDE TOOLS TO MANIPULATE PROTEASOME NANOMACHINERY

P.M. Juszczyk, M. Tokmina-Lukaszewska, S. Madabhushi, L. Endel,
M. Gaczynska, P.A. Osmulski

Department of Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center at San Antonio, TX, USA

The proteasome is an essential multicatalytic protease of the ubiquitin proteasome pathway. As a prime executor of regulated proteolysis, the proteasome controls almost all aspects of cell metabolism from signal transduction to cell cycle and differentiation. Pharmacological intervention into proteasome activity leads to cell apoptosis. This observation was applied to successfully treat multiple myeloma, since the cancer cells exhibit substantially higher sensitivity to competitive inhibition of proteasome than normal cells. However, the complete shutting down of the proteasome catalyzed proteolysis leads to serious side effects resulting from the disruption of proteolytic homeostasis even in noncancerous cells. Here, we show an alternative approach to control the proteasome activity using peptide based noncompetitive regulators. The cathelicidins derived peptides rich in proline and arginine (PR) residues have been found to affect activity of all the proteasome complexes both in vivo and in vitro, likely by binding to the face of the enzyme. Mechanism and structural constrains of the PR peptides dictating their influence on the proteasome remain elusive. Our results indicate that there are three sequence related properties of the PR peptides controlling their effectiveness as proteasome regulators: length of the peptide, distribution of a set of positive charges at the peptide N-terminus, and positioning of proline residues. Far UV CD spectroscopy demonstrates that these properties also correlate with the structure of PR peptides. In particular, it seems that structural propensity of the PR peptides to form beta-turns are required to bind to proteasome as regulatory competent molecules.

M244

CONFORMATIONAL STUDIES OF SELECTED BENZYLSULPHONYLACROYL PEPTIDES – PUTATIVE INHIBITORS OF CATHEPSIN B

R. Kasprzykowska, F. Kasprzykowski, A.S. KoŁodziejczyk, A. Kozak,
M. Kozarzewska, S. OŁdziej, S. Rodziewicz-MotowidŁo,
M. Tokmina-Łukaszewska, B. ŻoŁnowska

Faculty of Chemistry, University of GdańSk, Poland

Our work is focused on the search of selective, low-molecular cathepsin B peptide inhibitors acylated with the (E)-3-(benzylsulphonyl)acroyl group (Bsa). The double bond, embedded in the Bsa moiety is activated by two electron-withdrawing groups and may be a good target for the Michael-type addition of the catalytically active -SH group. Three series of peptide derivatives possessing general structures: Bsa-Phe-Asn(R)-OH, Bsa-Ile-X(OH)-N(CH3)2 and Bsa-X-Pro-OH were synthesized in solution and characterized by enzyme kinetic studies against papain, cathepsins B and K. It should be noted that all the investigated compounds were competitive and reversible inhibitors of the enzymes examined. Using 2D 1H NMR (TOCSY, COSY, ROESY) and 13C NMR spectroscopy along with theoretical calculations (ANALYSE program) we determined the conformational properties of two most potent and selective cathepsin B inhibitors.
This work was supported by grant DS/8350-5-0131-6.

HK2-INHIBITING PEPTIDES PREVENT ACTIVATION OF PSA AND DEGRADATION OF IGFBP-3 BY HK2

H. Koistinen[1], C. Hekim[1], A. Närvänen[2], U.-H. Stenman[1]

[1]Department of Clinical Chemistry, Biomedicum, University of Helsinki [2]Department of Chemistry, University of Kuopio, Finland

Background and aims: We have developed peptides inhibiting human kallikrein-2 (hK2) activity. As hK2 is overexpressed in prostate cancer tissue, these peptides are potentially useful for treatment and diagnosis of prostate cancer. Two of the potential physiological substrates for hK2 are proform of prostate specific antigen (proPSA) and insulin-like growth factor-binding protein-3 (IGFBP-3). Both of these might participate in the regulation of prostate cancer growth: IGFBP-3 by inhibiting IGF-dependent tumor growth and PSA by degrading extracellular matrix. We aimed to study whether our hK2-inhibiting peptides inhibit also hK2 activity towards natural protein substrates, i.e. activation of proPSA and degradation of IGFBP-3.
Methods: The effect of the peptides on the activation of proPSA by hK2 was studied by preincubating the peptides with hK2, followed by addition of PSA and specific PSA substrate. IGFBP-3 degradation was studied by two specific immunoassays, one detecting only native IGFBP-3, while the other one also detected proteolytically cleaved forms of the protein.
Results: hK2-inhibiting peptides were found to inhibit proPSA activation and IGFBP-3 degradation by hK2 in a dose dependent fashion.
Conclusions: We have developed new peptides inhibiting hK2 activity towards natural substrates, like proPSA and IGFBP-3. The peptides might be useful for treatment of prostate cancer and other diseases associated with increased hK2 activity.

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A NEW FAMILY OF SERINE PROTEINASE INHIBITORS FROM PLANTS

J. Kowalska[1], A. Wilmowska-Pelc[1], I. Lorenc-Kubis[1], A. Łęgowska[2], A. Kwiatkowska[2], M. Śleszyńska[2], A. Lesner[2], A. Walewska[2], E. Zabłotna[2], K. Rolka[2], T. Wilusz[2]

[1]Institute of Biochemistry and Molecular Biology, University of WroclAw [2]Department of Bioorganic Chemistry, Faculty of Chemistry, University of GdańSk, Poland

From the seeds of garden four-o'clock and spinach we isolated two serine proteinase inhibitors (MJTI I - Mirabilis jalapa trypsin inhibitor and SOTI I - Spinacia oleracea trypsin inhibitor), which are probably representatives of a new family of inhibitors.
The purification procedures of these inhibitors included affinity chromatography on immobilized methylchymotrypsin in a presence of 5 M NaCl, ion exchange chromatography and/or preparative electrophoresis and finally RP-HPLC on C18 column.
Their primary structures (Fig. 1) differ from those of known trypsin inhibitors, but showed significant similarity to one another, as well as to the antimicrobial peptides isolated from the seeds of Mirabilis jalapa (MJ-AMP1, MJ-AMP2), Mesembryanthemum crystallinum (AMP1) and Phytolacca americana (AMP-2 and PAFS-S) and from hemolymph of Acrocinus longimanus (Alo-1, 2 and 3).
The equilibrium association constants (Ka) of MJTI I and SOTI I with bovine □-trypsin were found to be about 107-109 M-1.
MJTI I and SOTI I have been synthesized using solid-phase method. The synthesized inhibitors and inhibitors isolated from plants have similar properties. The disulfide bridge pattern in both inhibitors was established after digestion with thermolysine, followed by the MALDI-TOF: Cys1-Cys-4, Cys2-Cys5 and Cys3-Cys6.

Figure 1. Sequence alignment of sequences of MJTI I and SOTI I with homological proteins.

```
MJTI I   EDEECAKTDQIC-PPNAPNYCCSGSCVPHPRLRIFVCA
SOTI I      KCSPSGAICSGFGPPEQCCSGACVPHPILRIFVCQ
MJ-AMP1    <ECIGNGGRCNENVGPPYCCSGFCLRQPGQGYGYCKNR
MJ-AMP2      CIGNGGRCNENVGPPYCCSGFCLRQPNQGYGVCRNR
PAFP-S     AGCIKNGGRCNASAGPPYCCSSYCFQIAGQSYGVCKNR
AMP-2       ACIKNGGRCVASGGPPYCCSNYCLQIAGQSYGVCKKH
AMP1       AKCIKNGKGCREDQGPPFCCSGFCYRQVGWARGYCKNR
Alo-1        CIKNGNGCQPDGSQGNCCSRYCHKEPGWVAGYCR
Alo-2        CIANRNGCQPDGSQGNCCSGYCHKEPGWVAGYCR
Alo-3        CIKNGNGCQPNGSQGNCCSGYCHKQPGWVAGYCRRK
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M247

NMR STUDIES OF THE INHIBITION OF PEPSIN BY PEPTIDE GLYOXAL INHIBITORS

S. Cosgrove, L. Rogers, C. Hewage, **J.P. Malthouse**

Centre for Synthesis and Chemical Biology, School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Ireland

Aspartyl proteases are required for the multiplication of the AIDS virus and for producing the amyloid protein which causes Alzheimer's disease. HIV protease inhibitors have been highly effective in treating Aids patients and it is hoped that potent inhibitors of the beta secretases will also prove effective in treating Alzheimer's disease. Therefore inhibitors of the aspartyl proteases have great therapeutic potential. We have shown that the peptide glyoxals are potent inhibitors of the thiol protease papain and of the serine proteases subtilisin and chymotrypsin. Using 13C-NMR we have been able to show that glyoxal inhibitors react reversibly with an active site nucleophile in these enzymes to form a tetrahedral adduct which is tightly bound by the enzyme.

In the present work we synthesise 13C-enriched peptide glyoxals, we assess their inhibitor potency, and use 13C-NMR to examine how the inhibitors interact with the aspartyl protease pepsin.
Z-Ala-Ala-[2-13C]Phe-glyoxal was synthesised from [1-13C]Phenylalanine which was converted to its methyl ester. This was then coupled with Z-Ala-Ala to give Z-Ala-Ala-[2-13C]Phe-OMe which was hydrolysed to the free acid. This was converted to the diazoketone and transformed into Z-Ala-Ala-[2-13C]Phe-glyoxal using dimethyldioxirane. NMR spectra at 11.75 T were recorded with a Bruker Avance DRX 500 standard-bore spectrometer.

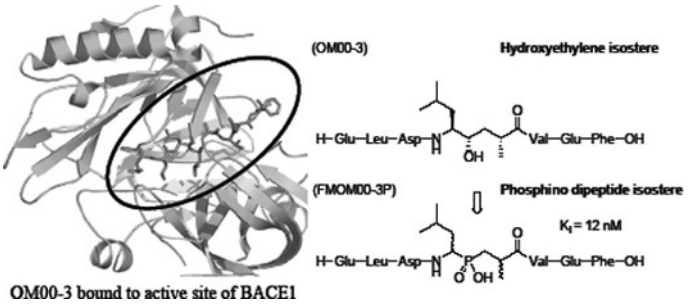
We show that peptide glyoxal inhibitors can be potent inhibitors of pepsin and that pepsin only binds one of the four glyoxal forms (one non-hydrated, one fully hydrated and two partially hydrated forms).

PHOSPHINO PEPTIDES AS INHIBITORS OF HUMAN β-SECRETASE (BACE1)

F. Manzenrieder[1], T. Huber[1], A. Frank[1], C. Dorner-Ciossek[2], H. Kessler[1]

[1]Institut für Organische Chemie II, Department Chemie, Technische Universität München, Garching [2]A ZNS Forschung, Boehringer-Ingelheim Pharma GmbH & Co.KG, Biberach, Germany

Alzheimer`s disease (AD) is the most common cause of dementia in older people. A major factor in the pathogenesis of AD is the cerebral deposition of amyloid fibrils, consisting of amyloid β peptides (Aβ), as senile plaque. The 40- to 42 amino acid long Aβ is generated by the proteolysis of β–amyloid precursor protein (APP) by β- and γ-secretases. Since BACE1, a unique member of the pepsin family of aspartyl proteases initiates the pathogenic processing of APP by cleaving at the N-terminus it is a molecular target for therapeutic intervention in AD [Haass, C. EMBO Journal, 23, 483 (2004).].
The phosphinic acid moiety is an excellent mimic of the tetrahedral transition state of amide bond hydrolysis. Therefore we used the sequence the inhibitor OM00-3 [Hong, L,; Turner, R. T.; Koelsch, G.; Shin, D.; Ghosh, A. K.; Tang, J. Biochemistry 41, 10963-10967 (2002).] and replaced the hydroxyethylene isostere with a phosphino dipeptide isostere to generate a phosphino peptide. Evaluation of these short phosphino peptides showed high BACE1 inhibition.



OM00-3 bound to active site of BACE1

Th249

PLASMIN INHIBITORS WITH AROMATIC OLIGOPEPTIDE STRUCTURE

K. Midura-Nowaczek, A. Pućkowska, I. Bruzgo

Department of Organic Chemistry, Medical University of BiałYstok, Poland

Plasmin, a key enzyme for fibrynolysis, plays an important role in variety of biological processes including pathological phenomena such as tumor growth and metastasis. The antitumor effect of some plasmin inhibitors was also reported. The most of antitumor drugs belongs to the great family of sequence-specific ligands nonintercalatively binding within minor groove of B-DNA. The cytotoxic effect of these antineoplastic agents and inhibition of many cellular processes is determined mainly by interference with catalytic activity of important regulatory proteins, such as topoisomerases and many of DNA proteases. In the course of our investigation of DNA minor groove binders we studied the effect of nine aromatic oligopeptides on amidolytic activity of plasmin, examined with the use of synthetic substrate S-2251. Reported compounds are built from 4-aminobenzoic and 5-amino-2-methoxybenzoic acids. Three of them are analogues of netropsin. Four oligopeptides have a structure of bis-analogue, in which two netropsin-like molecules were linked by tetra- or hexamethylene chain. Two compounds are analogues of pentamidine. Earlier, we reported that they inhibit catalytic activity of topoisomerase I and II and show antiproliferative effects in the standard cell line of mammalian tumor MCF7 .

This study demonstrates that six of these oligopeptides, among which four are netropsin bis-analogues, inhibit amidolytic activity of plasmin.
Examined compounds can potentially be a group of anticancer agents with wider spectre of working.

M250

PROTEOLYTIC SYSTEM OF THE COLD SEA INVERTEBRATES AS AN EXAMPLE OF BIOCHEMICAL ADAPTATION

V.A. Mukhin[1], V.Y. Novikov[1], E.B. Smirnova[2]

[1]Laboratory of Biochemistry and Technology, Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO) [2]Department of Biochemistry, Murmansk State Technical University (MSTU), Murmansk, Russia

Adaptation processes in an organism are reflected at the level of an enzymatic system.
From this viewpoint we studied digestive proteases in different northern sea invertebrates: crustaceans Paralithodes camtschaticus and Pandalus borealis; molluscs Chlamys islandicus, Buccinum undatum and Serripes groenlandicus; echinoderms Strongylocentrotus droebachiensis, Cucumaria frondosa, Asterias rubens and Crossaster papposus.
Proteolytic activity was found to occur, to a variable degree, in digestive organs of all studied organisms over the entire pH range. The common feature was the existence of two activity peaks, in the acid (pH 2.5 – 3.5) and alkaline (pH 7.5 – 8.5) zones, as well as a similar protease set containing E and D cathepsins, a trypsin-like enzyme, elastase, and collagenolytic proteases.
Proteolytic activity in the hepatopancreas of crab and sea star was found to be an order higher than in other study objects. High protease activity in crab hepatopancreas is an evolutionary mechanism compensating for a poor differentiation of digestive system, low substrate specificity of enzymes, and cold environment.
Trypsin activity in digestive organs of invertebrates suggests that a trypsin-like enzyme is a genetically old one, an evolutionary origin of all serine proteases. A difference of kind between vertebrates and invertebrates is that the latter have cathepsine activity (absent in vertebrates) and no pepsin activity.
Proteolysis in the alkalescent zone and homogenisation of nutrition occur in digestive cavities of invertebrates, while acidic proteolysis takes place in intracellular lysosomes. Cathepsine proteolysis possibly occurs in lysosomes of epithelium cells of digestive tract and/or in lysosomes of vagabonding coelomocytes.

Tu251

LIGHT CONTROLLED SWITCHING OF PROTEASE INHIBITION BY PEPTIDOMIMETICS

D.S. Pearson, N.A. Alexander, A.D. Abell

Department of Chemistry, University of Canterbury, New Zealand

It is of interest to develop enzyme inhibitors containing a light activated switch that can be used to control binding and inactivation of an enzyme. Several inhibitors containing the azobenzene photoswitch group have previously been developed and have shown changes in activity of around two times on photoswitching. This study aimed to improve this switching by more extensive derivatisation of azobenzene to closer resemble the peptide substrates of proteases.

A series of peptidomimetics containing the azobenzene photoswitch group were synthesized and assayed against the protease alpha-chymotrypsin. These compounds contained azobenzene, linked to a known chymotrypsin inhibitory group (either a trifluoromethylketone or boronate ester), and otherwise designed to be peptide-like. In some cases both ends of the azobenzene moiety were derivatized in order to increase the impact of photoswitching on the shape of the compound and thus its enzyme binding strength.

Assays showed that most compounds were reversible inhibitors of chymotrypsin, with low micromolar inhibition constants (Ki or IC50). Up to four times increase in enzyme inhibition on light activated switching of the azobenzene group conformation was obtained.

CONCLUSIONS: Chymotrypsin photoswitch inhibitors have been synthesized that are more highly derivatized than previously published examples. These new compounds show moderate inhibition and significantly improved photoswitching.

Th252

CONFORMATIONAL STUDIES AND COMPARISON OF Z-ARG-LEU-ARG-AGLY-ILE-VAL-OME – THE POTENT AND SELECTIVE CATHEPSIN B INHIBITOR AND Z-ARG-LEU-ARG-GLY-ILE-VAL-OME – THE PARENT PEPTIDE

E. Piotrowska, J. Ciarkowski, S. Rodziewicz-Motowidło, E. Wieczerzak, Z. Grzonka

Department of Chemistry, University of GdańSk, Poland

A number of peptidyl derivatives structurally based upon the inhibitory sites of cystatins has been synthesized. These compounds are prone to proteolytic degradation, are rapidly excreted and poorly bioavailable. The majority of this problems might be overcome by use of peptidomimetics with structures resembling those of previously synthesized peptidyl derivatives. Among the peptidomimetics are azapeptides, in which alpha-CH group of amino-acid residue is replaced by a nitrogen atom. The azapeptides have recently been demonstrated as potent and selective inhibitors of cathepsins B and K. It was shown that azapeptide inhibitors bind along the active site cleft of cathepsin B in a bent conformation. This bent structure is likely to result from the mobility of the bonds in the vicinity of the inserted azaamino acid residue as well as from the interaction with enzyme. In our present work we have studied the peptide of a sequence: Z-Arg-Leu-Arg-Gly-Ile-Val-OMe, which is characterized by one major and three minor conformations. The replacement of alpha-CH group in the Gly residue of peptide chain of Z-Arg-Leu-Arg-Gly-Ile-Val-OMe by the nitrogen atom likely results in rigid conformation. Our aim was a comparison of structure of the parent peptide Z-Arg-Leu-Arg-Gly-Ile-Val-OMe and a selective cathepsin B inhibitor Z-Arg-Leu-Arg-Agly-Ile-Val-OMe by using 1H-NMR.

Financially supported by KBN grant DS 8372-4-0138-6 and BW/2006.

M253

INHIBITION OF HUMAN TRYPTASE BY CYCLOTHEONAMIDE ANALOGS

N. Schaschke[1], C.P. Sommerhoff[2]

[1]Fakultät für Chemie, Universität Bielefeld [2]Klinische Chemie und Klinische Biochemie, Klinikum Innenstadt, LMU München, München, Germany

Cyclotheonamides are inhibitors of trypsin-like serine proteases that are characterized by an extended peptide conformation which is stabilized by macrolactamization. The X-ray structure of cyclotheonamide A in complex with trypsin has shown that this particular conformation allows to address in a substrate-like manner beside the S1 pocket also the S1', S2, and in a less pronounced manner the S3 pocket. The S1 ligand, (S)-3-amino-6-guanidino-2-oxo-hexanoic acid, interacts via its guanido function with Asp 189 at the bottom of the S1 pocket. In addition, the ketone covalently modifies the gamma-oxygen of Ser 195 by hemiketal formation [1]. Recently, two novel cyclotheonamides have been isolated from a marine sponge of the genus Ircinia. One of them, cyclotheonamide E4, is a potent inhibitor of human tryptase [2]. To convert cyclotheonamide E4 into a fully reversible acting tryptase inhibitor, the S1 ligand was structurally replaced by beta-homolysine. For the synthesis of this analog a solid phase approach was developed. Furthermore, this analog was starting point for systematical modifications to exploit the negatively charged Glu 217 within the S3 pocket of tryptase. The synthetic details as well as the inhibition profile of these novel cyclotheonamide E4 analogs will be discussed. [1] Lee, A. Y., Hagihara, M., Karmacharya, R., Albers, M. W., Schreiber, S. L., Clardy, J., J. Am. Chem. Soc. 1993, 115, 12619-12620. [2] Muramaki, Y., Takei, M., Shindo, K., Kitazume, C., Tanaka, J., Higa, T., Fukamatchi, H., J. Nat. Prod. 2002, 65, 259-261.

Tu254

AN EFFICIENT METHOD FOR THE SYNTHESIS OF PEPTIDE ALDEHYDE LIBRARIES EMPLOYED IN THE DISCOVERY OF SARS CORONA VIRUS MAIN PROTEASE INHIBITORS

M.F. Schmidt[1,4], S.I. Al-Gharabli[1,5], S.T. Ali Shah[1], S. Weik[1], J.R. Mesters[2], D. Kuhn[3], G. Klebe[3], R. Hilgenfeld[2], J. Rademann[1,4]

[1]Department of Medicinal Chemistry, Leibniz Institute for Molecular Pharmacology, Berlin [2]Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Luebeck [3]Institute of Pharmaceutical Chemistry, Philipps-University Marburg [4]Institute of Chemistry and Biochemistry, Free University of Berlin, Germany [5]Department of Medical Sciences, School of Medical Sciences, German-Jordanian University, Amman, Jordan

Severe acute respiratory syndrome corona virus associated main protease (SARS CoV Mpro protease), alternatively known as chymotrypsin-like protease (3CLpro), is a mediator of virus infection cyclus and from there a therapeutic target.

A peptide aldehyde library targeting the SARS corona virus main protease (SARS-CoV Mpro, alternatively known as 3CLpro) was designed on the basis of three different reported binding modes and supported by virtual screening. A set of 25 peptide aldehydes were prepared by a newly developed methodology and investigated in an inhibition assay against SARS-CoV Mpro.[1]

Protected amino acid aldehydes furnished by the racemization-free oxidation of amino alcohols with Dess-Martin periodinane were immobilized on threonyl resins as oxazolidines. Following Boc-protection of the ring nitrogen yielding the N-protected oxazolidine linker, peptide synthesis was performed efficiently on this resin releasing deprotected products under mild hydrolysis conditions.

The library was tested in a new fluorimetric enzyme assay for SARS CoV Mpro. Via immobilization of the fluorophor, 2-(7-amino-4-methyl-3-coumarinyl)-acetic acid, the substrate AcTSAVLQ-AMCA was prepared, surprisingly displaying a higher affinity than the native substrate. Several potent inhibitors were found with IC50 values in the low micromolar range. Interestingly, the most potent inhibitors seem to bind SARS-CoV Mpro in a non-canonical binding mode.

Currently, the initial screen is extended towards the discovery of small molecule inhibitors of SARS corona virus main protease.

Literature:

[1] Al-Gharabli, S.I., Ali Shah, S.T., Weik, S., Schmidt, M.F., Mesters, J.R., Kuhn, D., Klebe, G., Hilgenfeld, R., Rademann, J., submitted for publication.

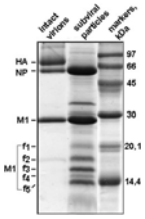
Th255

INFLUENZA A VIRUS PARTICLES AS A SUBSTRATE FOR BROMELAIN

L.V. Kordyukova[1], M.V. Serebryakova[2], **Y.A. Smirnova**[1], T.V. Ovchinnikova[3], E.N. Lysogorskaya[1], I.Y. Filippova[1], L.A. Baratova[1]

[1]Belozersky Institute of Physico-Chemical Biology and Department of Chemistry, Moscow State University [2]Orekchovich Institute of Biomedical Chemistry
[3]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

A method of bromelain cleavage of surface glycoprotein hemagglutinin (HA) from the Influenza A virions was initially employed for HA ectodomains crystallographic study [1]. The remaining spikeless subviral particles were used by us earlier for HA2 C-terminal fragment extraction and mass spectrometric (MS) investigation [2]. Now SDS-PAGE analysis of the subviral particle preparations revealed several additional bands in a range of 9-23 kDa together with major viral proteins comparing to intact virions (Figure, M1-matrix protein, f1-f5-M1 protein fragments, NP-nucleoprotein). MALDI-TOF MS analysis of the in-gel trypsin hydrolyzates has shown that the additional bands are fragments of M1 protein. This was confirmed by N-terminal sequencing of the protein fragments electroblotted from the bands. Concentration of SH-reducing reagent in bromelain digestion reaction influenced on the M1 fragment bands intensity. We conclude that due to membrane destabilization during HA spikes removing, M1 protein localized under viral membrane inside intact virions becomes accessible to limited proteolysis by bromelain. [1] Brand C.M., Skehel J.J. //Nat. New Biol., 238:145-147 (1972). [2] Kordyukova L.V. et al. //Peptides 2004, Proc. 3rd IPS and 28th EPS Prague, Czech Rep., M.Flegel, M.Fridkin, C.Gilon, J.Slaninova (Eds.) 435-436 (2005).This work was supported by the ISTC grant #2816p and RFBR grant #06-04-48728.



M256

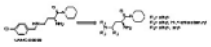
SYNTHESIS AND EVALUATION OF SUBSTITUTED 2,4–DIAMINOBUTYRIC ACID ANALOGUES AS POTENT AND SELECTIVE DPP II INHIBITORS

A. Soroka[1], P. Van der Veken[1], I De Meester[2], A.M. Lambeir[2], S. Scharpé[2], A. Haemers[1], K. Augustyns[1]

[1]Department of Medicinal Chemistry [2]Department of Medical Biochemistry, University of Antwerp, Antwerpen, Belgium

Dipeptidyl peptidases (DPP's) sequentially release dipeptides from polypeptides. Among those enzymes, DPPIV, FAPα, DPP8, DPP9 and DPPII cause the release of N-terminal dipeptides containing proline or alanine at the penultimate position. They are all members of clan SC, a group of serine proteases that contains proline-specific peptidases.
Dipeptidyl-peptidase IV (DPPIV) is the best studied member of this group of enzymes and has become a validated target for the treatment of type 2 diabetes over the last years. The development of inhibitors for the related enzymes (i.a. DPPII) has only started recently.
This poster presents selected products synthesised to further elaborate the STRUCTURE-ACTIVITY RELATIONSHIP for DPP II inhibitors with a 2,4-diaminobutyrylpiperidine basic structure. This class of compounds was described earlier by our group as the hitherto most potent and selective inhibitors of DPP II. Starting from N4-p-chlorobenzyl-substituted UAMC00039, our lead compound, two types of modifications were proposed:

- the synthesis of N4-(di)alkyl- and arylalkyl analogues;
- the synthesis of 3-methyl analogues.



DIFFERENTIAL EFFECTS OF N-PEPTIDYL-O-ACYL HYDROXYLAMINES ON DYNORPHIN-INDUCED ANTINOCICEPTION IN THE MOUSE CAPSAICIN TEST

K. Tan-No[1], A. Taira[1], M. Shimoda[1], O. Nakagawasai[1], F. Niijima[1],
H.U. Demuth[2], J. Silberring[3], L. Terenius[4], T. Tadano[1]

[1]Department of Pharmacology, Tohoku Pharmaceutical University, Sendai, Japan
[2]Probiodrug AG, Biocenter, Halle (Saale), Germany [3]Faculty of Chemistry and Regional Laboratory, Jagiellonian University, Krakow, Poland [4]Department of Clinical Neuroscience, Section of Alcohol and Drug Dependence Research, Karolinska Institute, Stockholm, Sweden

Background and aims: We have previously shown that p-hydroxymercuribenzoate (PHMB), a general cysteine protease inhibitor, when co-administered with dynorphin A or B, significantly prolongs antinociception induced by intrathecal (i.t.) injection of either dynorphin. This observation indicates that cysteine proteases may be important for terminating dynorphins A- and B-induced antinociception. Studies on N-peptidyl-O-acyl hydroxylamines have revealed that this novel class of inhibitors preferentially inactivates cysteine proteases in vitro. In the present study, we examined the antinociceptive effects of i.t. administered dynorphin A or B, in combination with Boc-Tyr-Gly-NHO-Bz or Z-Phe-Phe-NHO-Bz, the potent inhibitors of cysteine proteases in the mouse capsaicin test.
Methods: Experiments were performed on male ddY-strain mice weighing 22-25 g. The drugs were administered i.t. in a volume of 5 microliter. The antinociceptive effect was evaluated by capsaicin test.
Results: When administered i.t. 5 min before the injection of capsaicin (800 ng) into the plantar surface of the hindpaw, dynorphin A (62.5-1000 pmol) or B (0.5-4 nmol) produced a dose-dependent and significant antinociceptive effect. The effect of dynorphins A (1 nmol) and B (4 nmol) disappeared completely within 180 min and 60 min, respectively. PHMB (2 nmol) and Boc-Tyr-Gly-NHO-Bz (1 nmol) co-administered with dynorphin A or B significantly prolonged antinociception induced by both. On the other hand, Z-Phe-Phe-NHO-Bz (2 nmol) only prolonged antinociception induced by dynorphin A.
Conclusions: The present results suggest that Z-Phe-Phe-NHO-Bz is an inhibitor of cysteine proteases preferring cleavage of dynorphin A, with less specificity towards dynorphin B in the mouse spinal cord.

HIGHLY SENSITIVITY FRET SUBSTRATE FOR ASSAY OF MMPS

X.H. Tong, X.W. Zhang, Y. Tang, K. Ichihara, B. Sun, Y. Huang, Z.J. Diwu, A. Hong

AnaSpec Inc., San Jose, CA, USA

Matrix metalloproteinases (MMPs) are involved in the degradation of components of extracellular matrix and play an important role in apoptosis, embryogenesis, reproduction tissue remodeling and repair. The development of agonists or inhibitors in controlling MMP activity continues to be of great interest for drug discovery and life science research.

FRET peptide-based MMP assays have been widely used for measuring MMP activity. However, most of these FRET peptides use Mca/Dnp or Edans/Dabcyl pairs, which have relatively weak fluorescence signals with short wavelengths. We designed and synthesized sixteen MMP substrates that are incorporated with 5-FAM (donor) and QXL 520TM (quencher) by Fmoc solid phase synthesis method. QXL520TM is proven to be the most effective quencher for fluoresceins such as FAM and FITC. The new 5-FAM/QXL520TM FRET peptides offer the following advantages: a) the peptides are more readily adapted to high throughput screening since this pair is less interfered by autofluorescence of test compounds and cellular components with lower background. b) 5-FAM has stronger absorption and fluorescent signals with longer absorption and emission wavelengths (Ex/Em = 490nm/520nm) than Mca and Edans. c) QXL520TM is more water-soluble than Dabcyl and Dnp, which alleviates the problem caused by the hydrophobic nature of Dabcyl or Dnp.

These peptides were screened by MMPs. Each of these peptides was found to be cleaved by certain MMPs. The peptide, QXL520TM-g-Abu-Pro-Cha-Abu-Smc-His-Ala-Dab(5-FAM)-Ala-Lys-NH2, showed the highest proteolytic kinetics to all the tested MMPs. Submicromolar of this peptide is adequate to detect picomolar level of MMPs.

M259

NOVEL THIO-DERIVATIVE OF PHENYLNORSTATINE AND ITS APPLICATION TO THE BACE1 INHIBITORS DESIGN

Z. Ziora, S. Kasai, K. Hidaka, A. Nagamine, T. Kimura, Y. Hayashi, Y. Kiso

Kyoto Pharmaceutical University, Department of Medicinal Chemistry, Center for Frontier Research In Medicinal Science, 21st Century COE Program, Kyoto, Japan

In our previous study, we reported potent and small-sized BACE1 inhibitors containing phenylnorstatine [(2R,3S)-3-amino-2-hydroxy-4-phenylbutyric acid; Pns] at P1 position as a transition-state mimic. In developing more active compounds, we focused our attempts on the P1 position, where we replaced the Pns by its thio-derivative. Herein, we present the synthesis of a novel phenylthionorstatine [(2R,3R)-3-amino-2-hydroxy-4-(phenylthio)butyric acid; Ptns] as a P1 moiety with hydroxymethylcarbonyl (HMC) isostere, and then an application to the BACE1 inhibitors design.

We have synthesized Ptns starting from readily available N-benzyloxycarbonyl-serine and after multistep reaction (including Weinreb amide formation, thiophenyl group introduction, through cyanohydrin derivative the transformation into the 2-hydroxy ester and then acid). Purification was done by column chromatography and RP-HPLC. Peptides were synthesized by the Fmoc based solid phase method and characterized by MALDI-TOF MS. The peptide inhibitors were adopted to enzyme assay using a recombinant human BACE1 and a fluorescence-quenching substrate. BACE1 inhibitory activity was determined based on the decrease% of the cleaved substrate by the enzyme.We have synthesized Ptns and then the (2R,3R)-enantiomer was applied to SPPS (solid phase peptide synthesis). We synthesized octa- and pentapeptide-type inhibitors of BACE1 containing Pns or Ptns at the P1 position. These compounds were enzymatically tested and showed high BACE1 inhibitory activity. A novel derivative of Pns, Ptns, was synthesized, and evaluated in comparison to corresponding Pns. The inhibitors with Ptns exhibited a slightly higher inhibitory activity against BACE1 comparing to those with Pns. This study suggests possibilities of the application of Ptns to design other aspartyl proteases inhibitors.

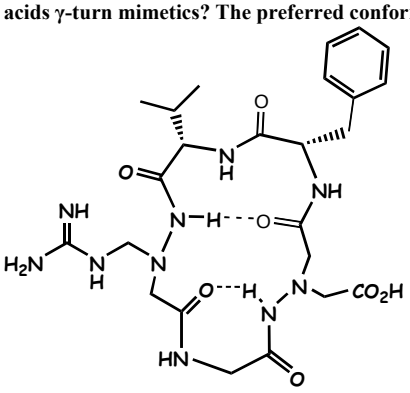
Tu260

STRUCTURE-ACTIVITY STUDIES OF CYCLIC AZA- BETA-3-RGD PEPTIDE ANALOGS

O. Busnel, I. Nicolas, A. Bondon, **M. Baudy-Floc'h**

UMR CNRS 6226, Université De Rennes 1, Rennes, France

The αv β3 integrin receptor plays an important role in human metastasis and tumor-induced angiogenesis, mainly by interacting with matrix proteins through recognition of an Arg-Gly-Asp (RGD) motif. Inhibition of the αv β3 integrins with a cyclic RGD peptide impairs angiogenesis, growth and metastasis of solid tumours in vivo. The aim of this study was to investigate the effects of replacement of amino acids by aza-β3-amino acid analogs in cyclic RGD-peptides as αv β3 -integrin antagonist on angiogenesis, microcirculation, growth and metastasis formation of a solid tumour in vivo. The selectivity profile of these antiadhesive cyclopeptide is rationalized by a special presentation of the pharmacophoric groups. Thr RGD motif resides in position i to i+2 of a regular γ-turn. We synthetized linear and cyclic aza-β3 RGD-peptide with the purpose to examine the effect on the conformation and the activity. Are aza-β3 amino acids γ-turn mimetics? The preferred conformations were determined by NMR.



CYCLIC OPIOID PEPTIDE AGONISTS AND ANTAGONISTS OBTAINED VIA RING-CLOSING METATHESIS

I. Berezowska, C. Lemieux, N.N. Chung, P.W. Schiller

Laboratory of Chemical Biology and Peptide Research,Clinical Research Institute of Montreal, Montreal, QC, Canada

The opioid peptide H-Tyr-c[D-Cys-Phe-Phe-Cys]NH2 cyclized via a methylene dithioether has recently been reported to be a potent and selective μ opioid agonist [M.J. Przydzial et al., J. Peptide Res. 66, 255-262 (2005)]. In the present study, we synthesized dicarba analogues of this peptide with Tyr or 2',6'-dimethyltyrosine (Dmt) in the 1-position. In an effort to develop μ-selective opioid antagonists, dicarba analogues containing 3-(2,6-dimethyl-4-hydroxyphenyl) propanoic acid (Dhp) or (2S)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl) propanoic acid [(2S)-Mdp] were also prepared. The syntheses were performed on solid-phase by assembly of the linear precursor peptide and subsequent ring-closing metathesis between D-allylglycine and (2S)-2-amino-5-hexenoic acid substituted in positions 2 and 5, respectively, using a second generation Grubbs catalyst . After cleavage from the resin the cyclic olefinic peptides were obtained as mixtures of cis and trans isomers and subsequent catalytic hydrogenation yielded the saturated -CH2-CH2- bridged peptides. In vitro opioid activities were determined in the guinea pig ileum (GPI) and mouse vas deferens (MVD) assays, and in opioid receptor binding assays. Nearly all Tyr- and Dmt cyclic olefinic and saturated peptides showed subnanomolar μ and δ agonist potencies in the GPI and MVD assays, and all of them were non-selective. Some of the Dhp- and (2S)-Mdp analogues were quite potent μ opioid antagonists with Ke values in the 25-40 nM range. Interestingly, all of them were partial or full δ agonists in the MVD assay and none of them showed μ vs. δ receptor binding selectivity.

EXPLORING THE RELATIONSHIP BETWEEN TURN GEOMETRY AND ALLOSTERIC ANTAGONISM OF PEPTIDE MIMIC LIGANDS FOR A PROSTAGLANDIN RECEPTOR

C.B. Bourguet[1], X. Hou[2], S. Chemtob[2], W.D. Lubell[1]

[1]Département de Chimie, Université de Montréal [2]Departments of Pediatrics and Ophthalmology and Pharmacology, Research Center of Hôpital Sainte-Justine, Montréal, QC, Canada

Prostaglandins are involved in a large number of biological activities mediated by their G-protein coupled receptors (GPCRs). The prostaglandins PGF2 alpha receptors are found specifically in uterine muscle, where they initiate parturition and labor. The PGF2 alpha receptor plays a key role in preterm labor, for which medical and social costs are estimated at $ 9 billion per year in the USA (the highest per patient cost of any disorder). Peptide mimics have been developed in our laboratory(1,2), that serve as allosteric antagonists of the PGF2 alpha receptor. The importance of the turn geometry of the central residue in these peptide mimics has been investigated using enantiomeric indolizidin-2-one beta-turn mimics which can respectively induce type II and II' geometry. Our presentation will discuss the synthesis and biology of these novel allosteric modulators of prostaglandin PGF2 alpha receptor activity.

1. Peri, K.G.; Quiniou, C.; Hou, X.; Abran, D.; Varma, D.R.; Lubell, W. D.; Chemtob, S. "THG113: a novel selective FP antagonist which delays preterm labor" Seminars in Perinatology 2002, 67, 389-397.

2. Peri, K.G.; Polyak, F.; Thouin, E.; Lubell, W.D., Chemtob, S. PCT Int. Appl. serial No. 60/387, 424, June 11, 2002.

Tu263

BETA-AMYLOID INTERACTIONS WITH METALS

K. Trześniewska, **M. Brzyska**

Laboratory of Bio-Physical Methods, Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Beta-amyloid is a 40-43 amino acid peptide found in the form of cortical deposits in the brains of Alzheimer's disease patients. Metal binding by beta-amyloid affects aggregation and redox properties of the peptide. In this work we investigated the relationship between the structural transformations of beta-amyloid - metal complexes during ageing in the solution and resulted changes in the redox and hydrolytic properties.

Conformational transitions induced by metals were documented by CD spectroscopy, followed by quantitative assesment of beta-amyloid structure. Aggregation was characterized by an increase of Thioflavin T fluorescence. Redox properties were expressed as the ability to oxidize dichlorofluorescin (H2DCF). Hydrolytic properties of beta-amyloid were measured as an increase of fluorescein fluorescence, released from fluorescein esters.

Presence of metals (copper, iron, zinc, aluminum, calcium and magnesium) affected the conformation and aggregation of beta-amyloid in solution. Beta-amyloid complexes with metals demonstrated different abilities to oxidize H2DCF. Hydrolytic as well as redox properties of beta-amyloid - metal complexes were reduced concomitantly with ageing in the solution, paralelly to an increase of the peptide aggregational state.

On the basis of the above results, we can conclude that an increase of beta-amyloid aggregation affects its redox and hydrolytic properties. Thus, "ageing" could reduce this part of beta-amyloid neurotoxic action that is linked to the oxidation and decomposition of the fluid and tissue components of the brain.

Acknowledgment: this work was supported by State Committee for Scientific Research, grant No. 3 P05F 00524.

LHRH ANALOGUES CONTAINING PALMITOYL MOIETY POSSESS CYTOTOXIC ACTION ON TUMOR CELLS IN VITRO

S. Burov[1], T. Yuablokova[1], M. Dorosh[1], M. Blank[2], N. Epstein[2], M. Fridkin[3]

[1]Institute of Macromolecular Compounds RAS, St. Petersburg, Russia [2]Chaim Sheba Medical Center, Tel-Hashomer [3]Weizmann Institute of Science, Rehovot, Israel

It was shown that luteinising hormone – releasing hormone (LHRH) receptors are overexpressed in the most of adenocarcinoma cells in contrast to their low content in normal tissues. These data create the basis for LHRH analogues application in therapy of breast, ovary, prostate, lung, intestine, liver and kidney cancers. Both agonists and antagonists utility for the targeting of cytotoxic moiety to the tumor cells is well documented. However, the number of LHRH analogues possessed their own cytotoxic activity is still very limited.

We investigated the influence of N-terminal modification on cytotoxic action of LHRH antagonists in vitro, using different human adenocarcinoma cell lines. Analogues, containing fatty acid residues were synthesized by solid phase technique and purified by means of SPE and HPLC. It was shown that attachment of palmitic acid significantly increases antitumor activity whereas introduction of lauryl, hexanoyl or pivaloyl moieties produces inactive compounds. This evidence suggests possible apoptosis-inducing role of palmitic acid in agreement with literature data. Subsequent modification of palmitoyl-containing analogues demonstrated the possibility of peptide chain elongation up to twelve amino acid residues without reduction of antitumor activity. Peptide efficiency in vitro can be substantially increased by the incorporation of nuclear localization sequence (NLS). Further biological studies emphasize important role of NLS position in analogue structure. Thus, it was demonstrated that attachment of palmitoyl moiety produce LHRH analogues possessed antitumor action in vitro. These data in conjunction with that for NLS-containing peptides are in favour of supposed apoptosis-inducing activity.

M265

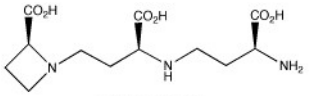
TOTAL SYNTHESIS OF NICOTIANAMINE AND OF A NON NATURAL ANALOGUE

M. Bouazaoui, J. Martinez, **F. Cavelier**

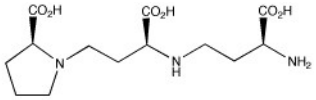
Lapp-Umr 5810, Montpellier, France

Nicotianamine (NA) that was first isolated from the leaves of Nicotiana tabacum L [1], is known as a key biosynthetic precursor of phytosiderophores. Various studies have proved that nicotianamine plays a significant role in plants as an iron, nickel, zinc ... transporter [2]. The aim of our study was to synthesize unnatural analogues of NA via peptide intermediates, to investigate the mechanisms of metal transport and accumulation within the plant. We found that the strategy developed for NA synthesis could not be applied when the azetidine ring was changed for pyrrolidine ring and we investigated a new route to synthesize such analogue. These synthetic pathways will be discussed.

[1] Noma M, Nogashi M, Tamaki E. 1971; A new amino acid, nicotianamine, from tobacco leaves. Tetrahedron Lett 22, 2017-2020.
[2] Stephan, U.W. and Scholz, G. 1993; Nicotianamine: mediator of transport of iron and heavy metals in the phloem. Physiol. Plant. 88, 522-529.



Nicotianamine



Non-natural analogue

Tu266

NEW ANALOGUES OF [ARG8]-VASOPRESSIN CONTAINING L-α-T-BUTYLGLYCINE IN POSITION 9

E. Pappa[1], V. Magafa[1], L. Borovičková[2], J. Slaninová[2], **P. Cordopatis**[1]

[1]*Department of Pharmacy, University Of Patras, Patras, Greece*
[2]*Group of Peptide Biochemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic, Prague, Czech Republic*

The primary physiological roles of arginine vasopressin (AVP), [cycle1-6 (H-Cys1-Tyr2-Phe3-Gln4-Asn5-Cys6-Pro7-Arg8-Gly9-NH2)], involve vasoconstriction of vascular smooth muscles, via V1a receptor, and antidiuretic action in kidney (blood osmolality regulation) via V2 receptor. Binding of AVP to the V1a receptor subtype also stimulates glycogenolysis in the liver and promotes platelet aggregation. In addition, activation of the V1b (also known as V3) receptor causes adrenocorticotropic hormone release from the anterior pituitary. V1b receptors are also present in the brain where AVP functions as a neurotransmitter.
The configuration and the hydrophobicity of the aromatic amino acid in position 2 are important factors for the antagonistic activity, while elimination of the N-terminal amino group plays an important role in prolongation of the activity. Furthermore the C-terminal Gly-NH2 can be deleted or replaced by a wide variety of substituents with excellent retention of V1-antagonistic potency. On the basis of these findings we set out the synthesis of new AVP analogues containing mercapto propionic acid (Mpa) in position 1, Tyrosine(O-Methyl) [Tyr(Me)] or 2-Naphtylalanine [Nal(2)] in position 2 and L-α-t-butyglycine [Gly(But)] in position 9. We also studied the impact of modified C-terminal amide on the biological potency of the new AVP analogues.
The analogues were synthesized by Fmoc/But solid phase methodology utilizing the Rink Amide MBHA resin, the [3-((Ethyl-Fmoc-amino)-methyl)-1-indol-1-yl]-acetyl AM resin and the 2-chlorotrityl-chloride resin. The new synthesized analogues were tested for their pressor, antidiuretic and uterotonic potencies.
Acknowledgement.
The work was partially supported by the research project No. Z40550506 of the Academy of Sciences of the Czech Republic.

Th267

ANALOGUES OF [MPA1, D-(ET)TYR2] OR [MPA1, D-NAL(1)2]-OXYTOCIN CONTAINING NON NATURAL AMINO ACIDS IN POSITION 3

S. Petraki[1], V. Magafa[1], L. Borovičková[2], J. Slaninová[2], **P. Cordopatis**[1]

[1]*Department of Pharmacy, University of Patras, Patras, Greece*
[2]*Group of Peptide Biochemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic, Prague, Czech Republic*

Oxytocin (OT) is a hypothalamic cyclic nonapeptide that is released into the general circulation from the posterior lobe of the pituitary. Generally known is its capability of inducing uterine contractions and facilitation of milk ejection during lactation. However widespread distribution of OT receptors in the brain and specific behavioral effects of centrally applied OT have firmly established OT role as a neurotransmitter modulating reproductive and social behaviors. The role of OT in preterm labor led to the search for and design of synthetic peptide antagonists as potential tocolytic agents. Of the many OT antagonists reported to date, only one, Atosiban, has been approved under the trade name Tractocile.

The design of new OT antagonists is based on data from structure-activity studies. Antagonistic activity depends on the configuration and the hydrophobicity of the amino acid at position 2. Based on these findings and for investigation of the role of position 3 in biological potency, we synthesized by the Fmoc/But solid phase methodology twelve new analogues of [Mpa1, D-(Et)Tyr2] or [Mpa1, D-Nal(1)2]OT containing L- or D-α-t-butylglycine [Gly(But)], L- or D-β-(2-thienyl)-alanine [Thi] and L-or D-3 pyridylalanine (Pya) in position 3. Electro-spray MS was in agreement with the expected results. The analogues were tested for uterotonic activity in the rat uterus in vitro test, for pressor activity in the rat pressor assay and for the affinity to human OT receptor using 3H.OT.

Acknowledgement.
The work was partially supported by the research project No. Z40550506 of the Academy of Sciences of the Czech Republic.

M268

NEW FRAGMENT ANALOGUES OF ANTISTASIN'S (ATS) ACTIVE SITE – DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP

D.L. Danalev, L.T. Vezenkov

Department of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

In the recent years by the salivary glands of several bloodsucking animals like, teaks, leeches, vampire bats and so forth are isolated plenty of proteins and peptides with different molecular weight and well established anticoagulant activity. Many of the strongest anticoagulants isolated by bloodsucking animals are found in the extract of salivary glands of different kinds of leeches. Such leech is the Haementeria officinalis, from which is isolated the most active inhibitor of factor Xa – ATS. In order to study the role of some amino acids in the process of interaction among peptides mimetics and the active site of serine proteinases, some fragment analogues of ATS's active site by replacement of some amino acids with the other with similar structure or with unnatural amino acids were synthesized. In the present work the synthesis and the anticoagulant activity according to the APTT and IC50 of the newly synthesized peptides and STRUCTURE-ACTIVITY RELATIONSHIP will be discussed.

Tu269

PEPLOOK: A NEW METHOD TO PREDICT SEQUENCE-FUNCTION RELATIONSHIP

M. Decaffmeyer[1], M.H. Van Eyck[2], A. Thomas[1]

[1]Centre de Biophysique Moléculaire Numérique, Faculté Des Sciences Agronomique de Gembloux [2]Biosiris SA, Gembloux, Belgium

Rational design of peptides is a challenge which would benefit from a better knowledge of their rules of sequence-structure-function relationships. Peptide structures can be approached by spectroscopy and NMR techniques but data from these approaches too frequently diverge. Structures can also be calculated in silico from primary sequence information using three algorithms: Pepstr, Robetta and PepLook.

The most recent algorithm, PepLook introduces indexes for evaluating structural polymorphism and stability. The method uses a de novo search of energy minima by an iterative Boltzmann-Stochastic procedure and using a combination of 64 phi/psi couples derived from the structural alphabet for protein structures proposed by Etchebest et al.

For peptides with converging experimental data, calculated structures from PepLook and, to a lesser extent from Pepstr are close to NMR models. The PepLook index for polymorphism is low and the index for stability points out possible binding sites. For peptides with divergent experimental data, calculated and NMR structures can be similar or, can be different. These differences are apparently due to polymorphism and to different conditions of structure assays and calculations.

The PepLook index for polymorphism maps the fragments encoding disorder and the Mean Force Potential score indicates which residues will be most available for interactions with partners. This should provide new means for the rational design of peptides.

CYCLIC PEPTIDES FOR TARGETING CXCR4 CHEMOKINE RECEPTOR EXPRESSION

O. Demmer[1], N. Koglin[2], H. Kessler[1], H.J. Wester[2]

[1]Institute of Organic Chemistry and Biochemistry, Technical University of Munich, Garching [2]Department of Nuclear Medicine, Klinikum Rechts der Isar, Technical University of Munich, Germany

Several diseases like cancer metastasis, rheumatoid arthritis and chronic lymphocytic B-cell leukemia are linked to the interaction of the CXCR4 chemokine receptor to its natural ligand, the 68 amino acid protein stromal cell-derived factor-1α (SDF-1α).[1] One strategy for the treatment of these diseases could be to block the interaction between CXCR4 and SDF-1α with small CXCR4 antagonists. Furthermore, radiolabeling of suitable compounds with appropriate radioisotopes could provide agents for imaging of CXCR4 expression in vivo via PET.
Previous studies by Fujii et al. on CXCR4 antagonists led to a high affinity cyclic pentapeptide with the sequence cyclo[Gly-D-Tyr-Arg-Arg-Nal].[2] To further improve this structure, different approaches have been chosen with respect to metabolic stability, bioavailability, conformational rigidity and chemical versatility for radiolabeling. First, an N-methyl scan of the backbone amides was performed to influence conformational freedom and to increase metabolic stability and bioavailability. N-methylation of arginine residues yielded peptides with moderate affinity (IC₅₀-values: 23nM (N-Me)Arg³ and 31nM (N-Me)Arg⁴, resp.) whereas N-methylation of other amino acids significantly decreased the affinity (IC₅₀>100nM). By substitution of Arg³ by ornithine, the affinity was mostly retained.[3] The amino group of Orn can be alkylated or acylated via radiolabeled groups containing short lived isotopes. Moreover, the bioavailability should be improved as the high basicity of the two guanidino groups could be reduced. First ornithine-acylated derivatives showed IC₅₀ values between 11-35nM enabling for the first time ¹⁸F-radiolabeling of small CXCR4 antagonists for PET imaging in vivo.

[1]Balkwill,F., Nature Reviews.2004(4)540; [2]Fujii,N. et al., Angew.Chem.2003(115)3373; [3]Tamamura,H. et al., J.Med.Chem.2005(48)3280

M271

CART (85-102) – CYCLIZATION AS A POSSIBLE MECHANISM OF TERMINATION OF BIOLOGICAL ACTIVITY

T. Dylag[1], J. Kotlinska[2], P. Rafalski[2], J. Silberring[1]

[1]Faculty of Chemistry and Regional Laboratory, Jagiellonian University, Krakow [2]Department of Pharmacodynamics, Medical University of Lublin, Poland

Background and aims: We have previously demonstrated that [Abu86,94]CART (85-102)red attenuates some effects of morphine and inhibits cocaine- and amphetamine-induced hyperlocomotion. In this study, its metabolism was assessed in rat cerebral cortex. Based on obtained results, in vivo activity of its derivatives was also evaluated.

Methods: Peptide metabolism in rat cerebral cortex was studied by MALDI-TOF mass spectrometry. Biological activity was measured in locomotor activity test in mice, following intracerebroventricular (icv) administration of the peptide together with amphetamine (5 mg/kg, sc).

Results: In cerebral cortex homogenate, [Abu86,94]CART (85-102)red was sequentially cleaved from N- and C-termini, and N-terminal cleavage was abolished by amastatin – an aminopeptidase inhibitor. This peptide contains two Cys residues in reduced form. It was found that, concomitant with cleavage, rapid cyclization occurred. Interestingly, the newly formed cyclic peptides were not further degraded.

[Abu86,94]CART (85-102)red (0.1 μg) inhibited amphetamine-induced hyperlocomotion, and its rapid cyclization prompted us to evaluate the activity of its cyclic analog as well. We found that [Abu86,94]CART (85-102)ox failed to inhibit amphetamine-induced hyperlocomotion. However, this ability was retained in [Abu86,88,94,101]CART (85-102), in which all Cys residues were replaced with 2-aminobutyric acid (Abu) to prevent their pairing. This suggested that only the linear version of the tested peptide could reverse amphetamine-induced hyperlocomotion.

Conclusions: Disulfide bridge formation might be an interesting mechanism that prevents proteolysis of [Abu86,94]CART (85-102)red and terminates its ability to reverse amphetamine-induced hyperlocomotion. Loss of biological activity might be due to limited conformational flexibility of the cyclic peptide, compared to its active linear version.

Tu272

CONFORMATIONAL STUDIES OF TWO NEW CYCLIC PEPTIDES WITH INHIBITORY ACTIVITY ON LIGAND-INTEGRIN-BINDING

K. Gaus[1], S. Urman[1], Y. Yang[1], U. Strijowski[1], S. De Pol[2], O. Reiser[2], N. Sewald[1]

[1]Department of Chemistry, Bielefeld University, Bielefeld [2]Institute of Organic Chemistry, University of Regensburg, Germany

Binding of ligands to integrins plays a major role in cell adhesion, migration, and signal transduction of cells. These interactions are important not only for normal cell functions, but also in pathogenic processes. The v 3 integrin for example is involved in tumor cell adhesion and osteoporosis. The association of ligands is specific and requires minimal recognition sequences. Therefore, suppression of integrin activity using competitive inhibitors bears great pharmacological potential.

The tri-peptide sequence RGD is a prominent recognition sequence of integrin ligands. Two new cyclic pentapeptides were synthesized containing the tripeptide sequence RGD as well as 3-amino-cyclopropane-1,2-dicarboxylic acid monomethyl ester (ACC) and valine varying only with respect to the stereochemistry of ACC. Both the (+) (all R) and (-) (all S) isomers of ACC were incorporated. ACC is a cyclic -amino acid as well as a cyclopropyl analogue of aspartic acid. Biological tests with cell lines expressing mainly v 3 and v 5 integrin show a higher inhibitory activity of cyclo-(-Arg-Gly-Asp-(+)ACC-Val-).

In order to derive a STRUCTURE-ACTIVITY RELATIONSHIP of these two isomers, solution structures in DMSO-D6 were investigated by NMR spectroscopy. Subsequently, structural information was obtained by applying distance restraints derived from the NMR spectra in distance geometry/simulated annealing and molecular dynamics calculations.

Due to the rigidity of the cyclopropyl unit in ACC, the structure of the cyclopeptide is significantly influenced by the integrated propane ring, thus explaining the different biological properties.

SYNTHESIS AND BIOLOGICAL EVALUATION OF SELECTED INSECT MYOTROPIC PEPTIDES

S. Grodecki[1], K. Szymanowska[1], M. Skonieczna[2], G. Rosiński[2], D. Konopińska[1]

[1]Faculty of Chemistry, University of Wroclaw [2]Department of Animal Physiology and Developmental Biology, A. Mickiewicz University, Poznan, Poland

In the last decade a number of insect peptides, structurally related to neuropeptide FMRF-amide has been identified. These peptides share the conserved X1X2RF-amide (where X1 = R or F and X2 = L) sequence at their C-terminal ends. Among them two peptides, Led-NPF-1 (ARGPQLRLRFamide) and Led NPF II (APSLRLRFamide) were isolated from the brain of Lepitonotarsa decemlineata as well as Mas-MT-I, II and III (PEDVHSFLRF-amide, GNSFLRF-amide, DPSFLRF-amide) which originated from the brain of Manduca sexta.. The object of our investigation was synthesis of two groups of peptides. There are: 1/ Led-NPF-1 (I), Led NPF-2 (II) and its 14 analogues with a fragment sequence such as: [2-10] - (III), [3-10] - (VI), [5-10] - (V), [6-10] - (VI), [7-10]- (VII) and [8-10] - (IX)-Led-NPF-1 and analogues where C-terminal Phe-amide was replaced by Phe-OH(X), and amides of D-Phe (XI), Tyr (XII), Phe(4-NO2) (XIII) and Phe(4-N.N-di-Me) (XIV) and 2/ Mas-MT III (XV) and its analogues with shortened sequence [4-7]- (XVI), [3-7] - (XVII) and [2-7] - Mas-MT-III (XVIII). The peptides were synthesized by the classical solid-phase method according to the Boc- or Fmoc- procedure. Biological effect was evaluated by the cardiotropic test on the semi-isolated heart of two insect species: Tenebrio molitor and Zophobas atratus. In the preliminary investigation we found, that the Led-NPF-1 and its analogues show cardioinhibitory effect, while Mas-MT-III and analogues stimulated heartbeat in two insect species, similar to proctolin.

M274

CYCLIC PEPTIDES COMPRISING BETA-HOMOAMINO ACIDS AS INTEGRIN ALPHA4BETA1 LIGANDS

E.W. Guthöhrlein[1], M. Malešević[2], S. Urman[1], N. Sewald[1]

[1]Department of Chemistry, Bielefeld University, Bielefeld [2]Max-Planck-Forschungsstelle für Enzymologie der Proteinfaltung, Halle (Saale), Germany

Integrins are an important family of cell adhesion molecules. Currently, 24 members are known. Among other functions, integrin $\alpha_4\beta_1$ is involved in inflammatory processes, leukocyte migration and tumor angiogenesis. The structure of its natural ligand VCAM-1, including the binding loop sequence TQIDSPLN, has been determined by X-ray crystallography. Therefore, it is possible to apply the concept of spatial screening: Using small cyclic peptides with structure inducing building blocks, the binding motif is presented in different well-defined structural arrangements.

For this study, a series of cyclic penta- and hexapeptides based on the TQIDSPLN sequence has been synthesized. β-Homoamino acids, i.e. β^3-amino acids with proteinogenic side-chains, have been incorporated as structure inducers for spatial screening. Although β^3-amino acids are supposed to prefer the central position of Ψγ-turns, less data exist than for e.g. D-amino acids. Apart from the structural characterization of potential high affinity ligands for integrin $\alpha_4\beta_1$, a major goal of this work is to provide a better understanding of the influence of β^3-amino acids on the structure of cyclic peptides.

The structures of the peptide library have been investigated by NMR spectroscopy, followed by DG/SA and MD calculations. The results substantiate the γ-turn inducing capability of β-homoamino acids, but also prove the formation of different turn structures in certain cases. A comparison to the X-ray structure of VCAM-1 shows that the structure of the binding sequence has been successfully approximated by some of the peptides. Biological activity tests should lead to meaningful structure-affinity relationships.

Tu275

INVESTIGATING THE ROLE OF N-TERMINAL POLYPROLINE II HELIX OF NEUROPEPTIDE Y FOR LIGAND BINDING USING ARTIFICIAL PYRIDONE DIPEPTIDE MIMETICS

M. Haack[1], P. Tremmel[3], S. Enck[2], A. Geyer[2], A.G. Beck-Sickinger[1]

[1]Institute of Biochemistry , University Of Leipzig [2]Faculty of Chemistry, Philipps-Universität Marburg [3]Institute of Organic Chemistry, University of Regensburg, Germany

Neuropeptide Y (NPY) is a 36-amino acid peptide amide and binds to the so-called Y receptors. Its most dominant element is the C-terminal alpha-helix spanning amino acid residues 12-36. Residues 1-10 form a polyproline helix with highly conserved proline residues at positions 2, 5 and 8, followed by a loop structure. The importance of the polyproline helix strongly varies between different receptor subtypes. It obviously plays no role in ligand binding at the Y2 receptor subtype, whereas the N-terminal segment is of importance for ligand binding at Y1 and Y5.

In order to further study the importance of the polyproline helix we introduced a conformationally constrained pyridone dipeptide mimetic at different single positions by solid phase peptide synthesis using Fmoc/tBu strategy. The resulting peptides have been investigated in cell lines that selectively express Y1 and Y5 receptor, respectively. Different methods including radioactive competitive binding assay, CD and NMR have been applied to investigate conformation and interaction of receptor and ligand.

Loss of affinity at the Y1 receptor is independent of the position and about 10-, 20- and 30-fold, respectively, when introduced once, twice and thrice. Introduction of the building block in position 8/9 leads to the most reduced affinity at the Y5 receptor subtype but, surprisingly, affinity can partially be regained by introduction of the dipeptide at two additional positions. The position of the dipeptide is of greater importance at Y5.

These novel peptides clearly indicate the importance of proline residues and the structure of the N-terminus for ligand binding.

SH2 DOMAIN DIRECTED EFFECTORS OF SHP-1 PHOSPHATASE ACTIVITY

K. Hampel[1], I. Kaufhold[1], M. Zacharias[2], F.D. Böhmer[3], D. Imhof[1]

[1]Friedrich-Schiller-University, Institute of Biochemistry and Biophysics, Jena [2]International University Bremen, School of Engineering and Science, Bremen
[3]Friedrich-Schiller-University, Research Unit Molecular Cell Biology, University-Hospital, Jena, Germany

Interactions of Src homology 2 (SH2) domains with phosphotyrosine (pY) containing ligands is critical for regulating cellular processes. The cytosolic protein tyrosine phosphatase SHP-1 contains two SH2 domains. An intramolecular interaction of the N-terminal SH2 domain with the catalytic (PTP) domain renders the enzyme inactive in the native state. Binding of a pY-ligand to SHP-1 N-SH2 leads to a conformational shift and the dissociation of the SH2-PTP complex [1]. In previous studies we investigated the topographical and conformational preferences of the N-SH2 domain of SHP-1 using conformationally restricted linear and cyclic peptides derived from the natural interaction partner Ros pY2267 [2]. We identified peptides that showed an increased binding affinity for the N-SH2 domain and partially inhibited Ros-mediated SHP-1 activity. On the basis of these results we hypothesized that an imperfect fit of the pY+1 and pY+3 side chains might be responsible for the inhibitory effect. In order to confirm this hypothesis we synthesized a new series of peptides and evaluated their biological activity. To better understand the role of each individual SH2 domain in the activation process we also determined the binding affinity against the C-SH2 domain and the activation profile of different SHP-1 mutants. Pull-down assays of the interactions of the pY-ligands with full length SHP-1 confirmed the results obtained for the binding to the individual SH2 domains.

[1] Yang, J., et al., J. Biol. Chem. 2003, 278, 6516-6520.
[2] Imhof, D., et al., J. Med. Chem. 2005, 48, 1528-1539.

M277

SINGLE AMINO ACID SUBSTITUTION ANALOGUES OF THE ANTIBACTERIAL PEPTIDE [A7]-ANOPLIN

P.R. Hansen, D. Ifrah, H.T. Lianee, C.A. Thing, T. Vitved

Department of Natural Sciences, Bioorganic Chemistry Group, Royal Veterinary and Agricultural University, Copenhagen, Denmark

Background and Aims: Anoplin, GLLKRIKTLL-NH2, is an antibacterial peptide isolated from the solitary spider wasp, Anoplius samariensis. Previously, we have reported a structure-activity study of anoplin based on 37 analogues (1). Alanine positional scanning showed restrictions on 6 out of the 10 residues, and improved MIC-values for the remaining 4 residues, including [A7]-anoplin. In the present paper, we report a series of analogues of [A7]-anoplin in which Ala was replaced by Val, Leu, Ile, Phe, Trp and Asn.

Methods: The analogues were synthesized by Fmoc chemistry, purified by preparative HPLC and characterized by MALDI-TOF-MS. A stock peptide solution in 1% DMSO was prepared and the peptide concentration of each peptide was determined by amino acid analysis. The anoplin analogues were tested for antibacterial activity against S. aureus ATCC 25923 and E. coli ATCC 25922. Furthermore, the cytotoxity towards red blod cells was evaluated using a hemolytic activity assay.

Results: The [V7], [I7], [L7], [F7] and [W7]-anoplin analogues displayed improved MIC-activities towards S. aureus and E. coli (2 µg/ml both strains) as compared with [A7]-anoplin (5 µg/ml both strains). However, the analogues also turned out to be more hemolytic. CD experiments indicated a high high α-helix content. In agreement with helical wheel predictions, [N7]-anoplin did not show any significant antibaterial activity (47 µg/ml both strains).

Conclusion: The results presented here suggest that anoplin analogues are promising lead structures for developing future antibacterial agents.

Reference:
[1] Ifrah D, Doisy X, Ryge TS, Hansen, PR. (2005). J. Peptide Science 11: 113-121.

Tu278

EFFECT OF NEAR UV IRRADIATION ON A SINGLE CHAIN VARIABLE FRAGMENT OF THE MONOCLONAL ANTIBODY 82D6A3 AND ITS MUTANTS

E. Illyés[1], S. Staelens[3], A. Vanhooren[4], H. Deckmyn[3], I. Hanssens[4], Zs. Majer[2]

[1]Research Group for Peptide Chemistry, Hungarian Academy of Sciences,Eötvös Loránd University [2]Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary [3]Catholic University of Leuven Campus Kortrijk IRC Laboratory for Thrombosis Research [4]IRC Protein Biophysics, Catholic University of Leuven Campus Kortrijk, Belgium

Proteins are targets for photo-destruction due to absorption of incident light by endogenous chromophores. Mass spectroscopic data presented evidence that structural modification observed upon irradiation of goat alpha-lactalbumin at 290 nm results from tryptophan (Trp) mediated cleavage of disulfide bonds [1].
The aim of the recent studies is to define structural elements that direct the destructive influence of near-UV light on the disulfide bridges of proteins.
Most of the proteins of the immunoglobulin superfamily contain a so called triad, consisting of two S atoms, forming a disulfide bridge, and a single Trp in their close vicinity [2]. We have indications that this arrangement gives rise to a photolytic degradation similar to that described in our earlier studies for goat alpha-lactalbumin [3]. We therefore investigated the influence of UV light on the single chain variable fragment (scFv) of a monoclonal antibody (82D6A3) [4] which contains two triads. The results showed that after irradiation of the wild type scFv (i) new bands (degradation products) appeared in electrophoresis experiments and (ii) the affinity for its antigen, von Willebrand factor decreased. By site-directed mutagenesis, we modified the critical Trp-residues to perform a parallel study on these mutants.

[1]. A. Vanhooren et al, Biochemistry 41, 36, 11035-11043 (2002)
[2]. T.R. Iorger et al. Molecular Immunology 36 373-386, (1999)
[3]. A. Vanhooren et al, Biochemistry 45 in press (2006)
[4]. S. Staelens et al. Molecular Immunology 43, 1243-1257 (2006)

Acknowledgements: The research was supported by Bilateral Research Grant B16/04 (Zs.M.), FWO-Flanders G-0180-03 (I. H.)

STRUCTURAL REQUIREMENTS OF SUBSTRATES FOR THE PTP DOMAIN OF EYA PROTEINS

A. Musharraf[1], N. Markschies[2], **D. Imhof**[2], C. Englert[1]

[1]Leibniz Institute of Age Research/Fritz Lipmann Institute [2]Institute of Biochemistry and Biophysics, Friedrich-Schiller-University, Jena, Germany

Animal Eya (Eyes absent) proteins are involved in the regulation of cell differentiation and organ development [1]. Recently, it has been shown that the evolutionarily conserved C-terminal segment of ~271-274 amino acids represents a PTPase catalytic domain (Eya domain). In contrast to classical protein tyrosine phosphatases that contain the signature motif HCXAGXGR(S/T) as indispensable part of the active site, hydrolysis of pY-containing substrates by the Eya domain was shown to be metal-dependent [2]. Here, we investigated the structural requirements of substrates for the Eya homologue in A. thaliana (AtEya) by two different approaches. A peptide derived from Src Y419 was chosen as a template for truncation and amino acid replacement studies. In a second approach we used a combinatorial peptide library containing phosphono methyl phenylalanine as pY-mimetic to screen for binding to the Eya domain. The contribution of individual residues on both sides of pY to binding and catalysis was assessed by kinetic analysis using HPLC and a spectrophotometric assay. [1] Rayapureddi, J. P., et al., Nature 2003, 426, 295-298. [2] Rayapureddi, J. P., et al., Biochemistry, 2005, 44, 751-758.

M280

ANTI-THROMBUS DIMER PEPTIDE DERIVED FROM THROMBOMODULIN

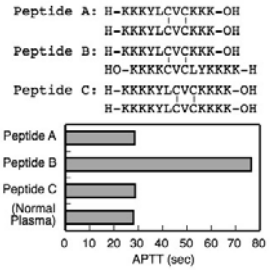
K. Inai[1,2], M. Oshita[2], T. Hashimoto[2], S. Yoshikawa[1]

[1]Molecular Assemblies Design Research Section, Institute of Advanced Energy, Kyoto University, Uji, Kyoto [2]Kuraray Medical Inc., Kurashiki, Japan

Background and aims: It is known that thrombomodulin has important function which prevents thrombus. We found KmYLCVCKn (m, n >= 2) peptides derived from thrombomodulin had strong anti-thrombus activity in our recent studies. These peptides formed two structures, parallel and anti-parallel, as dimers, we examined the relation between structure and activity.

Methods: Two peptides of KKKYLC(Acm)VCKKK and KKKKYLCVC(Acm)KKKK were synthesized by Fmoc chemistry. Dimer peptides were made by removing Acm with iodine, after dissolving in 0.1 M Tris HCl buffer (pH 8.0) and oxidizing the mixture of these synthesized peptides spontaneously. Then three peptides shown in figure were separated using RP-HPLC. The peptide concentration in normal human pooled plasma was 10 micro moles / l when measuring APTT (activated partial thromboplastin time).

Results: The anti-parallel formed peptide, Peptide B, was prolonged APTT approximately 2.7 times, although two parallel formed peptide, Peptide A and C, were not significantly different from the APTT of normal plasma.
Conclusions:
These peptides have STRUCTURE-ACTIVITY RELATIONSHIP, we observed that the anti-parallel formed peptide had strong anti-thrombus activity.



HOMOARGININE-CONTAINING OPIOID PEPTIDE ANALOGUES

J. Izdebski[1], D. Kunce[1], M. Grabek[1], N.N. Chung[2], P.W. Schiller[2]

[1]Peptide Laboratory, Department Of Chemistry, Warsaw University, Warsaw, Poland [2]Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, QC, Canada

Bioactive peptides containing pairs of basic amino acids are rapidly metabolized as a result of cleavage by trypsin-like enzymes. To increase the metabolic stability of opioid peptides containing Arg-Arg and Arg-Lys pairs, the Arg residues were replaced by homoarginine (Har). In the syntheses of these analogues we used Boc-Har{ω,ω'-[Z(2Cl)]2}-OH, which was developed in this laboratory [1]. Analogues of dynorphin A(1-13), dynorphin-32, α-neoendorphin and β-neoendorphin were synthesized. Their opioid activities in the GPI and MVD assays were compared with those of the native parent peptides.

[1] J, Izdebski, T. Gers, D.Kunce, in Peptides 2004, (Flegel M., Fridkin M., Gilon C., Slaninowa J, Eds.), Kenes International, 2005, pp.333-334.

Th282

SHORTENED AND FULL-LENGTH INSULIN ANALOGUES WITH MODIFICATIONS IN THE POSITION B26. BINDING AFFINITIES AND IN VIVO ACTIVITIES.

J. Jiracek[1], L. Zakova[1], I. Hanclova[1], E. Protivinska[1], L. Kazdova[2], M. Sanda[1], M. Budesinsky[1]

[1]Department of Biological Chemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic [2]Department of Metabolic Research, Diabetes Center, Institute of Clinical and Experimental Medicine, Praha, Czech Republic

Recently we published the synthesis and biological characterization of a series of des-tetrapetide(B27-B30)-insulin amides (DTIAs) with modifications in the position B26 (Zakova L. et al., Biochemistry 43, 2323-2331, 2004). The DTIA analogues with histidine and especially with N-methylated histidine in the position B26 were extremely potent in binding to rat adipose plasma membranes and in stimulating of glucose transport into isolated rat adipocytes if compared with human insulin.

Here we present the synthesis of and biological characterization of novel full-length insulin analogues with histidine, N-methylated histidine and N-methylated alanine in the position B26 and comparison of their properties with DTIA analogues with N-methylated histidine and N-methylated alanine in the position B26. For all presented analogues we determined the binding affinity to rat adipose plasma membranes, the ability to stimulate the incorporation of 14C-glucose into rat adipose tissue and the potency to influence the concentration of plasma glucose in normal and diabetic rats in vivo.

M283

EVALUATION OF INSECT KININS WITH STEREOCHEMICAL VARIANTS OF THE CIS-PEPTIDE BOND MOTIF 4-AMINOPYROGLUTAMATE

K. Kaczmarek[1,4], G.M. Coast[2], H.J. Williams[3], J. Zabrocki[1,4], R.J. Nachman[4]

[1]*Areawide Pest Management Research Unit, Southern Plains Agricultural Research Center, U.S. Department of Agriculture, College Station, TX, USA*
[2]*School of Biological and Chemical Sciences, Birkbeck College, London, UK*
[3]*Department of Chemistry, Texas A&M University, College Station, TX, USA*
[4]*Institute of Organic Chemistry, Technical University of ŁÓdź, Poland*

Insect kinins share a highly conserved C-terminal pentapeptide sequence Phe-Xaa-Xbb-Trp-Gly-NH2, where Xaa can be Tyr, His, Ser or Asn and Xbb can be Ala but is generally Ser or Pro. They are potent diuretic peptides that stimulate the secretion of primary urine by Malpighian tubules, organs involved in the regulation of salt and water balance (1). The insect kinins preferentially form a cis-Pro, type VI β-turn. Insect kinin analogs containing tetrazole (1) and 4-aminopyroglutamate (2), both cis-peptide bond, type VI β-turn motifs, demonstrate significant activity in the in a cricket diuretic assay.
In this study, we compare the diuretic activity of insect kinin analogs incorporating the four stereochemical variants of the 4-aminoglutamate (APy) motif. Three of the insect kinin analogs incorporating the stereochemical variants, (2S,4S)-APy, (2R,4S)-APy and (2R,4R)-APy, were found to have the same diuretic activity (EC50 values of between 7-14 x 10-8 M). However, the (2S,4R)-APy analog proved to be 10-fold more potent (EC50 value of 0.7 x 10-8 M). These analogs are more active than the (L,L)- and (D,D)-tetrazole insect kinin variants (EC50 values of 34 and 58 x 10-8 M, respectively). In contrast, the (L,D)-tetrazole variant demonstrated partial antagonism of the native achetakinins(1). Aqueous solution conformation structures will also be presented and correlated, where possible, with differences in diuretic activity.

References:
1. Nachman RJ, Zabrocki J, Olczak J, Williams HJ, Moyna G, Scott IA, Coast GM. Ci- Peptides 23 709-716.
2. Nachman RJ, Kaczmarek K, Williams HJ, Coast GM, Zabrocki J (2004) Biopolymers 75 412.

CONFORMATIONALLY RESTRICTED AT N-TERMINUS NOCICEPTIN/ORPHANIN OFQ/N(1-6) ANALOGUES: SYNTHESIS AND BIOLOGY

K. Kaczmarek[1], J. Kotlinska[2], M. Legowska-Lenard[2], A. Pachuta[2], J. Silberring[3], J. Zabrocki[1]

[1]Institute of Organic Chemistry, Technical University of Lodz [2]Department of Pharmacology, Medical School, Lublin [3]Department of PhFaculty of Chemistry, Jagiellonian University, Krakow, Poland

Our earlier studies demonstrated that nociceptin/orphanin FQ (1-17) inhibits morphine withdrawal in rats. This peptide is metabolized in the spinal cord, both in vitro and in vivo, to shorter fragments, including OFQ/N(1-11) and OFQ/N(1-6). These fragments are behaviorally active and modulate nociception in a bi-phasic process. In particular, OFQ/N(1-6) shows pronounced analgesia, followed by hyperalgesia. Removal of C-terminal Gly completely abolishes peptide activity. In the present work we have synthesized four OFQ/N(1-6) analogues having at the N-terminus all stereoisomers of 4-aminopyroglutamic acid as a replacement for Phe-Gly dipeptide unit. Due to expected importance of Phe side chain for biological activity, benzyl substituent was shifted to the peptide N-terminal amino group. The transformation into such derivative can be performed by reductive alkylation on solid support after finishing elongation of the peptide chain (Szardenings, A.K, Burkoth, T.S., Look, G.C., Campbell, D.A., J. Org. Chem. 61 (1996) 6720; Kaczmarek, K., Chung, N. N., Schiller, P.W., Zabrocki J., Peptides 2002 (Proceedings of the 27th European Peptide Symposium), Edizioni Ziino, Napoli 2003, p.158). Those sequences were tested for their analgesic activity using tail-flick and hot-plate tests.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SELECTED FRAGMENTS OF THE CRF-RELATED DIURETIC PEPTIDE OF LOCUSTA MIGRATORIA

Ch. Kaskani[1], C.P. Poulos[1], G.J. Goldsworthy[2]

[1]Department of Chemistry, University of Patras, Patras, Greece [2]Department of Biology, Birkbeck College, University of London, UK

Locusta Diuretic Hormone (Locmi-DH) is a neuropeptide that has been isolated from the locust Locusta migratoria. It belongs to the CRF-related diuretic peptides and has the following amino acid sequence: MGMGPSLSIVNPMDVLRQRLLLEIARRRLRDAEEQIKANKDFLQQI-NH2. Locmi-DH stimulates fluid secretion and cAMP production by Malpighian tubules in vitro and it is released into the haemolymph from corpora cardiaca . Structure-activity studies have shown that the C-terminal fragment Locmi-DH(32-46) is active in the feeding behavioral assay in Locusta migratoria while has very low response in stimulating cAMP production by Malpighian tubulus from Schistocerca gregaria [1]. In the present work we synthesized the C-terminal analogues Locmi-DH(34-46), Locmi-DH(36-46) and Locmi-DH(38-46) in order to determine whether the C-terminal fragment Locmi-DH(32-46) can be further truncated N-terminally with loss of its effects on feeding behavior in locust. We also synthesized the N-terminal fragments analogues [Hse(Me)1,3,13]-Locmi-DH(1-30), [Hse(Me)1,3,13]-Locmi-DH(1-30)-OH and [Hse(Me)13]-Locmi-DH(6-30)-OH to study the role of the N-terminal part of Locmi-DH on biological activity. The synthesis of the peptides was performed in solid phase on the 2-Chlorotrityl chloride Resin using either stepwise or fragment condesation strategy and the Fmoc/Bu-t methodology. References: [1] Goldsworthy G.J, Chung J.S., Simmonds S.J., Tatari M., Varouni S. and Poulos C.P, Peptides 24, 1607-1613 (2003).

M286

IN SEARCH OF SYNTHETIC LOW-MOLECULAR PEPTIDE DERIVATIVES WITH ACTIVITY AGAINST FUNGAL PATHOGENS

R. Kasprzykowska[1], **F. Kasprzykowski**[1], A. LeŚniewska[1], A. Pogorzelska[1],
W. Kamysz[2], A. Grubb[3], A. Jasir[4], C. Schalén[4]

[1]Faculty of Chemistry, University of GdańSk [2]Department of Physical Chemistry, Gen. J. Hallera 107, Medical University of GdańSk, Poland [3]Department of Clinical Chemistry [4]4Department of Microbiology, Dermatology and Infection, University Hospital, Lund, Sweden

The need for new effective and to mammalian cells non-toxic antifungal agents increases in parallel with the expanding number of immunocompromised patients at risk for invasive fungal infections. In our laboratory we have produced a serie of low-molecular peptide derivatives of the general structure: X-Arg-Leu-NH-CH(iPr)-CH2-NH-Y (where X and Y were acyl groups with aromatic carbocyclic system). We have found and earlier reported that some of these display high antimicrobial activities against several clinically important Gram-positive pathogenic bacteria. In this study we have by solution methods synthesized a group of low-molecular compounds and investigated their antifungal activity. The study included both Candida and Aspergillus species. We have found that some of the compounds were highly fungicidal. We also made a conformational study in which the residues were separately replaced by selected hydrophobic amino acids and their equivalents. The conformational study showed that the desirable stable intramolecular structure could only be formed in the presence of some vital components.

This work was supported by grant DS/8350-5-0131-6.

Tu287

INFLUENCE OF TERMINAL FRAGMENTS IN SELECTED LOW-MOLECULAR COMPOUNDS ON THEIR ANTIMICROBIAL ACTIVITY

R. Kasprzykowska[1], **F. Kasprzykowski**[1], A. LeŚniewska[1], A. Pogorzelska[1],
A. Grubb[2], A. Jasir[3], C. Schalén[3]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Department of Clinical Chemistry [3]Department of Microbiology, Dermatology and Infection, University Hospital, Lund, Sweden

Increased resistance of bacterial pathogens to currently employed antibiotics has resulted in efforts to develop antimicrobial compounds with new mechanisms of action. Previously, we have synthesized some high potent antimicrobial compounds based upon the N-terminal binding fragment of human cystatin C. Some derivatives of the general structure: X-Arg-Leu-NH-CH(iPr)-CH2-NH-Y (1) (where X and Y were acyl groups with aromatic carbocyclic system) have displayed the broad antibacterial spectrum and high activity against several clinically important Gram-positive pathogens, including multi-resistant staphylococci. Herein, the synthesis and structure - antibacterial properties relationship for two series of analogues of 1 are presented. The X and Y groups in 1 were replaced by selected substituents with various geometry and distance between aromatic moieties and carbonyl. We have established the general structural features which the discussed class of peptide derivatives should possess in order to displaying the particular antimicrobial activity.
This work was supported by grant DS/8350-5-0131-6.

STUDY OF NON-OPIOID BETA-ENDORPHIN RECEPTOR

Y.A. Kovalitskaya[1], A.A. Kolobov[2], E.A. Kampe-Nemm[2], V.M. Lipkin[1], E.V. Navolotskaya[1]

[1]Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy Of Science, Pushchino, Moscow Region [2]State Research Center for Institute of Highly Pure Biopreparations, Ministry of Health of The Russian Federation, St. Petersburg, Russia

We have synthesized beta-endorphine-like decapeptide immunorphin SLTCLVKGFY which corresponds to the 364-373 sequence of the heavy chain of human IgG. Immunorphin was found to be a selective agonist of non-opioid (naloxone-insensitive) beta-endorphin receptor. The purpose of this study was to prepare [3H]immunorphin and characterize by its using the non-opioid beta-endorphin receptor on mouse peritoneal macrophages and membranes isolated from various rat organs. By use of tritium-labeled immunorphin ([3H]SLTCLVKGFY) with specific activity of 24 Ci/mmol, non-opioid beta-endorphin receptors were revealed and characterized on mouse peritoneal macrophages and rat myocardium, spleen, adrenal, and brain membranes. The non-opioid beta-endorphin receptor of macrophages has in addition to immunorphin (Kd of the [3H]immunorphin-receptor complex was 2.4 nM) and beta-endorphin (Ki of the [3H]immunorphin specific binding was 2.9 nM) a high affinity for Fc-fragment of human IgG1, pentarphin (VKGFY), cyclopentarphin [cyclo(VKGFY)], and [Pro3]pentarphin (VKPFY) (Ki were 0.0060, 2.7, 2.6, and 2.8 nM, respectively) and is insensitive to naloxone and [Met5]enkephalin (Ki > 100 µM). Treatment of macrophages with trypsin resulted in the loss of their ability for the specific binding of [3H]immunorphin. Values of the specific binding of 8.4 nM [3H]immunorphin to rat adrenal, spleen, myocardium, and brain membranes were determined to be 1146.0, 698.6, 279.1, and 172.2 fmol/mg protein, respectively. Unlabeled beta-endorphin, pentarphin, [Pro3]pentarphin, cyclopentarphin, cyclodipentarphin [cyclo(VKGFYVKGFY)], and Fc-fragment of IgG1 inhibited the binding of [3H]immunorphin to membranes from these organs. No specific binding of [3H]immunorphin to rat liver, lung, kidney, and intestine membranes was found.

M289

INFLUENCE OF INSECT OOSTATIC PEPTIDE NEB-COLLOSTATIN ON HEARTBEAT OF TWO INSECT SPECIES TENEBRIO MOLITOR AND ZOPHOBAS ATRATUS

M. Kuczer[1], M. Skonieczna[2], G. Rosinski[2], D. Konopinska[1]

[1]Faculty of Chemistry, University of Wroclaw [2]Department of Animal Physiology, A. Mickiewicz University, Poznan, Poland

Oogenesis in insects is a well studied and complex process. At present, three oostatic factors called Aea-TMOF (H-Tyr-Asp-Pro-Ala-Pro-Pro-Pro-Pro-Pro-Pro-OH), Neb-TMOF (H-Asn-Pro-Thr-Asn-Leu-His-OH), and Neb-collostatin (H-Ser-Ile-Val-Pro-Leu-Gly-Leu-Pro-Val-Pro-Ile-Gly-Pro-Ile-Val-Val-Gly-Pro-Arg-OH), isolated from Aedes aegypti (Aea-TMOF) and fleshfly Neobellieria bullata (Neb-TMOF and Neb-collostatin)
The subject of our paper is structure/function relationship studies of insect oostatic factor Neb-collostatin originating in Neobellieria bullata and its analogues.
We performed the synthesis of Neb-collostatin and its 29 analogues. Two analogues were modified in position 13 by Ala or D-Pro and other were oligopeptides with a shortened peptide sequence such as [1-13]-, [14-19]-, [15-19]-, [16-19]-, [13-19]-, [12-19]-, [11-19]-, [10-19]-, [9-19]-, [8-19]-, [7-19]-, [6-19]-, [5-19]-, [4-19]-, [3-19]-, [2-19]-, [1-11]-, [1-4]–, [1-6]-, [1-8]-,[1-9]-, [3-9]-, [3-11]-, [4-8]-, [2-6]- and [2-9]-collostatin. Synthesis of these peptides was carried out by classical solid-phase method according to the Boc- or Fmoc- procedure.
Biological effects of the Neb-collostatin and its analogues were determined in vivo on ovarian growth and oocyte maturation of yellow mealworm, Tenebrio molitor. Moreover, the mentioned peptides were evaluated in vitro by cardiotropic bioassays with the semi-isolated heart of T. molitor and Zophobas atratus. In this paper we discuss a gonadoinhibitory and cardiotropic effect of the investigated peptides.

Tu290

THEORETICAL AND SPECTROSCOPIC STUDIES OF PHOSPHONODEHYDROPEPTIDES AND THEIR ACTIVITY TOWARDS CATHEPSIN C

R. Latajka[1], M. Jewginski[2], M. Pawelczak[2], M. Makowski[2], P. Kafarski[1,2]

[1]Faculty of Chemistry, Wroclaw University of Technology, Wroclaw [2]Institute of Chemistry, University of Opole, Poland

Since dehydroamino acids are quite reactive and various thiol nucleophiles are known to add to their double bonds [1,2], we hoped that these compounds might act as alkylating inhibitors of cathepsin C (dipeptidyl-peptidase I). Its main function is protein degradation in lysosymes, but it is also found to participate in the activation of neuraminidase and proenzymes of serine proteinases (leukocyte elastase, cathepsin G, granzyme A) [3,4].
It is well known, that phosphonodipeptides structural analogues of synthetic substrates of cathepsin C are the model substances in designing the new inhibitors of this enzyme. For that reason we have undertook the synthesis, theoretical and structural investigations of phosphonic analogues of dehydropeptides.

Gly-ΔZPhe-AbuPO(OMe)2
Gly-ΔZPhe-AlaPO(OEt)2
Gly-ΔZPhe-LeuPO(OMe)2
Gly-ΔZPhe-ValPO(OEt)2
Gly-ΔZPhe-GlyPO(OMe)2
Gly-ΔZPhe-nBuPO(OEt)2

The structure and conformational preferences in this group of peptides had been investigated by mean of NMR techniques. In order to find the interactions between compounds-enzyme (cathepsin C) and interpret the results of biological test, the molecular modelling methods had been used.
[1] Breitholle, A.O., and Stammer, C.H., Tetrahedron Lett. 1975, 28, 2381
[2] Turk, B., Dolenc, I., and Turk, V., Handbook of Proteolytic Enzymes, Barrett A.J. (ed), Academic Press 1998
[3] Butler, R., Michel, A., Kunz, W., and Klinkert, M.O., Protein Pept. Lett. 1995, 2, 313

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SILAPROLINE-CONTAINING ODN ANALOGS

J. Leprince[1], F. Cavelier[2], P. Gandolfo[1], M. Diallo[1], H. Castel[1], L. Desrues[1], J. Martinez[2], H. Vaudry[1], M.C. Tonon[1]

[1]Inserm U413, Laboratory of Cellular And Molecular Neuroendocrinology, European Institute for Peptide Research IFRMP 23, University of Rouen, Mont-Saint-Aignan [2]CNRS UMR 5810, Laboratory of Amino-Acids Peptides and Proteins, University of Montpellier, France

The octadecaneuropeptide (ODN), increases [Ca2+]i in cultured rat astrocytes through activation of a GPCR coupled to PLC. The action of ODN on [Ca2+]i is mimicked by the C-terminal octapeptide RPGLLDLK (OP), partially antagonized (-40%) by the linear analog [DLeu5]OP and totally suppressed by the cyclic analog cyclo[DLeu5]OP. In vivo, ODN and OP inhibit food intake and their anorexigenic effect is blocked by cyclo[DLeu5]OP. Silaproline (Sip) is a silylated proline-surrogate which influences both the physicochemical and pharmacodynamic properties of peptides without affecting their native 3D-structure. The aim of this study was to substitute the Pro2 residue of OP and [DLeu5]OP, that is involved in the bioactive conformation of these peptides, by Sip and to evaluate the Ca2+-mobilizing activity of [Sip2]OP and [Sip2,DLeu5]OP in astrocytes. Application of [Sip2]OP provoked a transient increase in [Ca2+]i with an amplitude significantly higher than that induced by ODN. Repeated pulses of [Sip2]OP resulted in sequential increases with gradual attenuation of the response. Preincubation of astrocytes with cyclo[DLeu5]OP partially abolished the effect of [Sip2]OP. [Sip2,DLeu5]OP did not affect the basal [Ca2+]i, but reduced by 37% the ODN-evoked [Ca2+]i increase. In conclusion, these data indicate that the substitution of Pro2 by Sip enhances the efficacy of OP but does not affect the antagonistic properties of [DLeu5]OP. Owing to their better stability and enhanced hydrophobicity, silylated ODN analogs may cross more easily the blood-brain barrier and thus may prove useful for the development of novel appetite-regulating drugs. Supported by Inserm (U413), CNRS (UMR-5810) and the Conseil Régional de Haute-Normandie.

M292

AGONIST AND ANTAGONIST ACTIVITIES OF PEPTIDES RELATED TO 26RFA, THE LAST MEMBER OF THE RFAMIDE PEPTIDE SUPERFAMILY

O. Le Marec[1], C. Dubessy[1], J.C. Do-Régo[2], O. Nosjean[3], V. Audinot[3], J.A. Boutin[3], N. Chartrel[1], L. Guihlaudis[4], I. Segalas-Milazzo[4], D. Davoust[4], M. Tena-Sempere[5], J. Costentin[2], H. Vaudry[1], M.C. Tonon[1], **J. Leprince**[1]

[1]Inserm U413, Laboratory of Cellular and Molecular Neuroendocrinology, European Institute For Peptide Research (IFRMP 23), University of Rouen, Mont-Saint-Aignan [2]CNRS FRE 2735 Laboratory of Experimental Neuropsychopharmacology, European Institute for Peptide Research (IFRMP 23), University of Rouen [3]Department of Cellular and Molecular Pharmacology, Servier Research Institute, Croissy-sur-Seine [4]CNRS UMR 6014, Laboratory of Nuclear Magnetic Resonance, European Institute for Peptide Research (IFRMP 23), University of Rouen, Mont-Saint-Aignan, France [5]Physiology Section, Faculty of Medicine, University of Cordoba, Spain

26RFa, a novel neuropeptide of the RFamide superfamily characterized in our laboratory, is the endogenous ligand of the orphan receptor GPR103. Consistant with the hypothalamic expression of the peptide and its receptor, icv injection of 26RFa induces a potent orexigenic effect in mouse. Recently, it has been shown that GPR103-deficient mice exhibit a kyphotic hump and suffer from osteopenia. Analysis of the human 26RFa (h26RFa) precursor indicates that it may generate several additionnal RFa-peptides including an N-terminal extended form (43RFa), a truncated form (26RFa(20-26)) and a 9-amino acid peptide located upstream of 43RFa (9RFa). Molecular modeling under 1H-NMR constraints of h26RFa shows that the N-terminal part encompasses an □-helix. The aim of this work was to study the Ca2+-mobilizing activity of human and rodent 26RFa-related peptides in a CHO cell line over-expressing the human GPR103. Human and rodent 26RFa and 43RFa induced a dose-dependent increase in [Ca2+]i with nanomolar potencies. Conversely, 26RFa(20-26) and 9RFa were about 400 times less potent than 26RFa, suggesting that these peptides are not endogenous ligands of GPR103. Despite their well-defined conformation within the native peptide, the human and rodent N-terminal fragments were devoid of agonistic and antagonistic activities indicating that the C-terminal part is of paramount importance for receptor activation. These data constitute the first step toward the development of new GPR103-analogs that could prove useful for the treatment of feeding or bone disorders. **Supported by Inserm (U413) and the Conseil Régional de Haute-Normandie. O.L.M was recipient of a fellowship from MEN.**

Tu293

CONJUGATION OF A CYCLIC RGD DERIVATIVE TO BRANCHED CHAIN POLYMERIC POLYPEPTIDE: SYNTHESIS AND BIOLOGICAL STUDY OF AK-(CYCLO[RGDFC])

M. Ligeti[1], G. Mező[1], K. Markó[2], E. Madarász[2], F. Hudecz[1,3]

[1]Research Group of Peptide Chemistry, Eötvös L. University [2]Laboratory of Neural Cell Biology, Institute of Experimental Medicine, Hungarian Academy of Sciences [3]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

The interaction of □v□3 integrin receptor with its ligands is selectively implicated in various processes, like angiogenesis, bone-formation, tumor genesis and tissue-genetic migration of embryonic cells. Several cyclic RGD pentapeptides are known as selective ligands for □v□3 integrin receptor. The aim of this study was to prepare a new conjugate, composed of the cyclo[RGDfC] derivative and a branched chain polycationic polypeptide, poly[Lys(DL-Alam)] (AK). The cyclopeptide was prepared on 2-Cl-trityl chloride resin by Fmoc/tBu strategy. The "head-to-tail" cyclisation was achieved in a diluted solution of DMF in the presence of BOP and HOBt coupling reagents and DIEA base. Coupling of the cyclopeptide to AK polymer was carried out by thioether linkage.

Adhesion properties of soluble cyclic RGD peptides and their plate-immobilized forms were studied. Free cyclopeptides evoke aggregation of cultured primary neural and cloned neural stem cells, while their plate-immobilized forms fail to support cell adhesion. On the contrary, in case the newly synthesized AK-c(RGDfC) conjugate such induction of cell aggregation was not observed. Whereas immobilizing this derivative to either glass, or plastic was found to support cell-attachment in case of various cell types. In addition, all cell lines investigated - including also the primary neural cells - attached to AK-c(RGDfC) coated surface and survived, grew or differentiated even in the absence of serum. Our data suggest that cyclic RGD - polypeptide conjugates represent a new tool to investigate selective cell adhesion and may provide a novel scaffold-material for directed cell-seeding.

These studies were supported by OTKA T038456.

STRUCTURAL BASIS FOR PIP2 REGULATIONS OF PH-SENSITIVE GATING IN ROMK1 CHANNELS

K.-L. Lou[1], J.-X. Li[2], P.-T. Huang[1], H.-H. Liou[2]

[1]Biochemistry Department [2]Pharmacology Department, NTUMC, Taipei, Taiwan-R.O.C.

ROMK1 (Kir1.1) channels are widely distributed in different tissues and regulate many important cellular processes, including the membrane resting potentials, cell and synaptic excitability, and the renal K+ transport. Kir1.1 is mediated by intracellular pH (pHi), cAMP-dependent protein kinase (PKA) and phosphatidylinositol 4, 5-bisphosphate (PIP2). Previous studies have suggested the crucial roles of several intracellular N- and C-terminal basic residues in the pH-induced channel closure in combination with the PIP2 interactions. However, their detailed regulations are still remaining unclear. Therefore, in the present study, we investigate these crucial residues with electrophysiological recordings and rationally designed mutagenesis based upon our structural analysis of Kir1.1 tetramer. Lys-80 is located fairly close to the intracellular channel gate and protrudes its long side chain positive charge into the pore. This may interfere with the potassium flow by providing repulsion charge while pH is lowered, which pushes the channel towards its closed state. Mutation to Met-80 therefore reduces such pH-sensitivity. On the other hand, Arg-188 is supposed to be responsible for the maintenance of channel opening in the presence of PIP2. Loss of positive charge at this site may lead to the enhanced pH-sensitivity due to an abolished or reduced PIP2 interaction. More interestingly, the double mutant for both sites reveals a compensation scenario. In combination with the discussion for the role of previously known R-K-R triad, our data provide very clear structural explanation for the exact functional roles of these basic residues in the regulation of pH-sensitive channel gating.

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FEEDING-RELATED EFFECTS OF CART (COCAINE AND AMPHETAMINE REGULATED TRANSCRIPT) PEPTIDES AND CHOLECYSTOKININ IN MOUSE OBESE MODELS

L. Maletínská[1], J. Maixnerová[1], R. Shamas Toma[1], R. Haugvicová[3], J. Slaninová[1], B. Železná[2]

[1]Institute of Organic Chemistry and Biochemistry [2]Institute of Molecular Genetics [3]Institute of Microbiology, Academy of Sciences of The Czech Republic, Prague, Czech Republic

CART peptides are neurotransmitters involved in feeding, stress and endocrine regulation. Leptin, a long-term adiposity signal, upregulates expression of CART in the hypothalamus. Recent findings of co-localization of CART and cholecystokinin (CCK)-A receptor (responsible for satiety effect of CCK) in brain and gastrointestinal tract suggest a neurochemical link between CART peptides and CCK.

In normal fasted mice, CART(61-102) peptide decreased food intake after intracerebroventricular (ICV) administration in a dose-dependent manner. Anorectic effect of CART peptide was enhanced by peripherally administered CCK-8, while CCK-A receptor antagonist, devazepide blocked the effect of CART peptide on food intake.

We used two mouse obesity models in this study: monosodium glutamate (MSG) and diet-induced obese (DIO) C57Bl mice. Both DIO and MSG mice had substantially increased fat to body mass ratio compared to their controls and were hyperleptinemic. MSG mice were hypophagic and neither CART peptide nor CCK-8 and devazepide had any effect on food intake of these mice. DIO mice fed high-fat diet showed slightly decreased sensitivity to central administration of CART peptide, effect of CCK-8 on food intake was preserved.

In conclusion, CART peptide and CCK-8 showed a synergistic effect on feeding in control mice that pointed to their probably integrated action in the central nervous system. Analogously, devazepide suppressed CART anorectic effect. In MSG obese mice, effects of both CART peptide and CCK-8 on food intake were diminished due to disrupted signaling in hypothalamus. In DIO mice, additive effects of CART and CCK-8 were partly preserved inspite of hyperleptinemia and increased adiposity.

THE FIRST BIVALENT LIGANDS FOR OXYTOCIN AND VASOPRESSIN RECEPTORS

B. Chini[1], S. Stoev[2], L.L. Cheng[2], **M. Manning**[2]

[1]CNR Cellular and Molecular Pharmacology Section, Institute of Neuroscience, Milan, Italy [2]Department of Biochemistry and Cancer Biology, Medical University of Ohio, Toledo, OH, USA

The bivalent ligand approach has been an invaluable tool for the design of potent and selective agonists and antagonists for receptors of a wide variety of biologically active peptides and non-peptides [1-3]. Remarkably, to-date, this approach has not been utilized in the neurohypophysial peptide design field [4 and references therein]. We now report the syntheses and some preliminary pharmacological properties of the first bivalent ligands for the human (h) oxytocin (OT) and vasopressin VP (V1a) receptors. Suberic acid (X), utilized as reported in [1], served as the spacer joining Lys or Orn residues in an OT agonist, dLVT(A), an OT/V1a antagonist, d(CH2)5Tyr(Me)2OVT (B) and two linear V1a/OT antagonists, HO-Phaa-D-Tyr(Me)Phe-Gln-Asn-Arg-Pro-Lys-NH2 (C) and HO-Phaa-D-Tyr(Me)Phe-Gln-Asn-Arg-Arg-Lys-NH2 (D). The resulting dimers: XA2, XB2, XC2 and XD2 exhibit high affinities, in the nanomolar range, for the hOTR expressed in etherologous cell systems. These findings point to the promise of the bivalent ligand approach for the design of selective OT and VP ligands.

[1] Gera, L., Stewart, J.M, Whalley, E., Burkard, M., Zuzack, J.S. Immunopharmacology 33, 178 (1996). [2] Halazy S, Expert Opinion on Therapeutic Patents 9, 431 (1999). [3] Daniels D.J., Lenard, N.R., Etienne,C.L., Law, P.Y., Roering, S.C., Portoghese, P.S., Proc. Natl. Acad. Sci. USA 102, 19208 (2005). [4] Manning, M. in "Peptides 2004" Flegel, M., Fridkin, M., Gilon, C., Slaninova, J., eds. Kenes International Geneva 101 (2005).

SELECTIVE AGONISTS FOR HUMAN AND RAT VASOPRESSIN V1B RECEPTORS

G. Guillon[1], B. Murat[1], A. Pena[1], M. Trueba[1], M.A. Ventura[2], N.C. Wo[3], H.H. Szeto[3], S. Stoev[4], L.L. Cheng[4], **M. Manning**[4]

[1]Institut de Génomique Fonctionnelle, UMR5203-CNRS, U661-INSERM, Université Montpellier I & II, Montpellier [2]Institute Cochin, Paris, France [3]Department of Pharmacology, Weill Medical College of Cornell University, New York, NY [4]Department of Biochemistry and Cancer Biology, Medical University of Ohio, Toledo, OH, USA

We recently reported a breakthrough in the search for vasopressin (VP) agonists which are selective for the human V1b (pituitary) receptor with respect to the V1a (pressor), V2 (renal) and OT (oxytocin) receptors [1]. This was accomplished by replacements of the Gln4 residue in [deamino] arginine-vasopressin (dAVP). The resulting peptides, d[Cha4]AVP (A), d[Leu4]AVP (B), d[Orn4]AVP (C) and d[Arg4]AVP (D) [1], were subsequently shown not be selective for the rat V1b receptor [2]. Peptides A-D served as excellent leads to the design of selective agonists for the rat VP V1b receptor [3]. Replacement of the Arg8 residue in A-D by Lys, Orn, Dap and Dab, led to the first potent and selective agonists for the rat V1b receptor [3]. We now report that three of these; d[Cha4,Dab8]VP, d[Leu4,Dap8]VP and d[Leu4,Lys8]VP, are the first ligands which exhibit potent and selective agonism for both the human and the rat VP V1b receptors. [1] Cheng, L.L., Stoev, S., Manning, M., Derick, S., Pena, A., Ben Mimoun, M., Guillon, G. J. Med. Chem. 47, 2375 (2004). [2] Guillon, G., Pena, A., Murat, B., Derick, S., Trueba, M., Ventura, M.A., Szeto, H.H., Wo, N.C., Stoev, S., Cheng, L.L., Manning, M. J. Pep. Sci. (in press) epublication (August 30, 2005). [3] Manning, M., Cheng, L.L., Stoev, S., Wo, N., Szeto, H.H., Pena, A., Murat, B., Trueba, M., Ventura, M.A., Guillon, G. in "Understanding Biology Using Peptides" (Ed. S.E. Blondelle), American Peptide Soc. 2005 (in press).

STRUCTURE ACTIVITY STUDIES ON HIGHLY POTENT VASOPRESSIN HYPOTENSIVE PEPTIDE

L.L. Cheng[1], S. Stoev[1], **M. Manning**[1], N.C. Wo[2], H.H. Szeto[2]

[1]Department of Biochemistry and Cancer Biology, Medical University of Ohio, Toledo, OH [2]Department of Pharmacology, Weill Medical College of Cornell University, New York, NY, USA

Besides its well known antidiuretic, vasopressor, ACTH releasing and uterine contracting effects, mediated respectively by the V2, V1a, V1b and OT (oxytocin) receptors, vasopressin (VP) also causes vasodilation (for reference see 1). We serendipidously discovered the first analog of VP, d(CH2)5[D-Tyr(Et)2,Arg3,Val4]AVP (A), which exhibits selective hypotensive activity and is devoid of the characteristic activities of VP [1]. We recently reported that the D-Tyr(iPr)2, Lys7, Lys8, Eda9 (ethylenediamine) analog of (A), d(CH2)5D-Tyr(iPr)2, Arg3,Val4,Lys7,Eda9]LVP (B) exhibits a 30-fold increase in hypotensive activity relative to (A) [2]. (B) has the following structure: (CH2)5C(S) CH2-CO1-Tyr(iPr)2-Arg3-Val4-Asn5-Cys6-Lys7-Lys8-Eda9 (with a disulphide bridge between positions 1 and 6). In this study, we investigated the effects of modifications of (B) at positions 3 (replacing Arg with Lys and Nar), 4 (replacing Val with Abu, Nva, Leu, Nle, Cha, Thr and Har), 3 and 9 (replacing Arg and Eda with Nar and 1-amino, 2-guanadinoethane (EdaG) respectively and at positions 7 and 10 (replacing Lys and Eda with Arg and Eda retro Tyr respectively). We now report that, with few exceptions, all changes were well tolerated. The Cha4 and the Nar3, EdaG9, analogs of (B) exhibit almost full retention of hypotensive activity. **[1] Chan, W.Y., Wo, N.C., Stoev, S., Cheng, L.L., Manning, M. Br. J. Pharmacol. 125, 803 (1998). [2] Stoev S, Cheng, L.L., Manning, M., Wo, N.C., Szeto, H.H. J. Peptide Science (in press).**

Tu299

INTERACTION OF HEME- AND CHLOROPHYLL-BASED COFACTORS WITH DE NOVO SYNTHESIZED PEPTIDES

A. Mennenga, M. van Gastel, H. Görner, I. Heise, W. Gärtner, W. Lubitz

Max Planck Institute for Bioinorganic Chemistry, Mülheim an der Ruhr, Germany

The aim of the de novo peptide synthesis and the incorporation of cofactors is the construction of artificial protein models. These model systems can be used for understanding the structure-function relationship of native proteins and might open a way for possible applications.

For the current project, de novo-peptides, containing 62 amino acids, were synthesized with a Multiple Peptide Synthesizer using Fmoc-protected amino acids on a PAL-PEG-PS resin. The design of the peptides is based on the heme binding sites of the cytochrome b subunit of cytochrome bc1, originally designed by DeGrado, Dutton and coworkers [Nature, 1994]. The purification and identification of the water-soluble peptides were done by HPLC and MALDI-TOF-MS. With UV-Vis- and fluorescence spectroscopy the interactions of the heme and chlorophyll derivatives with the peptides were investigated and circular dichroism studies gave information about the stability of the peptides. The coordination number of the cofactors was studied by Resonance Raman spectroscopy. Redox titrations of the heme-peptide complex showed two redox potentials of Em(7.0) = -92 mV and Em(7.0) = -192 mV vs NHE. Initiated by the work of Razeghifard et al. [Biochemistry, 2003], an electron transfer could be observed by time resolved EPR and transient absorption spectroscopy between the chlorophyll-based cofactors and a noncovalently bound quinone. The interaction between both cofactors was demonstrated from longer triplet lifetimes under quenching conditions, selectively in cases, when the pigments are bound to the peptide and not for a mixture of both cofactors in solution, indicating a protective envelope created by the protein.

Th300

CONFORMATIONAL SOLUTION STUDIES OF ANTIMICROBIAL PROTEGRIN-1 ANALOGUES BM-1 AND BM-2 BY USING NMR SPECTROSCOPY

B. Mickiewicz[1], W. Kamysz[2], K. Greber[2], S. Rodziewicz-Motowidlo[1]

[1]Faculty of Chemistry, University of GdańSk [2]Department of Pharmacy, Medical University of GdańSk, Poland

Protegrin-1 (PG-1) is an 18-amino acid peptide with an amidated C-terminus, which forms an antiparallel beta-sheet, constrained by two disulfide bridges. The native sequence of PG-1 is highly cationic, containing six positively charged arginine residues. It was found that the structural features such as amphiphilicity, charge and shape are important for the cytolitic activity of PG-1. In this study we investigate the SAR (Structure Activity Relationship) of two PG-1 analogues: RGLCYCRGRFCVCVG-NH2 (BM-1) and RGLCYRPRFVCVG-NH2 (BM-2). Our antimicrobial activity studies of these peptides show that the BM-1 peptide is active against microbe species as well as the native PG-1, whereas the BM-2 is completely inactive. The BM-1 analoque is shorter than native PG-1 and contains only three arginine residues, therefore is much cheaper in the chemical synthesis, what could be an advantage of this antimicrobial peptide.
The conformational studies of both analogues were performed by using 2D 1H-NMR technique (in DMSO-d6) and molecular dynamics studies. The 3D solution structure of both analogues was established using interproton distances and torsion angles. For simulated annealing calculations the XPLOR program was used. Our conformational studies show that the BM-1 forms a regular beta-hairpin structure, which is very similar to that of the native PG-1 peptide, whereas the BM-2 analogue is very flexible, what could be a reason of the antimicrobial inactivity.

Acknowledgments:
This work was financially supported by the Ministry of Scientific Research and Information Technology of Poland DS 8372-4-0138-6. B.M. thanks to EFS project ZPORR/2.22/II/2.6/APR/U/2/05.

M301

THE KINETIC STUDY ON THERMAL DENATURATION OF LENTIL SEEDLING AMINE OXIDASE

S.Z. Moosavi-Nejad[1], M. Seifi[1], A.A. Moosavi-Movahedi[2], G. Floris[3]

[1]Department of Biology, Faculty of Science, Alzahra University [2]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran [3]Department of Applied Sciences in Biosystems, University of Cagliari, Italy

Copper amine oxidases (EC 1.4.3.6) catalyze the oxidative deamination of primary amines to the corresponding aldehydes, ammonia and hydrogen peroxidase. These enzymes are ubiquitous, occurring in micro-organisms, plant and animals. Activity of this enzyme increases under various stress conditions including thermal and water stresses. Although LSAO is not a thermostable enzyme, it is in maximum stability and activity above physiological temperatures.

In this study we have investigated the kinetics of thermal denaturation of lentil seedling amine oxidase (LSAO) by measuring its denaturation constant (kden) at various temperatures from 37 to 67 degrees centigrade in 100 mM phosphate buffer, pH 7.0. The results of thermal inactivation curves as well as measuring of A280 at various temperatures were used to calculate kden. Moreover, activation energy (Ea) for denaturation reaction was obtained from corresponding Arrhenius plot. Our results showed that unfolding process started to occur at 56 degree centigrade and Ea of denaturation was changed at 65 degree centigrade proving a dominant conformational change of the enzyme at this temperature. The results of the kinetic study are coincident with previously reported equilibrium studies denoting the optimum and melting temperature of the enzyme are 56 and 65 degree centigrade, respectively.

Tu302

ENZYMATIC DEPOLYMERIZATION OF CHITIN AND CHITOSAN

V.Y. Novikov

Laboratory of Biochemistry and Technology, Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO), Murmansk, Russia

Development and advancing of enzymatic processes used for production and modification of natural polysaccharides are now major biochemistry challenges. The paper investigates enzymatic systems in invertebrates, in particular, an enzymatic complex obtained from the hepatopancreas of red king crab Paralithodes camtschaticus, and clarifies its effect on the mechanism of chitin and chitosan hydrolysis.

Chitinolytic activity was estimated with spectrophotometer using 4-(dimemylamine)-behzaldehyde method by the concentration of N-acetyl-D-glucosamine which is educed under chitinolysis. Total glycolytic activity was defined by the sum of N-acetyl-D-glucosamine and D(+)-glucosamine in the reaction with potassium hexaferricyanide (III). Content of D(+)-glucosamine in the hydrolysates of chitin and chitosan was estimated by highly effective reverse-phase liquid chromatography (HELC) of aminosaccharides with ortho-phthalaldehyde.

The paper studies the process of chitin and chitosan glycolysis and the effects of different factors (pH, temperature and time of incubation, enzyme/substrate ratio) on the total glycolytic activity of the enzymatic complex from crab hepatopancreas, which is compared with a previously studied proteolytic and exochitinase activities. A mechanism of enzymatic hydrolysis of chitin and chitosan is suggested.

Study results allowed the following conclusions concerning glycolytic and deacetylase activity of EP:

1) EP induces the formation of a monomer (N-acetyl-D-glucosamine) and oligomers (chitin and chitosan) with low deacetylation. Thus, EP is characterised by a marked endochitinase (endochitosanase) activity;

2) N-acetylglucosamine deacetylase and, apparently, exochitosanase activity was not revealed;

3) It was found that chitinase and protease activities of EP are associated with different **enzymes.**

DEVELOPMENT OF UNIQUE AND POTENT μ-OPIOID AGONISTS AND ANTAGONISTS CONTAINING DMT

Y. Okada[1,2], T. Li[2], A. Miyazaki[1], Y. Fujita[2], K. Shiotani[2], Y. Tsuda[1,2], T. Yokoi[1,2], A. Ambo[3], Y. Sasaki[3], Y. Jinsmaa[4], S.D. Bryant[4], E. Marczak[4], Q. Li[5], H.S. Swarztwelder[6]

[1]Faculty of Pharmaceutical Sciences [2]The Graduate School of Food and Medicinal Sciences, Kobe Gakuin University, Nishi-ku [3]Department of Biochemistry, Tohoku Pharmaceutical University, Aoba-Ku, Sendai, Japan [4]Medicinal Chemistry Group, LPC, National Institute of Environmental Health Sciences, Research Triangle Park, NC [5]Department of Pharmacology and Cancer Biology and 6Department of Psychiatry, Duke University Medical Center [6]Neurobiology Research Laboratory, VA Medical Center, Durham, NC, USA

[Background] In opioids, the N-terminal amino acid 2',6'-dimethyl-L-tyrosine (Dmt) enhances bioactivity by orders of magnitude. C-Terminal modification of the Dmt by a methyl group, H-Dmt-NH-CH3, exhibited μ-opioid receptor affinity (Kiμ = 7.5 nM) equivalent to that of morphine; however, antinociception was only 0.64-0.85% [1]. Dmt plays an important role in the message domain to anchor opioid ligands into the active site of opioid receptors, specifically to trigger biological activity by the μ-opioid receptor.

[Methods] Dimerization of Dmt through diaminoalkanes [2] or 3,6-bis- (aminoalkyl)-2(1H)- pyrazinone produced potent opioidmimetics with high affinity for μ-opioid receptors (Kiμ = 0.02–0.115 nM), agonism (GPI, IC50 = 1.3–1.9 nM), and antinociception in mice after systemic and oral administration, which verified passage through the epithelial membranes of the gastrointestinal tract and blood-brain barrier [3]. [Results and Conclusion] Furthermore, tail-to-tail condensation of the Dmt-Tic pharmacophore with these linkers yielded potent δ-opioid receptor antagonists that exceeded that of the prototype (H-Dmt-Tic-OH) by orders of magnitude (pA2 = 10.30-11.22) and N,N-dimethylation produced potent μ-opioid antagonists (pA2 = 8.34) . In addition, N-allylation converted the potent μ-opioid agonists [Dmt1]endomorphin-1 and -2 into μ-opioid antagonists (pA2 = 8.18, 8.59) with potential application for the alleviation of alcohol dependency.

[1] Fujita, Y., et al. (2005), Bioorg. Med. Chem. Lett., 15, 599-602.
[2] Okada et al. (2003) J. Med. Chem. 46, 3509-3516.
[3] Jinsmaa, Y., et al. (2004) J. Med. Chem., 47, 2599-2610.

M304

NEW BRADYKININ ANALOGUES MODIFIED WITH 1-AMINOCYCLOPENTANE-1-CARBOXYLIC ACID

O. Labudda-Dawidowska[1], D. Sobolewski[1], M Śleszyńska[1], I. Derdowska[1],
J. Slaninowa[2], T. Wierzba[3], **A. Prahl**[1]

[1]Department of Chemistry, University of GdańSk, Poland [2]Department of Biological Chemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic, Prague, Czech Republic [3]Department of Phisiology, Medical Academy of GdańSk, Poland

In the present work, sterically constrained non-coded amino acid, 1-aminocyclopentane-1-carboxylic acid (Apc), was substituted in positions 7 or 8 of its B2 receptor antagonist [D-Arg0,Hyp3,Thi5,8,D-Phe7]BK, previously synthesized by Stewart's group. The Apc should reduce the flexibility of the peptide backbones by restricting conformational freedom. This modification is an example of the Cα- Cα cyclization whereby a dialkylated glycine residue is converted into a cyclic side chain (1-aminocycloalkane-1-carboxylic acid). In this case the ring consists of five atoms. Knowing that acylation of the N-terminus of several known B2 blockers with a variety of bulky groups has consistently improved their antagonistic potency in the rat blood pressure assay, the Apc substituted analogues were also synthesized in N-acylated form (with 1-adamantaneacetic acid (Aaa)). The activity of eight new analogues was assayed in isolated rat uterus using a modified Holton method in Munsick solution and in rat blood pressure tests. The results clearly demonstrated the importance of the position in the peptide chain into which the sterically restricted Apc residue was inserted. Apc at positions 7 led to preservation or reduction of antagonistic qualities, respectively. Acc at position 8 enhanced antagonistic qualities in blood pressure test and led to preservation of activity in antiuterotonic test.. In most cases acylation of the N-terminus led to enhancement of antagonistic potencies. Our findings offer new possibilities for designing new potent and selective B2 blockers.

THE ROLE OF 2',6'-DIMETHYL-L-TYROSINE (DMT) FOR MANIFESTATION OF HIGH MU-OPIOID RECEPTOR BINDING AFFINITY IN OPIOIDMIMETICS

K. Shiotani[2], T. Li[2], A. Miyazaki[1], Y. Fujita[2], Y. Tsuda[1,2], T. Yokoi[1,2], A. Ambo[3], Y. Sasaki[3], Y. Jinsmaa[4], S.D. Bryant[4], E. Marczak[4], L.H. Lazarus[4], Y. Okada[1,2]

[1]Department of Medical Chemistry, Faculty of Pharmaceutical Sciences [2]The Graduate School of Food and Medicinal Sciences, Kobe Gakuin University [3]Department of Biochemistry, Tohoku Pharmaceutical University, Sendai, Japan

[4]Medicinal Chemistry Group, LPC, National Institute of Enviromental Health Sciences, Research Triangle Park, NC, USA

Background: During the course of developing opioidmimetic analgesics, data revealed that the N-terminal residue 2',6'-dimethyl-L-tyrosine (Dmt) plays an important role in anchoring opioid ligands in the active site of opioid receptors. As a single residue C-terminally extended with an aminomethyl group exhibited μ-opioid receptor affinity (Kiμ = 7.5 nM) similar to morphine; however, antinociception was only 0.64-0.85% [1]. In order to develop potent μ-opioid agonists, the dimerization of Tyr or Dmt through diaminoalkanes [2] or 3,6-bis-(aminoalkyl)-2(1H)-pyrazinones [3] resulted in production of unique opioidmimetics with high receptor affinities and potent biological activities [3].
Methods: The synthesis of opioids and opioidmimetics and the determination of their receptor binding characteristics were performed as described previously [1-3].
Results and Conclusion: Newly synthesized 3-(Tyr-NH-butyl)-6-(Tyr-NH-propyl)-2(1H) pyrazinone and 3-(Tyr-NH-propyl)-6-(Tyr-NH-butyl)-2(1H) pyrazinones (I and II) exhibited fairly high binding affinity towards μ-opioid receptor (Kiμ = 7.6 and 27.4 nM, respectively). Replacement of Tyr with Dmt in I and II gave opioidmimetics III and IV (Kiμ = 0.021 and 0.051 nM, respectively); they exhibited 361- and 537-fold higher binding affinity than the Tyr derivatives. While III is a dual μ-/δ-opioid agonist, IV is only a μ-opioid agonist. These findings pave the way to design additional μ-opioid receptor agonists and antagonists for therapeutic application.

[1] Fujita, et al. (2005) Bioorg. Med. Chem. Lett., 15, 599-602.
[2] Okada, et al. (2003) J. Med. Chem. 46, 3509-3516.
[3] Jinsmaa, et al. (2004) J. Med. Chem., 47, 2599-2610.

OXYTOCIN, DIVALENT CATIONS AND MECHANISM OF ACTION

J. Slaninová[1], L. Borovicková[1], L. Maletinská[1], T. Elbert[1], J. Hlavácek[1], J. Cvacka[1]

[1]*Institute of Organic Chemistry and Biochemistry, Academy Science Czech Republic, Prague, Czech Republic*

Divalent cations have been known for a long time to influence significantly binding to receptors and biological activity of the peptide oxytocin (OT). There is very low binding of 3H-OT to the receptors in the absence of these ions. It has been speculated where the divalent cations work. Recently an article appeared showing formation of a complex divalent cation-OT and stressing the importance of N-terminal amino group for binding and activity [1]. However deamino analogues of OT are also very active and their binding is also influenced by divalent cations. We have studied OT, deaminooxytocin (dOT) and an OT antagonist (Antag) by means of electrospray MS and we have observed that all these compounds form molecular adducts with Zn2+, Mg2+, Mn2+ and Ca2+. In binding experiments using 125-I Antag, the quantity of tracer bound to membranes of HEK cells having stable expressed human OT receptor strongly depends on the character and concentration of divalent ions. Displacement curves using unlabelled Antag do not change in the absence or presence of 10 mM of tested divalent ions. On the other hand, displacement curves using unlabelled OT and dOT are shifted to the left in the presence of Mg2+ and Mn2+, and to much lesser extent by Zn2+ and Ca2+. All this points to the idea that the divalent ions do work on the site of membrane receptors.

[1] D. Liu et al., JACS, 127, 2024-2025 (2005).

The work was supported by the research project No. Z40550506 of the Acad. Sci. Czech Rep.

M307

THE EFFECTS OF N-TERMINAL MODIFICATION OF ARGININE VASOPRESSIN (AVP) ANALOGUES WITH 2-AMINOINDANE-2-CARBOXYLIC ACID. HIGHLY POTENT V2 AGONISTS

W. Kowalczyk[1], **D. Sobolewski**[1], A. Prahl[1], I. Derdowska[1], A. Kwiatkowska[1], L. Borovičková[2], J. Slaninová[2], B. Lammek[1]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic, Prague, Czech Republic

In this study we present the synthesis and some pharmacological properties of nine new analogues of arginine vasopressin (AVP) modified in the N-terminal part of the molecule with 2-aminoindane-2-carboxylic acid (Aic). All the peptides were tested for their in vitro pressor, antidiuretic and uterotonic activities. The substitution of position 2 with 2-aminoindane-2-carboxylic acid, compared to the previously studied modification using 1-aminocyclopentane-1-carboxylic acid (Apc), did not result in any dramatic change of activity. The enlargement of the cyclic side chain moiety, restoring its aromatic character from the aliphatic one, however placing the aromatic ring closer to the backbone led either to retention or a slight decrease of antidiuretic activity. All the analogues had prolonged action. One of the new peptides, namely [Mpa1, Aic2, Val4] AVP, exhibits an antidiuretic activity similar to that of dDAVP, thus being one of the most potent V2 agonists reported to date. The remaining five new peptides substituted at position 3 are moderately potent antidiuretic agents with prolonged activities. These analogues also exhibit different range of antioxytocin activity which is enhanced as compared to that of the Apc analogues. Summing up, our studies provide new useful information about structure – activity relationships and open new possibilities for designing potent V2 agonists.

Tu308

INFLUENCE OF CONFORMATIONALLY CONSTRAINED AMINO ACIDS AT POSITIONS 2 AND 3 OF ARGININE VASOPRESSIN (AVP) AND ITS ANALOGUES ON THEIR PHARMACOLOGICAL PROPERTIES

D. Sobolewski[1], A. Prahl[1], I. Derdowska[1], A. Kwiatkowska[1], J. Slaninová[2], B. Lammek[1]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic, Prague, Czech Republic

Biologically active peptides exhibit multiple conformations in solution. Thus, the synthesis of conformationally restricted analogues is a valuable approach for determining structure – activity relationships. Restrictions can be imposed e.g. through the formation of cyclic structures within the peptide framework by disulfide bridges, or by substitution of chosen amino acid residues that limit conformational freedom, thus forcing the peptide backbone and/or side chains to adopt specific orientations.

The present work is a part of our studies aimed at clarifying the influence of conformational constraints in the N-terminal part of arginine vasopressin (AVP) and its analogues on pharmacological activity of the resulting peptides. We describe the synthesis of thirteen new peptides. First three compounds were designed by substitution of positions 2 and 3 of AVP with 4-aminobenzoic acid (Abz), cis-4-aminocyclohexanecarboxylic acid (ach) or its trans-isomer (Ach), while the next analogues had the above-mentioned modifications at the same positions of [3-mercaptopropionic acid (Mpa)1]AVP, [Mpa1,Val4]AVP and [Mpa1,Val4,D-Arg8]VP. The last compound was designed by substitution of positions 2 and 3 of a potent V1a antagonist, [1-mercaptocyclohexaneacetic acid (Cpa)1]AVP, with Abz. Unfortunately, all new peptides were inactive in bioassays for the pressor, antidiuretic and uterotonic in vitro activities in the rat.

Th309

ANALOGUES OF ARGININE VASOPRESSIN (AVP) MODIFIED IN THE N-TERMINAL PART OF THE MOLECULE WITH STEREOISOMERS OF 4-AMINOPYROGLUTAMIC ACID

D. Sobolewski[1], A. Prahl[1], I. Derdowska[1], J. Slaninová[2], K. Kaczmarek[3], J. Zabrocki[3], B. Lammek[1]

[1]Faculty of Chemistry, University of GdańSk [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic, Prague, Czech Republic [3]Institute of Organic Chemistry, Technical University of ŁÓdź, Poland

In recent years, conformationally constrained analogues of bioactive peptides seem to be a feasible approach to providing useful informations concerning three-dimensional structure of such compounds which, in turn, could rationalize our knowledge about structure-biological activity relationships and thus help to design peptides with desired pharmacological properties. Steric restrictions can be introduced by the formation of cyclic structures within the peptide backbone or by incorporation of amino acids with limited conformational freedom, which in turn results in specific orientations of the peptide backbone and its side chains. Another approach to reduce the flexibility of the analogue is substitution of chosen amino acids with various types of pseudopeptides prepared trough short-range cyclizations. **The present work is a part of our studies aimed at clarifying the influence of sterical constraints in the N-terminal part of arginine vasopressin (AVP) and its analogues on the pharmacological activity of the resulting peptides. We describe the synthesis of four new analogues of AVP substituted at positions 2 and 3 or 3 and 4 with two diastereomers of 4-amino-pyroglutamic acid and four peptides in which we combined the above modification with the placement of 3-mercaptopropionic acid (Mpa) at position 1. All the peptides were tested for their in vitro uterotonic, pressor and antidiuretic activities in the rat.**

M310

DIFFERENT STRATEGIES TO MODULATE SHP-1 ACTIVITY

K. Teichmann, K. Hampel, D. Imhof

Friedrich-Schiller-University, Institute of Biochemistry and Biophysics, Jena, Germany

Protein tyrosine phosphatase SHP-1 consists of two SH2 domains N-terminal to the catalytic (PTP) domain and a short C-terminal tail. The binding of a pY-ligand to the N-SH2 domain is required for an efficient activation of SHP-1 phosphatase activity. The specificity of the SHP-1 SH2 domains is determined by the pY-residue (position 0) and residues at positions -2, +1 and +3. Combinatorial peptide library methods revealed different classes of consensus sequences for both SH2 domains [1,2]. In addition, the importance of residues C-terminal to pY+3 (+4 to +6), in particular for binding to the N-SH2 domain, has been demonstrated [3]. Together with investigations of the determinants for optimal SH2 domain binding and stimulation/inhibition of SHP-1 activity [4], these informations were useful for the generation of different strategies for effectors of SHP-1 activity. Peptides cyclized between different positions of the general consensus pY-2 to pY+3 were synthesized and evaluated with respect to N-SH2 domain binding and stimulation of phosphatase activity.

[1] Beebe, K.D., et al., Biochemistry 2000, 39, 13251-13260.
[2] Sweeney, M. C., et al., Biochemistry 2005, 44, 14932-14947.
[3] Imhof, D., et al., J. Biol. Chem., submitted.
[4] Imhof, D., et al., J. Med. Chem. 2005, 48, 1528-1539.

STRUCTURE-ACTIVITY STUDIES OF RGD-CONTAINING PEPTIDES DERIVED FROM ADHESIVE PROTEINS. APPLICATION OF STRUCTURAL CRITERIA EVALUATED FROM BIOACTIVE SYNTHETIC RGD PEPTIDE ANALOGUES

S. Kostidis[1], E. Thanou[1], D. Tsoukatos[1], M.T. Cung[2], **V. Tsikaris**[1]

[1]Department of Chemistry, University of Ioannina, Ioannina, Greece [2]Laboratory of Macromolecular Physical Chemistry, ENSIC-INPL, Nancy, France

Structure-activity studies have revealed that the specificity of an integrin towards its RGD-containing ligands can be evaluated through the distances between the Cβ atoms and/or the distance between the charged centers of arginine and aspartic acid as well as, the pseudo-dihedral angle (pdo), composed by the R-Cζ, R-Cα, D-Cα and D-Cγ atoms, which defines the relative orientation of the Arg and Asp side chains. In a previous study [1], the antiaggregatory activity of RGD peptide analogues, i.e. their ability to act as fibrinogen receptor αIIbβ3 antagonists, was correlated with the above structural criteria. Our results suggested that the fulfillment of the criterion -45o < pdo < +45o is a prerequisite for an analogue to exhibit activity. In the present study, we examine the above criteria to RGD-containing 15-peptides, derived from the active sites of the ECM proteins fibrinogen, fibronectin and vitronectin, as well as, from the cryptic RGD site of von Willebrand factor. The correlation of the structural data with the biological activity of compounds, are in good agreement with the previously mentioned -45o < pdo < +45o criterion. Furthermore, our results show that the differences in activity of compounds, which display similar distances between the charged centers of Arg and Asp, can be better evaluated by the pdo structural criterion. Acknwolegments : This work was supported by grants from EU and the Hellenic Ministry of Education (Heraklitos).
References :

1. Kostidis S. et al. (2004), J. Peptide Sci. 10, 494-509.

STRUCTURE ACTIVITY RELATIONSHIP OF PEPTIDE ANALOGUES DERIVED FROM INTEGRIN SUBUNIT GPIIB

R.-M. Stanica[1], D. Benaki[3], D. Tsoukatos[1], A. Tselepis[1], E. Mikros[2], **V. Tsikaris**[1]

[1]Department of Chemistry, University of Ioannina [2]Department of Pharmacy, University of Athens [3]Institute of Biology, NCSR "Demokritos", Athens, Greece

The GPIIb/IIIa receptor, which is a member of the integrin family, is the most abundant receptor in the surface of platelets and can interact with a variety of adhesive proteins including fibrinogen, fibronectin and Von Willebrand factor. Fibrinogen binding on GPIIb/IIIa is an event essential for platelet aggregation and thrombus formation. Mapping of the fibrinogen binding domains on GPIIb subunit suggested the sequence 313-332 as a putative binding site [1]. This region was restricted to sequence GPIIb 313-320 (YMESRADR) using synthetic octapeptides overlapping by six residues [2]. The YMESRADR octapeptide inhibits ADP stimulated human platelets aggregation and binds to immobilized fibrinogen. In this study we present the conformational analysis of three synthetic analogues YAESRADR (A2) YMESAADR (A5), and YMESRAAR (A7), using NMR spectroscopy and distance geometry calculations. Common structural characteristic of peptides A2 and A7 is the interaction between the side chains of Arg5 and Glu3, however in A2 the guanidino group of Arg5 seems to form salt bridges with both Glu3 and Asp7. Peptide A5 is stabilized only by a week interaction between Arg8 and Glu3 side chains. The interactions between the residue side chains provoke different overall shape of the three molecules. The most populated structural family of A2 exhibits a π backbone shape, A5 a turn around -S4A5-, while A7 an almost extended shape.

References:

1. Biris N. et al. (2003), Eur. J. Biochem. 270, 3760-3767.

2. Mitsios J.V. et al. (2004), Eur. J. Biochem. 271, 855-862.

M313

SYNTHESIS AND BINDING PROFILES OF ENDOMORPHIN-2 ANALOGUES CONTAINING CONFORMATIONALLY CONSTRAINED MOIETY

Y. Tsuda[1,2], T. Kawakami[1], Y. Fujita[2], T. Yamada[3], K. Isozaki[4], Y. Shimohigashi[4], L.H. Lazarus[5]

[1]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences [2]The Graduate School of Food and Medicinal Sciences, Kobe Gakuin University, Nishi-ku [3]Department of Chemistry, Faculty of Science and Engineering, Konan University, Higashinada-ku, Kobe [4]Laboratory of Structure-Function Biochemistry, Faculty and Graduate School of Science, Kyushu University, Fukuoka, Japan [5]Medicinal Chemistry Group, LPC, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Background and aims: Endomorphin-2 (EM-2: H-Tyr-Pro-Phe-Phe-NH2), endogenous opioid peptide isolated from bovine and human brain, has high affinity and selectivity for the mu receptor and produces potent and prolonged analgesia in mice [1]. In this presentation, the incorporation of ethylene-bridged Phe-Phe unit (eb[Phe-Phe]) or piperidine carboxylic aid (Pic) in position 2 was carried out to obtain more potent agonist or antagonist with stability against dipeptidyl peptidase IV (DPP IV).
Methods: The synthesis of eb[Phe-Phe] unit was achieved according to the procedure of Lammek B. et al. [2] Protected peptides were synthesized by a solution method using Boc-chemistry. The final products were identified by MALDI-TOF mass spectrometry and elemental analyses. The receptor binding affinity of peptides was assessed by radio-ligand receptor binding assay using mu and delta opioid receptors from rat brain membranes or COS-7 cell membranes expressing each opioid receptors.
Results and conclusions: The substitutions of Pro in [Dmt1]EM-2 (IC50=0.15 nM) with eb[Phe-Phe] or Pic gave the mu receptor ligands in a nanomolar range. Among them, H-Dmt-Pip(2)-Phe-Phe-NH2 exhibited the highest mu receptor affinity (IC50=0.41 nM). Furthermore, the substitution of Pro in EM-2 (IC50=3.61 nM) with Ala, eb[Phe-Phe] or Pic(2) resulted in Ki values of 3,700 and 381 nM and IC50 value of 2.13 nM, respectively, for mu receptor. This implies that the incorporation of conformationally constrained moiety into position 2 increases the mu receptor affinity.

[1] Zadina J. E. et al. (1997) Nature, 386, 499-502.
[2] Lammek B. et al. (1998) J. Peptide Res., 51, 149-154.

Tu314

THE INFLUENCE OF SIDE CHAIN GLYCOSYLATION AND FLANKING REGION MODIFICATION ON THE ANTIBODY RECOGNITION OF A MUC2 EPITOPE

K. Uray[1], M. Mizuno[2], E. Windberg[1], T. Inazu[2], A. Perkins[3], F. Hudecz[1,4]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Budapest, Hungary [2]Noguchi Institute, Tokyo, Japan [3]Queen's Medical Centre, University of Nottingham, UK [4]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

MUC2 glycoprotein, produced by the epithelium of the colon, built up mainly of repeat units of 1PTTTPITTTTTVTPTPTPTGTQT23, can be underglycosylated in colon carcinoma. We have been studying the epitope structure of the MUC2 repeat unit with the mucin peptide specific MAb 996 monoclonal antibody. This antibody recognizes the 18PTGTQ22 sequence as minimal, and 16PTPTGTQ22 as optimal epitope. Our interest lies in the modification of this epitope with maintained or enhanced specificity, and we aim to clarify the effect of different epitope modifications on MAb 996 antibody binding: A) amino acid changes in the flanking region, B) glycosylation in the epitope core and in the flank. For this we have prepared A) libraries of AX(1)PTGTQAA and ATPTGTQX(2)A peptides, and X(1)PTGTQX(2) heptapeptides based on the antibody binding properties of the libraries; and B) glycopeptides PT(GalNac)PTGTQ, PTPT(GalNac)GTQ and PTPTGT(GalNac)Q. The peptides were prepared by solid phase synthesis; after purification, ESI-MS and amino acid analysis characterisation their antibody binding properties were studied by competitive ELISA. Our results show that A) although all amino acids in positions X(1) and X(2) resulted in antibody binding; in position X(1) hydrophobic, in X(2) aromatic residues provided stronger binding than that of the native peptide; B) glycosylation on Thr(17) did not influence the binding of MAb 996, but on Thr(19) the presence of N-acetyl-galactosamine, interestingly, slightly increased the antibody recognition. These findings could be useful in designing synthetic peptide vaccines for tumour therapy.

Supported by: Hungarian Research Fund (OTKA F 034886, T037749), Ministry of Culture (FKFP 0153/2001)

Th315

A NOVEL POLYHISTIDINE TYPE LIGAND FOR ZINC(II) AND COPPER(II) BINDING

I. Vosekalna[1], B. Gyurcsik[2], A. Kolozsi[2], E. Larsen[3]

[1]Latvian Institute of Organic Synthesis, Riga, Latvia [2]Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary [3]Chemistry Department, The Royal Veterinary and Agricultural University, Copenhagen, Denmark

Histidines play essential role in binding of biological metal ions, either in small or macromolecular chelating molecules, e.g. in metalloenzymes. Therefore the low molecular weight polyhistidine type ligands are of potential importance as model substances.

Continuing our investigations on a novel branched oligopeptide type ligand - (His)4(Lys)2Lys-NH2 - prepared by solid phase peptide synthesis, we investigated the metal ion binding properties with zinc(II) and copper(II). The eight primary metal-binding sites are the four imidazole and four ammine groups on the ligand. pH-potentiometric titrations revealed, that up to pH 8 all these donor atoms loose their protons on increasing pH. The competition between the protons and the metal ions results the decrease of pKa values to about 1-3 in the case of copper(II) and to about 4-6 in case of zinc(II) ion. This reflects the higher stability of the complexes formed with copper(II) in spite of the weak axial coordination that seems to occur in zinc(II) complexes. Combined potentiometric, spectrophotometric, CD and NMR spectroscopic methods were utilized to investigate the speciation and the structure of the complexes formed in aqueous solution. The prepared Cu(II) complexes cleaved DNA, but it is not known whether in oxidative or in hydrolytic manner. Because of this ambiguity further studies with Zn(II) complexes will be undertaken.

This work has received support through SAPSTCLG97697 NATO Collaborative Linkage Grant and from the Hungarian Science Foundation (OTKA T43232).

M316

STRUCTURE-FUNCTION RELATIONSHIP OF INSULIN-LIKE PEPTIDE 3, A REGULATOR OF GERM CELL MATURATION

S. Zhang[1], F. Lin[1], A. Hossain[1], F. Shabanpoor[1,2], L. Hung[1], C. Guo[3], B. van Lierop[3], L. Otvos[4], R.A. Hughes[2], R.A. Bathgate[1], **J.D. Wade**[1]

[1]Howard Florey Institute [2]Department of Pharmacology, University of Melbourne, Parkville, Vic. [3]School of Chemistry, Monash University, Melbourne, Vic., Australia
[4]College of Science and Technology, Temple University,
Philadelphia, PA, USA

Background and aims:
Insulin-like peptide 3 (INSL3) is a member of the insulin superfamily. We have identified the receptor for INSL3 as being a G protein-coupled receptor (LGR), LGR8. Further studies have shown that in both male and female gonads, INSL3 and LGR8 represent a paracrine system important for meiosis induction in the ovary and male germ cell survival in the testis. Thus INSL3 may have clinical applications in fertility management. We undertook to determine the key structural elements responsible for its unique actions.
Methods:
Alanine-scanned analogues of human INSL3 and mimetics of the B-chain alone were prepared by solid phase peptide synthesis. Each was subjected to CD spectroscopy for secondary structure analysis and assayed for in vitro LGR8 binding and activation activity.
Results:
Full length linear INSL3 B-chain (1-32) was a weak LRG8 binder but not activator. N-terminal chain shortening could be tolerated to residue 5 and C-terminal by 4 residues only. The B28 Trp residue was crucial for activity; its replacement or removal caused complete loss of activity. Three mid-B-chain residues confer important binding properties. N-terminal A-chain truncation of 8 residues abolished receptor activation but not binding.
Conclusion:
The principal receptor binding site of INSL3 resides in the C-terminal half of the B-chain while receptor activation is mediated via the N-terminal region of the A-chain. This will enable the design and preparation of potentially clinically useful specific agonists and antagonists for, respectively, fertility management and contraception.

SYNTHESIS AND OPIOID ACTIVITY PROFILES OF PEPTIDES CONTAINING 3,4-O-CARBORANYLENEPIPERIDINE AT THE C-TERMINUS

G. Weltrowska, N.N. Chung, C. Lemieux, P.W. Schiller

Laboratory of Chemical Biology and Peptide Research,Clinical Research Institute of Montreal, Montreal, QC, Canada

We prepared opioid peptide analogues that contain tetrahydroisoquinoline (Tiq) or 3,4-o-carboranylenepiperidine (Cbp) at the C-terminus. Cbp is an analogue of Tiq containing the carboranyl icosahedron in place of the phenyl ring. It is slightly bulkier and more lipophilic than Tiq. Aside from being interesting probes for opioid receptor binding sites, Cbp-containing opioid peptides may have potential as Boron Neutron Capture Therapy (BNCT) agents in the treatment of cancer patients. Cbp was synthesized as described [C.H. Lee et al., Synlett, pp. 1799-1801 (2004)] and peptides were prepared either in solution or by a combination of solid-phase and solution techniques. The dipeptide H-Dmt-Cbp was a selective δ opioid antagonist with a Ke of 301 ± 76 nM in the mouse vas deferens (MVD) assay, being somewhat less potent than H-Dmt-Tiq (Ke = 165 ± 43 nM). Interestingly, the tripeptide H-Dmt-D-Arg-Cbp turned out to be a more potent μ opioid agonist (IC50 = 365 ± 67 nM) in the guinea pig ileum (GPI) assay than the corresponding Tiq3-analogue H-Dmt-D-Arg-Tiq (IC50 = 1890 ± 230 nM). Both peptides were μ receptor-selective, as indicated by their weak δ agonist potencies in the MVD assay (IC50 > 5 μM). The tetrapeptide H-Dmt-D-Arg-Phe-Cbp was found to be a selective μ agonist [IC50 (GPI) = 78.8 ± nM] with 40-fold lower potency than the corresponding, highly potent Tiq4-tetrapeptide, but with still 3-fold higher potency than leu-enkephalin. In conclusion, we developed selective, Cbp-containing δ antagonists and μ agonists with significant potency.

Th318

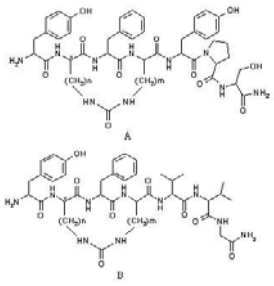
CYCLIC DERMORPHIN AND DELTORPHIN ANALOGUES

E. Witkowska[1], A. Zieleniak[1], K. Filip[1], N.N. Chung[2], P.W. Schiller[2], J. Izdebski[1]

[1]Faculty of Chemistry, Warsaw University, Warsaw, Poland [2]Clinical Research Institute of Montreal, Montreal, QC, Canada

Recently, we described the syntheses and biological activities of several opioid peptide analogues that contained the N-terminal sequence 1-4, common to dermorphin and deltorphin. Some of them showed very high agonist potency both in the GPI assay and in the MVD assay [1,2].
In this work, we designed new analogues in which the sequences were elongated at the C-terminal to obtained the full sequences of dermorphin (A) and deltorphin (B).
The syntheses of compounds and their biological activity profiles will be discussed.

References
[1] K.Filip, M.Oleszczuk, D.Pawlak, J.Wójcik, N.N.Chung, P.W.Schiller, J.Izdebski; J. Peptide Sci. 9, 649-657, 2003
[2] K.Filip, M.Oleszczuk, J.Wójcik, N.N.Chung, P.W.Schiller, D.Pawlak, A.Zieleniak, A.Parcińska, E.Witkowska, J.Izdebski; J. Peptide Sci. 11, 347-352, 2005



M319

SYNTHESIS AND BINDING ACTIVITIES FOR OPIOID RECEPTORS OF ENDOMORPHIN AND MORPHICEPTIN ANALOGS CONTAINING CONFORMATIONALLY CONSTRAINED 1-AMINOCYCLOALKANE-1-CARBOXYLIC ACIDS

T. Yamada[1], S. Hakogi[1], M. Iino[1], T. Murashima[1], T. Miyazawa[1], Y. Tsuda[2,3], T. Kawakami[2], Y. Okada[2,3], K. Isozaki[4], Y. Shimohigashi[4]

[1]Department Of Chemistry, Faculty Of Science And Engineering, Konan University [2]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences [3]The Graduate School of Food and Medical Sciences, Kobe Gakuin University, Kobe [4]Laboratory of Structure-Function Biochemistry, Faculty and Graduate School of Science, Kyushu University, Fukuoka, Japan

Background and aims: Endomorphin-2 (EM-2: Tyr-Pro-Phe-Phe-NH2) is very potent endogenous opioid peptide, which exhibits high affinity and selectivity for the mu-opioid receptor [1]. Previously, we had reported that [Ac3c2]-EM-2 containing 1-aminocyclopropane-1-carboxylic acid (Ac3c) exhibited higher affinities than EM-2 for the mu-opioid receptor [2]. In order to clarify that the substitution of 1-aminocycloalkane-1-carboxylic acids (Acnc: n indicates the number of carbon atoms in a ring) for Pro in position 2 of EM-2 is efficient to obtain higher affinity for the mu-receptor, we synthesized [Acnc2]-analogs of morphiceptin (MC: [Pro4]-EM-2) and other EM-2 analogs, which has been reported in the literatures.

Methods: Various analogs containing Acnc (n=3-6) instead of Pro in position 2 of MC and other EM-2 analogs, in which Tyr1, Phe3 and Phe4 are replaced to 2',6'-dimethyl-Tyr (Dmt), N-methyl-Phe (MePhe) and phenylalaninol (Pheol), respectively, were synthesized by the solution method. The receptor binding potency of these analogs was assessed by radio-ligand receptor binding assay using COS-7 cell membrane expressing mu- and delta-opioid receptors.

Results and conclusions: Binding potencies (IC50: nM) for the m-receptor were 3.61 (EM-2), 0.66 ([Ac3c2]-EM-2), 1.55 ([Ac4c2]-EM-2); 87.6 (MC), 58.8 ([Ac3c2]-MC), 66.1 ([Ac4c2]-MC); 2.55 ([Pheol4]-EM-2), 1.23 ([Ac3c2, Pheol4]-EM-2); 0.15 ([Dmt1]-EM-2), 0.21 ([Dmt1, Ac3c2]-EM-2). Therefore, the replacement of Pro to Ac3c and Ac4c will be efficient to make these analogs adopt bioactive conformation and exhibit high affinity for mu-receptor.

References: [1] Zadina, J. E., et al., (1997) Nature, 386, 499-502. [2] Yamada, T., et al., (2003) Peptides 2002, 324-325.

Tu320

EFFECT OF GLYCINE B23 AND PHENYLALANINE B24ON INSULIN ACVTIVITY

M.A. Zewail

National Research Centre, Dokki, Cairo, Egypt

In the past few years, many attempts have been made to prepare a synthetic insulin. The biological activity of insulin is known to be closely related to the C-terminal octapeptide fragment of its B-chain.It was found that B23 gly and B24 phe were present in all insulins so far obtained from various animal species indicating the significance of these two residues.It would therefore seem desirable to study the effect of each of these two amino acid residues or both on biological activity of the octapeptide fragment of the B-chain.

A heptapeptide Arg-Phe-Tyr-Thr-Pro-Lys-Ala-OCH3, corresponding to (B22-B30) insulin des Gly23-Phe24, and an octapeptide Arg-Phe-Phe-Tyr-Thr-Pro-Lys-Ala-OCH3, des Gly23 were synthesized using the solid phase method.

The C-terminal ends of both peptide were converted to methyl ester by transesterification cleavage from the resin. The side chain protecting groups were removed by HF. Manual counter current distribution method was used for purification of the free peptides. The way to solve the evaluation of tyrosine containing peptide was studied. The free methyl ester peptides were administered for insulin-like activity test by glucose metabolism in the rat fat cells technique in vitro.

Th321

DESIGN AND SYNTHESIS OF HEME PEPTIDES FOR BIOREMEDIATION

N. Alexandris[1], M.C. Alcaro[1,2], A.M. Papini[1,2], R. Bianchini[1]

[1]Department of Organic Chemistry "Ugo Schiff", University of Florence [2]Laboratory of Peptide & Protein Chemistry & Biology, Sesto Fiorentino (FI), Italy

Aim of this study is to develop peptides as useful tools for degradation of synthetic dyes, which are often pollutants. We focused our interest in peroxidases, a class of enzymes reported to efficiently degrade azo and anthraquinonic dyes. In particular, the fungus Versatile Peroxidase (VP) of Pleurotus Eryngii can perform this degradation. Therefore, our goal is the synthesis of a peptide based on this peroxidase able to emulate its biological function.
The linear and cyclic peptide sequences were derived by the theoretical model of VP [PDB: 1A20], which determined the amino acids fundamental for the desired function of the active sites. In particular, the residues instrumental for the coordination of the heme, the Mn binding site, and the Long Range Electron Transfer pathway [1], were pin-pointed. Moreover, we calculated the radius of the heme cavity. The next step was the synthesis of these peptides in order to verify the coordination of the heme and optimize their sequences. The syntheses were carried out by solid-phase following the Fmoc/tBu strategy. Because of purification difficulties of the fully-protected peptide, we undertook an alternative synthetic pathway, based on a solid phase head-to-tail cyclisation strategy, following the Fmoc/tBu/allyl three-dimensional protection scheme [2].
Next steps will be to test the coordination properties of the synthetic peptides, with respect to the heme, and further computational studies based on the new model of Pleurotus Eryngii [PDB: 2BOQ].

[1] Ruiz-Duenas FJ et al. Biochemical Society Transactions 2001, 29, 116-122
[2] Papini AM et al. Tetrahedron Letters, 2003, 44, 5217-5219

M322

PHOTOPROBE-PEPTIDES FOR THE ELUCIDATION OF THE CONTINUOUS INTERACTIONS OF ANGIOTENSIN II WITH ITS RECEPTOR AT1

D. Fillion, G. Lemieux, M. Clément, **E. Escher**

Department of Pharmacology, Faculty of Medicine & Health Sciences, Université de Sherbrooke, QC, Canada

Heptahelical G protein-coupled receptors (7TM-GPCRs) are the largest family of cell surface receptors and are implicated in various pathologies. Approximatively 50 % of all current drugs target these receptors, making them privilegeous targets in pharmacotherapy. The rational design of drugs targeting 7TM-GPCRs requires a molecular-based knowledge of those proteins that may be acquired by identification of ligand-receptor interactions through photoaffinity labeling. In the present contribution, a systematic introduction of the carbene-generating amino acid p–[3–(trifluoromethyl)–3H–diazirin–3–yl]–L–phenylalanine (Tdf) into the octapeptide angiotensin II (AngII) sequence was carried out to produce eight different AngII photoprobe-peptides with agonistic properties. On the AngII type 1 receptor (AT1), nM affinities (15 nM; comparable to AngII) were found for Tdf1-, Tdf2-, Tdf3- and Tdf8-AngII, whereas the others analogues were in the 10 M range and above. On the constitutively active receptor mutant N111G-hAT1, all analogues except Tdf4- (150 M) and Tdf6-AngII (300 nM) displayed also nM affinities (10 nM; comparable to AngII). These analogues produced excellent photoaffinity labeling yields on both wt- and N111G-hAT1 receptors. Chemical and enzymatic cleavage of the photolabeled receptors will allow us to gain insight on the precise molecular structure of the ligand-activated AngII receptor. The results up to now demonstrate that the C- and N-terminal residue of AngII adopts an extended peptide conformation within the transmembrane domains 3, 6 and 7 of the hAT1 receptor. **This work was funded by grants from CIHR and CHSF Québec to G. Guillemette, R. Leduc and E. Escher.**

Tu323

THERMODINAMIC STUDIES ON THE CALCIUM BINDING OF ASPARTIC ACID RICH CYCLOPEPTIDES

V. Farkas[1], I. Hanssens[2], Z. Majer[1]

[1]*Institute of Chemistry, Eotvos L. University, Budapest, Hungary*
[2]*Interdisciplinary Research Centre, K.U. Leuven Campus Kortrijk, Leuven, Belgium*

The calcium plays an important role in biochemical pathways. It binds to enzymes and proteins in a different process. Aspartic (Asp, D) and glutamic (Glu, E) acid side chains are the main ligands of calcium, but the contribution of the backbone carbonyl groups in the binding is also important. Generally the binding places in the proteins are an unstructured loop between two helixes (310- or alpha-helix). The common sequence is the so-called EF-hand motif, which contains 12 amino acids [1]. It is already known that some proteins also bind calcium with a non-EF-hand loop. For example alpha-lactalbumins have a ten amino acid long sequence for binding [2]. It is an Asp rich sequence where 5 Asps are closer to each other than in EF-hand motif (-K79FLDDDLTDD88-) but only 3 Asps side chains take part in calcium coordination.
We constructed a series of cyclopeptides to mimic the loop structure of alpha-lactalbumin [3]. In this study we focus on determining the importance of conservative amino acids within the Ca2+ binding loop of this protein, using microcalorimetry (ITC). The ITC measurements were performed in different organic solvents and at different temperature.

[1] http://www.structbio.vanderbilt.edu/cabp_database/cabp.html
[2] Calcium binding proteins, Volume II, Biological functions; CRC Press, Inc., (2000) 79
[3] V. Farkas, E. Vass, I. Hanssens, Zs. Majer, M. Hollósi; Bioorg. Med. Chem. (2005) 13 (17): 5310-5320

The research was supported by Bilateral Scientific Cooperation (B-16/04), FWO-Flanders G-0180-03 (I. H.) and Reanal Fine Chemicals Co.

NMR AND MODELLING STUDIES OF GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE (GIP)

I. Alana[1], J.C. Parker[2], V.A. Gault[2], F.P.M. O'Harte[2], J.P.G. Malthouse[1],
C.M. Hewage[1]

[1]UCD School of Biomolecular and Biomedical Science, Centre for Synthesis and Chemical Biology, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland [2]School of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland, UK

Due to its glucose-sensitive insulinotropic activity, there has been a considerable interest in utilising the hormone as a potential treatment for type 2 diabetes. Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone that stimulates the secretion of insulin after ingestion of food. GIP also promotes the synthesis of fatty acids in adipose tissue. In this article, we present the solution structure of GIP in water and TFE/water determined by NMR spectroscopy. The calculated structures are characterised by the presence of an □-helical motif between residues Ser11-Gln29 and Phe6-Gln29 respectively. The helical conformation of GIP is further supported by CD spectroscopic studies. Six GIP(1-42)Ala1-7 analogues were synthesised by replacing individual N-terminal residues with alanine. Alanine scan studies of these N-terminal residues showed that the GIP(1-42)Ala6 was the only analogue to show insulin secreting activity similar to that of the native GIP. However, when compared with glucose its insulinotropic ability was reduced. For the first time, these NMR and modelling results contribute to the understanding of the structural requirements for the biological activity of GIP. A knowledge of the solution structure of GIP and of the role of its individual residues will be essential in the understanding of how they interact with the GIP receptor.

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MEASUREMENT AND APPLICATION OF RESIDUAL DIPOLAR COUPLINGS IN MOLECULAR MECHANICS SIMULATIONS ON EFRAPEPTIN C

T. Huber, N. Sewald

Department of Chemistry, Bielefeld University, Bielefeld, Germany

Efrapeptins are pentadecapeptides produced as a mixture of six closely related analogues (efrapeptin C-G) by the fungus Tolypocladium niveum and other members of this species. They consist predominantly of the nonproteinogenic amino acids -aminoisobutyric acid (Aib), isovaline (Iva), -alanine (Ala) and pipecolic acid (Pip), have an acetylated N-terminus and bear an unusual cationic C-terminal headgroup derived from leucinol and 1,5-diazabicyclo[4.3.0]non-5-ene.
Efrapeptin C is a competitive inhibitor of the F1-ATPase and active against the malaria pathogen Plasmodium falciparum. An anti-proliferative effect was also reported. Conformational analysis of efrapeptin C in trifluoroethanol and dimethylsulfoxide was conducted to obtain structure-affinity relationships. The absence of amide- and -protons resulted in an imperfect assignment and unsatisfying conformational study. Specific deuteration of methyl groups in Aib did not simplify the assignment.
CD and FT/IR spectra hint to helical or beta-turn secondary structures as main structure elements. Residual dipolar couplings (RDC) were measured in a stretched cross-linked poly(dimethylsiloxane) gel in dichloromethane. The impact of the RDC on the conformational analysis led to an improved high resolution structure from simulated annealing protocols and consolidated the formation of a helical structure of efrapeptin C in nonpolar solution which is comparable with the binding pocket of the F1-ATPase. Finally, the dynamics of the resulting structures was studied using the GROMOS96 force field in explicit solvent.

MOLECULAR MECHANISM OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRI'S) INTERACTIONS WITH SEROTONIN TRANSPORTER

M. Jarończyk[1], Z. Chilmonczyk[1], A.P. Mazurek[1], M. Dybała[2], A.W. Ravna[3], K. Kristiansen[3], I. Sylte[3]

[1]National Insititute of Public Health, Warsaw [2]Department of Cytobiology and Histochemistry, Collegium Medicum, Jagiellonian University, Cracow, Poland
[3]Department of Pharmacology, Institute of Medical Biology, University of Tromsø, Tromsø, Norway

Serotonin selective reuptake inhibitors (SSRIs) are currently among the most frequently prescribed therapeutic agents of depression. Their therapeutic use includes also obsessive-compulsive disorder, panic disorder, bulimia. The serotonin transporter (SERT) is the target of serotonin selective reuptake inhibitors (SSRIs). Altough the inhibition is the proximal event in antidepressant action, the clinical benefit of antidepressant medications requires weeks of continuous dosing, indicating that their mechanism of action involves events downstream from acute transporter blockade. Long-term effects of SSRI treatment may be due to changes in intrinsic properties of SERT structure, function, or regulation. Thus, understanding the mechanism of action of SERT remains a primary goal in the search for developing novel treatments for diseases associated with serotonergic dysfunction.

In the present study experimentally determined ligand selectivity of the buspirone analogues toward the serotonin transporter was theoretically investigated on the molecular level. The model of serotonin transporter based on the crystal structure of bacterial homologue from Aquifex aeolicus (LeuTAa) was constructed using the traditional homology modelling approach. A series of docking experiments with SSRI's were conducted, using interactive molecular graphics techniques combined with energy calculations and analysis of the transporter-ligand complexes.

Structural information about the serotonin transporter and its molecular interactions with SSRI's is important for understanding the mechanism of action of these drugs and for development of drugs with improved potency and selectivity.

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THEORETICAL MODELING OF FOUR PRKC PROTEIN KINASE COMPLEXES WITH THE ATP ANALOGS

R. Kaźmierkiewicz[1], P. Gruszczyński[1], B. Śmiech[1], M. Obuchowski[2]

[1]Department of Chemistry [2]Department of Molecular Biology, University of GdańSk, Poland

The protein kinase C (PrkC) is a member of a super-family of the eukaryotic receptor protein kinases. It forms dimers and is anchored in the membrane, with a cytoplasmic kinase domain and an external domain, presumably acting as a sensor. PrkC enables formation of biofilms of Bacillus subtilis which show a high degree of spatial organization. They colonize various surfaces and produce complex antibiotic resistant communities. PrkC acts as a Ser/Thr kinase with features of the receptor kinase family of eukaryotic Hanks kinases.

Our current study involved theoretical modeling of the protein kinaze PrkC complexes with the modified ATP. The ligands were selected from a set of molecular probes developed by K. Shah and coworkers [1]. Each modified ATP molecule was docked to the active site of the kinase molecule using Autodock genetic algorithm procedure. The optimized structures of the complexes were submitted to the molecular dynamics simulations in the Amber force field. We obtained four optimized structures of PrkCc complexes in water. The results suggest the great similarity of our complexes with human cyclin-dependent kinase 2 [1] complexes.

[1] K. Shah, Y. Liu, C. Deirmengian, K. M. Shokat, PNAS, 94, 3565–3570, 1997.

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CONFORMATIONAL PREFERENCES OF NON-PROLYL AND PROLYL RESIDUES

Y.K. Kang

Department of Chemistry, Chungbuk National University, Chungbuk, South Korea

The conformational study on Ac-Ala-NHMe (the alanine dipeptide) and Ac-Pro-NHMe (the proline dipeptide) is carried out using ab initio HF and density functional methods with the self-consistent reaction field method to explore the differences in the backbone conformational preference and the cis-trans isomerization for the non-prolyl and prolyl residues in the gas phase and in solution. For both the dipeptides, the relative free energy of the cis conformer to the trans conformer decreases and the rotational barrier to the cis-trans isomerization increases as the solvent polarity increases. The cis populations of the alanine dipeptide are calculated to be 0.1%, 0.1%, and 0.4% in the gas phase, chloroform, and water, respectively, whereas the corresponding values of the proline dipeptide are 2.5%, 9.0%, and 23.3%, respectively. It has been known that the cis-trans isomerization proceeds in common through only the clockwise rotation with ω´ ≈ +120° about the non-prolyl and prolyl peptide bonds in the gas phase and in solution. In particular, the barrier to the trans-to-cis isomerization for the non-prolyl peptide bond is higher by 0.51, 2.29, and 1.42 kcal/mol than the prolyl peptide bond in the gas phase, chloroform, and water, respectively. The pertinent distance d(N···H-N) could successfully describe the increase in the rotational barriers for the non-prolyl and prolyl trans-cis isomerization as the solvent polarity increases, and the higher barriers for the non-prolyl residue than the prolyl residue, as seen in experimental and calculated results.

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CONFORMATIONAL ANALYSIS OF THE ANTIMICROBIAL PEPTIDE, INDOLICIDIN CONTAINING CIS OR TRANS XXX-PRO PEPTIDE BONDS

Á. Kerényi[1], G. Rákhely[1,2], B. Leitgeb[1]

[1]Institute of Biophysics, Biological Research Center of The Hungarian Academy of Sciences [2]Department of Biotechnology, University of Szeged, Hungary

Background and aims. Indolicidin is a 13-residue antimicrobial peptide, which was isolated from bovine neutrophils. This molecule possesses a wide spectrum of antibacterial, antifungal and antiviral activity, furthermore it has also haemolytic effect. Data derived from structural investigations led to considerably diverse conclusions regarding the secondary structure of this peptide, therefore the aim of this study was to examine the effect of cis-trans isomerization on the conformational properties of this antimicrobial peptide.

Methods. The conformational analysis of indolicidin containing cis or trans Xxx-Pro peptide bonds was performed by simulated annealing calculations with the use of AMBER force field.

Results. For the conformers of indolicidin with cis or trans Xxx-Pro peptide bonds, the evolving secondary structural elements were examined and poly-proline II helix and type VI beta-turn were identified. In the case of this peptide, various intramolecular interactions may play an important role in stabilizing the structure of conformers. Therefore the presence of the H-bonds between backbone atoms, the aromatic-aromatic interactions between the side-chains of Trp amino acids and the proline-aromatic interactions between the side-chains of Trp and the pyrrolidine rings of Pro amino acids was investigated.

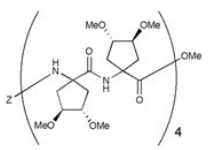
Conclusions. The conformational comparison of the peptides possessing cis or trans Xxx-Pro peptide bonds resulted in different secondary structural elements for both isomers, which are the poly-proline II helix and type VI beta-turn for the trans and cis isomers of indolicidin, respectively. The occurrences of various intramolecular interactions are in agreement with the observed secondary structures.

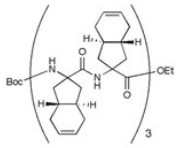
COMPUTATIONAL STUDY ON HELICAL STRUCTURES OF OLIGOPEPTIDES CONTAINING CHIRAL CYCLIC ALPHA-AMINO ACIDS

M. Kurihara[1], W. hakamata[1], Y. Sato[1], H. Okuda[1], Y. Demizu[2], M. Nagano[2], K. Anan[2], M. Doi[3], M. Tanaka[2], H. Suemune[2]

[1]Division Of Organic Chemistry, National Institute of Health Sciences, Tokyo [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka [3]Osaka University of Pharmaceutical Sciences, Osaka, Japan

Prediction of the conformation of peptides using computational simulation is an interesting challenge for the design of functionalized and bioactive peptides. We have shown the Monte Carlo conformational search using MacroModel is useful for conformational study of oligopeptides prepared from alpha, alpha-disubstituted alpha-amino acids. Moreover, we have studied conformational analysis of oligopeptides containing chiral alpha, alpha-disubstituted alpha-amino acids to predict the helical screw sense of helical structures. Here we report computational study on conformation of oligopeptides containing cyclic alpha, alpha-disubstituted alpha-amino acids with side-chain chiral centers. **[1] Tanaka, M., Demizu, Y., Doi, M., Kurihara, M., Suemune, H. (2004) Angew. Chem. Int. Ed., 43, 5360-5363. [2] Kurihara, M., Sato, Y., Hakamata, W., Okuda, H., Demizu, Y., Anan, K., Takano, Y., Oba, M., Doi, M.,Tanaka, M., Suemune, H. (2005) Peptide Science 2004, 297-298. [3] Tanaka, M., Anan, K., Demizu, Y., Kurihara, M., Doi, M., Suemune, H. (2005) J. Am. Chem. Soc., 127, 11570-11571.**





M331

INVESTIGATION OF SECONDARY STRUCTURAL ELEMENTS AND INTRAMOLECULAR H-BONDING PATTERNS OF POLY-(ALA) AND POLY-(GLN) PEPTIDES BY MOLECULAR MODELLING METHODS

B. Leitgeb[1], F. Bogár[2], G. Paragi[2], Á. Kerényi[1], B. Penke[2,3], G. Rákhely[1,4]

[1]Institute of Biophysics, Biological Research Center of The Hungarian Academy of Sciences [2]Protein Chemistry Research Group of The Hungarian Academy of Sciences [3]Department of Medical Chemistry [4]Department of Biotechnology, University of Szeged, Hungary

Background and aims. The homopolymeric amino acids (HPAAs) are polypeptides consisting of the same amino acids. Some of them play a relevant role in the formation of several neurodegenerative diseases. Most probably the poly-(Ala) and poly-(Gln) are the best representatives of these peptides because of their important biological effects. Our aim was to perform conformational analysis and structural investigation of these two HPAAs.
Methods. To explore the conformational spaces of the peptides, simulated annealing (SA) and random search (RS) calculations were carried out using AMBER force field. Two different forms of the HPAAs were modelled: either with charged N-terminal amino group and C-terminal carboxyl group, or with the N- and C-termini blocked by acetyl and N-methyl amide groups, respectively.
Results. For the conformers obtained by SA and RS calculations, the occurrences of various secondary structural elements like different types of beta-turns, gamma- and inverse gamma-turns, alpha-helix, 310-helix, poly-proline II helix and beta-strand were investigated. In the cases of various helices and beta-strand, segments with different lengths characterized by these secondary structures were determined along the entire sequence of peptides. For the conformers of the HPAAs, the intramolecular H-bonds formed between the backbone atoms as well as between the backbone and side-chain atoms were identified.
Conclusions. For poly-(Ala) and poly-(Gln) peptides, different secondary structural elements including mainly beta-turns, alpha-helix, 310-helix and beta-strand were found along the entire sequence of molecules. In accordance with the secondary structures occurred, characteristic H-bonding patterns were observed for the HPAAs.

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VASOPRESSIN AND OXYTOCIN RECEPTORS INTERACTIONS WITH AGONISTS AND ANTAGONISTS - MOLECULAR MODELING STUDY

M.J. Ślusarz, R. Ślusarz, J. Ciarkowski

Faculty of Chemistry, University of GdańSk, Poland

The vasopressin and oxytocin receptors (V1aR, V2R and OTR) are membrane–embedded proteins belonging to the large family A G protein-coupled receptors (GPCRs). They are involved in crucial physiological functions as the regulation of water metabolism, control of blood pressure and stimulation of labor and lactation, mediated via V2R, V1aR and OTR, respectively. As such, they are involved in a number of pathological conditions and are important drug targets. Understanding their inhibition and activation mechanisms may improve design of ligands capable of selective stimulation or blockade of the respective receptors presenting the therapeutic targets.

To investigate the OTR, V1aR and V2R interactions with agonists and antagonists thirty computer models of receptor–ligand complexes have been modeled via docking and molecular dynamics (MD) and analyzed in details. The receptor models were built on RD crystal structure template or using the coordinates of MII–Gtα(338-350), for non-active and activated models, respectively. The ligands (arginine vasopressin, oxytocin, desmopressin, atosiban ([Mpa1,D-Tyr(Et)2,Thr4,Orn8]OT) and barusiban (Mpa1,D-Trp2,Ile3,allo-Ile4,Asn5,Abu6,Mol7) were docked into the receptors. The complexes have been embedded into the hydrated POPC bilayer and submitted to 1ns unconstrained MD in the Amber force field. The relaxed systems have been obtained and analyzed in details. The receptor residues responsible for agonists/antagonists binding have been identified and mechanism of binding involving the highly conserved residues has been proposed.

MOLECULAR MODELING-BASED STUDY OF NEW VASOPRESSIN ANALOGS AFFINITY TOWARD HUMAN NEUROHYPOPHYSEAL HORMONE RECEPTORS

R. Ślusarz, M.J. Ślusarz, B. Lammek

Faculty of Chemistry, University of GdańSk, Poland

The main physiological roles of arginine vasopressin (CYFQNCPRG-NH2, AVP) are the control of blood pressure, adrenocorticotropin hormone (ACTH) secretion and the regulation of water balance, mediated via three different subtypes of vasopressin receptors: V1aR, V1bR and V2R. Moreover, AVP can modulate many social and behavioral functions. In last decades numerous AVP analogs have been extensively investigated for better understanding of the functional architecture of vasopressin receptors and rationally design the new AVP analogs. Molecular modeling methods could be an alternative for more expensive and time–consuming clinical tests. In this study nine vasopressin analogs have been investigated: [Aic2]AVP, [Mpa1,Aic2]AVP, [Aic2,D-Arg8]VP, [Mpa1,Aic2,D-Arg8]VP, [Aic2,Val4]AVP, [Mpa1,Aic2,Val4]AVP, [Aic3]AVP, [Mpa1,Aic3]AVP and [Aic3,D-Arg8]VP (Aic: 2-aminoindane-2-carboxylic acid) to determine their presumable biological activity using docking and molecular dynamics methods.

A three-dimensional models of the neurohypophyseal hormone receptors were constructed using a multiple sequence alignment and either the crystal structure of bovine rhodopsin or the complex of activated rhodopsin with Gta C-terminal peptide of transducin RD*–Gt(338-350) prototype to obtain non–active or activated receptor models, respectively. Analogs were docked to V1aR, V2R and OTR, both non-active and activated models. The low–energy receptor–ligand complexes, with properly docked analogs were submitted to the constrained simulated annealing (CSA), in vacuo. The relaxed receptor–analog models were obtained. The residues responsible for analogs binding to V1aR, V2R and OTR have been identified and presumable biological activity of these compounds was determined.

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N-METHYLDEHYDROAMINO ACIDS STRONGLY PROMOTE CONFIGURATION CIS OF N-METHYLAMIDE BOND

D. Siodłak, **A. Macedowska**, K. Paczkowska, J. Grondys, B. Rzeszotarska

Institute of Chemistry, University of Opole, Poland

N-Methyldehydroamino acids belong to non-standard amino acids found in nature. N-Methyl-(Z)-dehydrophenylalanine was found in tentoxin, a selective weed killer, having been produced by several phytopathogenic fungi of the Alternaria genus. N-Methyl-(Z/E)-dehydrobutyrine and N-methyldehydroalanine are components of nodularins and microcystins, families of hepatoxins produced by species of freshwater cyanobacteria, primarily Nodularia spumingena and Microcystis aeruginosa.

The simplest N-methyl dehydropeptides, Ac-delta(Me)Xaa-NHMe (where Xaa = Ala, (Z/E)-Abu, (Z/E)-Phe, and Val) and, for comparison, the saturated Ac-L-(Me)Ala-NHMe analogue were investigated using computational methods. Cis-trans B3LYP/6-31+G**//HF/3-21G Ramachandran potential energy surfaces were created. The conformers found were optimised at the B3LYP/6-31+G** level. The effect of the electrostatic solute/solvent (water) interaction on the solute energies was investigated within the SCRF method using the polarisable continuum model (PCM) on the geometries of solutes in vacuo.

It was found that for all the studied dehydropeptide molecules the lowest conformer (phi, psi = ~ -109°, 10°) has the cis N-methyl amide bond. This feature seems to be independent of the dehydroamino acid moieties, the C-beta substituent and the Z/E configuration. The pi-electron conjugation as well as the N-H···N hydrogen bond play the dominant role in the stability of this conformer (see Figure). The preliminary NMR investigations into the conformational preferences of the studied molecules in solution confirm the theoretical results obtained.

The strong tendency of the N-methyl amide bond to adopt the cis configuration seems to be the reason why N-methyldehydroamino acids are found in small natural cyclic peptides, where they ensure the conformational flexibility necessary for biological action.

DETERMINATION OF POTENTIALS OF MEAN FORCE DEPENDENT ON ORIENTATION IN HYDROPHOBIC SYSTEMS MODELING SIDE-CHAINS OF BIOMOLECULES

M. Makowski[1,2], E. Sobolewski[1], A. Liwo[1,2], J. Makowska[1,2], L. Chmurzyński[1], H.A. Scheraga[2]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA

The purpose of this study was to determine the potentials of mean force (PMF) of the interactions between models of nonpolar amino acid side chains in water. The potentials of mean force (PMF's) dependent on orientation were determined for systems forming hydrophobic and diagonal complexes composed of side-chain models of alanine, valine, leucine, proline and iso-leucine, respectively, in water. For each hydrophobic pair in water a series of umbrella-sampling molecular dynamics simulations with the AMBER force field and explicit solvent (TIP3P water model) were carried out and the PMFs were calculated by using the Weighted Histogram Analysis Method (WHAM). In all cases a characteristic shape of PMF plots for hydrophobic association were found, which was manifested as the presence of contact minima and solvent separated minima. Depths of contact minima for all systems studied were about 1 kcal/mol.

VALUATION OF TWO THEORETICAL METHODS TO ESTIMATE POTENTIOMETRIC-TITRATION CURVES OF PEPTIDES: COMPARISON WITH EXPERIMENT

J. Makowska[1,2], K. Bagińska[1], **M. Makowski**[1,2], A. Liwo[1,2], L. Chmurzyński[1],
H.A. Scheraga[2]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA

In this work we compared the ability of two theoretical methods of pH-dependent conformational calculations to reproduce experimental potentiometric-titration curves of two models of peptides: Ac-K5-NHMe in 95% methanol (MeOH)/5% water (H2O) mixture and Ac-XX(A)7OO-NH2 (XAO) (where X is diaminobutyric acid, A is alanine, and O is ornithine) in water, methanol (MeOH) and dimethylsulfoxide (DMSO), respectively. In theory, in all three solvents, the first pKa of XAO is strongly downshifted compared to the value for the reference compounds, the water and methanol curves have one, and the DMSO curve has two jumps characteristic of remarkable differences in the dissociation constants of acidic groups.

The predicted titration curves of Ac-K5-NHMe are in good agreement with the experimental ones; better agreement is achieved with the MD-based method. The titration curves of XAO in methanol and DMSO, calculated using the MD-based approach, trace the shape of the experimental curves, reproducing the pH jump, while those calculated with the EDMC-based approach, and the titration curve in water calculated using the MD-based approach, have smooth shapes characteristic of the titration of weak multifunctional acids with small differences between the dissociation constants. Quantitative agreement between theoretically predicted and experimental titration curves is not achieved in all three solvents. The poorer agreement obtained for water than for the non-aqueous solvents suggests a significant role of specific solvation in water, which cannot be accounted for by the mean-field solvation models

ANGIOTENSIN-I CONVERTING ENZYME (ACE) – SUBSTRATE INTERACTION THROUGH MOLECULAR DYNAMICS SIMULATIONS

A. Papakyriakou[1], G.F. Vlachopoulos[2], G.A. Spyroulias[2], **E. Manessi-Zoupa**[3], P. Cordopatis[2]

[1]Institute of Physical Chemistry, NCSR "Demokritos", Attikis [2]Department of Pharmacy [3]Department of Chemistry, University of Patras, Greece

Angiotensin-I converting enzyme (ACE) belongs to the M2 family of the MA clan of zinc metallopeptidases and can act either as a dipeptidyl carboxypeptidase, which catalyses the proteolytic cleavage of dipeptides from the carboxy terminus of a wide variety of peptides, or as an endopeptidase, which hydrolyses peptides bearing amidated C-termini. Among the former category of ACE peptide substrates, the most distinguished are those involved in blood pressure regulation, such as angiotensin I (AngI) and bradykinin (BK). In the latter category falls the Gonadotropin-releasing hormone (GnRH)

In an attempt to analyze molecular interactions at atomic level we simulated the ACE-substrate complexes, using the recently determined 3D crystal structure of ACE testis isoform and a knowledge-based docking method in order to insert the peptide substrate (AngI, BK and GnRH) of ACE into its catalytic cleft. In order to introduce the effect of protein mobility and gain information about enzyme-substrate recognition and interaction we have sampled the conformational space of these complexes via molecular dynamics simulations with explicit solvent representation. We have also performed molecular dynamics calculations with tACE-inhibitor complexes, such as lisinopril, as well as with tACE mutated at specific sites, such as the ligands of the two buried chloride ions that have been shown to affect substrate activity. Our results provide new insights into the role of specific domains of tACE and their implication in the enzyme activity, which is not readily apparent from the available crystal structures.

COMPARISON OF PROPOSED PUTATIVE BIOACTIVE CONFORMATIONS OF ALTERED PEPTIDE LIGANDS OF MYELIN BASIC PROTEIN BY SPECTROSCOPIC AND MODELLING STUDIES

E.D. Mantzourani[1,2], T.M. Mavromoustakos[1], S. Golič-Grdadolnik[3], J.A. Platts[4], G.N. Deraos[2], J.M. Matsoukas[2], T.V. Tselios[2]

[1]Institute of Organic and Pharmaceutical Chemistry , National Hellenic Research Foundation, Athens [2]Department of Chemistry, University of Patras, Rion, Patras, Greece [3]Laboratory for Molecular Modeling and NMR Spectroscopy, National Institute of Chemistry, Ljubljana, Slovenia [4]Department of Chemistry, Cardiff University, Cardiff, Wales, UK

This work focuses on the conformational analysis and proposes a structural motif for the linear altered peptide ligand (APLs) of myelin basic protein (MBP) and inhibitor of experimental autoimmune encephalomyelitis (EAE), [Ala91,96] MBP87-99. The APL is rationally designed by substituting two residues that are considered as T-cell receptor (TCR) contact sites. A combination of molecular dynamics (MD) calculations with nuclear magnetic resonance (NMR) structure determination was applied, distinct populations of conformations present in solution were generated, and a putative bioactive one identified among them. This approach is extremely valuable as no crystallographic data is available for the complex of the APL with the receptor. The conformational differences and similarities between [Arg91,96] MBP87-99 and [Arg91, Ala96] MBP87-99 were sought in an attempt to correlate the APLs' activity with the conformation they adopt. Despite the differences caused by the substitution of Arg91 with Ala, in both conformations the distance between the major histocompatibility complex (MHC) anchors Val87, Phe90 is similar. On the other hand TCR contact residue Phe89 has an altered topology with respect to the native peptide as found via crystallography. More specifically, it is no longer prominent and solvent exposed and is therefore not accessible for interaction with the TCR. It is proposed that the antagonistic activity of the APLs is due to their binding to MHC, preventing the binding of self myelin epitopes, with the absence of an immunologic response as the loss of some interactions with the TCR hinders activation of T-cells.

MODELING GAP JUNCTION BEHAVIOR IN MYOCYTES :ELECTRICAL FIELD VERSUS ELECTRICAL COUPLING

S.H. Moghadam Nia[1], S. Mahdavi[2], F. Towhidkhah[2], M. Rezaei-Tavirani[3], Sh. Gharibzadeh[4], A. Shabani[5]

[1]Shaheed Beheshti University [2]Department of Cellular and Molecular Biology, Khatam University [3]Biological Systems Modeling Laboratory, Faculty of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Neuromuscular Systems Laboratory [4]Faculty of Medicine, Ilam Medical Sciences University; & Asre Novin Institute of Research and Industrial Services [5]Neuromuscular Systems Laboratory, Faculty of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic) [6]Department of Biochemistry, Faculty of Science, Azzahra University, Tehran, Iran

Two main mechanisms for the propagation of action potential in myocytes are: 1) the free flow of local circuit current through gap junctions and 2) the effect of electrical field. Here we study effect of each mechanism and their importance during action potential propagation.
Method: We simulated the cardiac myocyte by the Orcad software, then used the model of sinoatrial node to stimulate the myocytes model and studied the propagation of action potential with and without gap junction.
Result: Our results show that, although gap junction solely is not able to mimic physiological condition, but it is necessary for normal cardiac functioning. On the other hand, electric field is not sufficient for successful propagation of action potential and the existence of gap junction is necessary.
Conclusion: Gap junction has a dynamic behavior in each cardiac cycle, managing different routes of propagation in the diverse moments. Our results support that gap junctions could be open in phases 0, 1, 3, 4 and close in phase 2 of action potential. Whenever gap junction is open, conduction can be fulfilled rapidly by current flow and whenever it is closed, the electrical field will be the main route of propagation. When the prejunctional cell is in the peak of AP, gap junction is closed and the postjunctional cell should use the electrical field to be stimulated.

M340

ANTHRAX LETHAL FACTOR (ALF) - SUBSTRATE INTERACTION THROUGH MOLECULAR DYNAMICS SIMULATIONS

G.A. Dalkas[1], A. Papakyriakou[2], A. Vlamis-Gardikas[3], G.A. Spyroulias[1],
G. Pairas[1], P. Cordopatis[1]

[1]Department of Pharmacy, University of Patras [2]Institute of Physical Chemistry, NCSR "Demokritos", Attikis [3]Foundation for Biomedical Research of the Academy of Athens, Greece

Anthrax is a disease of animals and humans, caused by the bacterium Bacillus anthracis. Anthrax toxin (AT) consists of three proteins, one of which is the anthrax lethal factor (ALF). ALF is a gluzincin Zn-dependent highly specific metalloprotease (~90.000 kDa), which belongs to the M34 family of the MA clan of zinc metalloproteases. ALF cleaves most isoforms of mitogen-activated protein kinase (MAPK)-kinases (MEKs) close to their amino termini, leading to the inhibition of one or more signaling pathways. No data are available on the enzyme-substrate interaction at the molecular level.

Therefore, we performed classical molecular dynamics simulations on the ALF-MKK/MEK complexes in order to probe protein-substrate interactions. The simulations pinpointed specific hydrophobic as well as electrostatic ALF-peptide substrate interactions and these data were exploited in the building of virtual combinatorial libraries of di- and tri-peptides using the twenty native aminoacids. By applying docking simulations to Anthrax Zn-metalloprotease around 1.000 peptide substrates were virtually screened according to their binding affinity. Data suggest that complexes of ALF with peptides substrates bearing Arg, Trp, Lys and Phe aminoacids, exhibit the highest binding affinity providing evidence for electrostatic interactions between negatively charged residues of ALF's active site and positively charged side-chains of di/tri-peptides. New libraries of substrates were built incorporating non-protein residues, organic moieties and chelating groups. ALF-substrate complexes with the best score (in terms of binding energy) are further analysed.

Tu341

NEW BRADYKININ ANALOGUES ACYLATED ON THEIR N-TERMINUS: EFFECT ON RAT BLOOD PRESSURE AND RAT UTERUS

O. Labudda-Dawidowska[1], D. Sobolewski[1], M Śleszyńska[1], I Derdowska[1],
J. Slaninowa[2], T. Wierzba[3], **A. Prahl**[1]

[1]Department of Chemistry, University of GdańSk, Poland [2]Department of Biological Chemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic, Prague, Czech Republic [3]Department of Phisiology, Medical Academy of GdańSk, Poland

In the present studies we designed and synthesised seven new bradykinin (BK) analogues and evaluated them in the in vivo rat uterotonic assay using a modified Holton method in Munsick solution on a strip of rat uterus and in blood pressure test. We used [Arg0, Hyp3, Thi5, 8, D-Phe7]BK, the B2 antagonist of Vavrek and Stewart as a model, when designing our analogues. In all cases, the N-terminus of our peptides is acylated with bulky substituent. We previously reported that acylation of the N-terminus of several known B2 antagonists with various kinds of bulky acyl groups has consistently improved their antagonistic potency in rat blood pressure assay. On the other hand, our earlier results seem to suggest that effects of acylation on the contractility of isolated rat uterus depend substantially on the chemical character and size of the acyl group, as we observed that this modification may either change the range of antagonism or even transform it into agonism. The peptides were synthesized by the solid-phase method using the Fmoc-strategy

The modifications proposed either preserved or increased the antagonistic potency in the rat blood pressure test. On the other hand, the seven substituents, differently influencend the interaction with the rat uterine receptors and except one led to decrease of antiuterotonic activity. In both cases acylation of the N-terminus led to enhancement of antagonistic potencies. Our results may be of value in the design of new B2 agonists and antagonists.

PREDICTION OF FIBRIL CORE REGIONS IN AMYLOID FORMING PROTEINS

M. Saiki[1], T. Konakahara[2], Y. Hidaka[1], H. Morii[3]

[1]Department of Life Science, School of Science And Engineering, Kinki University, Higashi-Osaka [2]Faculty of Science and Technology, Tokyo University of Science (RIKADAI), Noda, Chiba [3]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

The formations of amyloid fibrils have been reported as for various amyloidosis. Several structural models of fibrils are proposed for respective proteins so far. However, their common basic structures and universal features to induce amyloid fibril formations are not known in detail. Previously, we examined intermolecular interactions among the several amino acid residues in barnase, which is known to form amyloid-like fibril. Based on the experimental results using a series of mutant barnase, we discovered that the interactions between hydrophobic side-chains are the most essential driving force to form the fibrils and that both intermolecular and inter-sheet interactions in the fibril maintain highly ordered molecular packing. In the present paper, we describe a novel prediction method for core regions of various fibril-forming proteins and show the verification of the above possible structural principle. At first, we calculated the interaction's score between side-chains in the antiparallel orientation of beta-strands. Next, the peptides with predicted sequences of fibril cores, a couple of high-scored regions with a designed turn moiety to induce a hairpin-like form, were chemically synthesized by SPPS. As a result, the formation of amyloid fibrils was confirmed for most of high-scored sequences. In addition, we also applied this method to prion protein, we could predict 4 possible beta-strands with hetero-paired orientation. Some synthetic peptides involving these strands were proved to have fibril-forming ability. Thus, we have developed the novel method to predict the core regions that induce amyloid fibrils.

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MOLECULAR DYNAMICS SIMULATIONS OF SIX POTENTIAL T CELL EPITOPES OF THE LA/SSB AUTOANTIGEN COMPLEXED WITH HLA-DQ7

A. Kosmopoulou[1], A. Stavrakoudis[1,2], C. Sakarellos[1], **M. Sakarellos-Daitsiotis**[1]

[1]Department of Chemistry [2]Department of Economics, University, Ioannina, Greece

In this study, molecular dynamics (MD) simulations were performed with TINKER molecular modeling software package to examine the binding of the modeled HLA-DQ7 complexed with six potential T cell epitopes of the La/SSB autoantigen. The proposed epitopes were determined by combining three different computational methods including the binding motifs of Taylor, the TEPITOPE software and the MULTIPRED program. MD simulations at 300K of 150ps duration were carried out for the six modeled HLA-DQ7-epitope complexes and compared with the crystal structure of HLA-DQ8-insulin-B peptide complex used as template. A gross overview of the MD simulations is provided by the root mean square deviation (rmsd) plots. The rms deviations were computed only for the Cα atoms of the complexes as a function of time. The potential energy of interaction, ΔEint, between the modeled HLA-DQ7 and each one of the potential T cell epitopes, the variations of the solvent accessible surface areas (SASA) of the predicted epitopes during the MD simulations within the binding groove of DQ7 and the free energy of binding ΔGbind of the complexes were also calculated. The proposed T cell epitopes, classified according to their binding efficiency, could act as immunotherapeutic drug-like molecules.

EXAMINATION OF THE MD TRAJECTORY OF THE OPENING OF THE NF-KB USING PFA

B. Smiech, R. Kazmierkiewicz, B. Lammek

Faculty of Chemistry, University of Gdansk, Poland

Principal factor analysis (PFA) is a very efficient way of identifying patterns in the data sets even if the patterns are hard to find (e.g. in the high dimensional data sets). This is the reason why the PFA method can be powerful tool for analyzing molecular dynamics (MD) trajectories. It is possible to reduce dramatically the trajectory size without loosing significant structural information by applying the PFA procedure. We used this tool for interpretation of results from the molecular dynamics simulations of the model of the transcription factor NF-kB. NF-kB is a protein involved in the numerous biological processes such as regulation of immune response, inflammation, various autoimmune diseases and is used by many viruses, including human immunodeficiency virus (HIV), to activate transcription of their own genes. Only the trajectory of the backbone atoms of the NF-kB were subjected to the further analysis.

Using PFA, we found that only first few degrees of freedom per trajectory are necessary and sufficient to describe in full the motions relevant for the function of the protein. The examination of the extreme protein conformations generated using PFA reveals the asymmetry of the process of opening of the protein molecule.

STEREOCHEMISTRY INFLUENCE ON PROTON TRANSFER IN PROTONATED TRIPEPTIDE MODEL

N.A. Soliman, P. Kulhanek, J. Koca

National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic

Peptides contain many basic sites such as side chains of basic amino acid residues, oxygen and nitrogen atoms of amide groups, and terminal amino groups. These parts can interact with protons. This interaction can change conformational behaviour of peptides and, consequently, their biological functions. The interaction becomes even stronger in the gas phase. In that case, the stability of the peptide chain is influenced, which may have impact on peptide fragmentation during mass spectroscopy analysis of peptide structures.

In this study, we will present the interaction of proton with carbonyl oxygens in the model of alanine tripeptide. Quantum chemical calculations employing density functional theory using hybrid B3LYP functional and 6-31++G basis set were used to describe this interaction and also to find possible pathways of proton transfer among interaction sites. Two different mechanisms of proton transfer were found. The first mechanism is represented by an isomerization of the proton around the double bond of the carbonyl group. The second mechanism is based on the large conformational flexibility of the tripeptide model where all carbonyl oxygens cooperate. The later mechanism exhibits nearly half energy barrier of the rate-determining step compared to the first one. We focus our attention on situation, in which methyl groups attached to alpha atoms in tripepetide model influence the conformational behavior. Results will be presented for all four possible stereochemical configurations.**

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STRUCTURE VARIATION IN E3 LIGASES UBIQUITINATION PLATFORM

A. Papakyriakou[1], P. Galanakis[2], P. Gazonis[2], **G.A. Spyroulias**[2]

[1]Institute of Physical Chemistry, NCSR "Demokritos", Attikis [2]Department of Pharmacy, University of Patras, Patras, Greece

p53 protein is one of the most effective defensive weapons of human body against carcinogenesis, due to its tumor suppression properties. It has been noticed, in many types of cancer, that the functions of p53 are being downgraded or even suppressed and this fact is ought to the presence of mutated forms of p53 or to the complete absence of the protein. The suppression of p53 levels is being indirectly regulated by the protein itself, which activates the expression of a gene, the oncogene mdm2 (murine double minute 2), which expresses the MDM2 protein, known as human-MDM2 or just HDM2. HDMX protein is a homologue protein to HDM2 and is being implicated, through various biological processes, in the suppression of p53. However, recent experimental evidence suggests that HDM2 and HDMX proteins are not the only ubiquitin ligases that negatively regulate p53 through ubiquitin pathway. Two recently discovered E3 ligases, COP1 and Pirh2, are also proposed to promote p53 for degradation. All these proteins function as E3 ligases bearing a Ring finger domain. These domains are characterized by their high content in cysteines and the binding of two Zn(II) ions while they catalyze the latter stage of protein signaling for proteolysis by the 26S proteasome, through the ubiquitin pathway. The structure variation and the stability of these Ring fingers is studied through molecular dynamics simulations of 2-5 ns and structure variations are analyzed in a Structure-Function correlation basis.

COMPARATIVE STUDY OF NOOTROPIC AGENT SEMAX AND ITS ANALOGOUS USING MOLECULAR DYNAMIC METHOD

K.B. Tereshkina, K.V. Shaitan

Department of Bioengineering, Faculry of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia

Semax is a synthetic analogue of adrenocorticotropic hormone ACTH 4–10. It is a nootropic agent containing seven amino acids Met- Glu -His-Phe-Pro-Gly-Pro without hormonal (adrenocorticotrophic) activity. Semax is neuroprotective via a mechanism involving the regulation nitric oxide (NO) and lipid peroxidation. Semax proved to be highly effective in abating the rise in NO and restoring neurologic functioning [1]. It was found to improve intellect and memory in healthy human. It is effective in rehabilitation of people with memory and motor disorders, Parkinson's and Hantington's diseases, after cerebral stroke and head trauma [2].

To study conformation dynamics in connection with in vivo activity of Semax the molecular dynamics method of standard protocol was applied [3]. Semax and about twenty its analogs were studied. Using cluster analysis method Semax was found to be more labile among various synthesized analogous (Met- Gln -His-Phe-Pro-Gly-Pro; Gly-Glu-His-Phe-Pro-Gly-Pro; Lys-Glu-His-Phe-Pro-Gly-Pro; Glu-His-Phe-Pro-Gly-Pro; His-Phe-Pro-Gly-Pro). Because of collective degree of freedom it has one more stable configuration that is unreachable in analogs. Singularities of Semax and analogous were studied using 2-D, 3-D Poincare maps, auto and cross-correlation functions of special type in terms of topological structure of energy hypersurface.

This work was supported by RFBR (pr. 04-04-49645), Russian Ministry of Education and Science, Moscow Government and CRDF.

[1]V.G.Bashkatova at al.//Brain Research(2001),894,145–9; [2]I.P.Ashmarin at al.//Pathophysiology(2005),11,179-185; [3]K.B.Tereshkina at al.//Biophysics(2005),6 (in press).

SIDE CHAIN AND BACKBONE CONFORMATIONAL PREFERENCES OF –CXC- CONTAINING PEPTIDES EVIDENCED BY COMPUTATIONAL STUDIES

A. Stavrakoudis, **V. Tsikaris**

Department of Chemistry, University of Ioannina, Greece

Cyclization via a disulfide bond is a widely used strategy to design constrained peptide analogues. In this study we continue our previous work on the conformational preferences of highly constraint peptide analogues containing the (S,S)-CDC- motif [1]. We have performed molecular dynamics calculations of the linear peptides: Ac-RCDC-NH2, Ac-RCNC-NH2, Ac-CDCR-NH2, Ac-CNCR-NH2, Ac-CRCD-NH2, Ac-CRCD-NH2 and their corresponding cyclic (S,S) analogues. The main goals of this study is to explore the influence of a) the (S,S)-CXC- cyclic motif and b) the charge-charge interactions to the relative orientation of the X and X-2 or X+2 amino acid residue side chains. Our findings indicate that independently of the nature of the X residue, there is a preference for an almost cis coplanar orientation of the X and either one of the adjacent to cysteine residue side chains. The conformation of the X residue is constrained in the region (-80o, 60o) for the Φ and Ψ backbone dihedral angles. We did not observed any b-turn conformation neither in linear nor in cyclic peptides. It is concluded that the (S,S)-CXC- motif can be incorporated in peptide analogues in which the cis coplanar orientation of the corresponding amino acid side chains is desirable. **Acknowledgments: This work was supported by grants from EU and the Hellenic Ministry of Education (Pythagoras II).**
References: 1. Stavrakoudis, A. et al. (2001) Biopolymers 56, 20-26.

M349

MOLECULAR SYMULATIONS OF RHODOPSIN TETRAMER

M. Witt, J. Ciarkowski, C. Czaplewski

Faculty of Chemistry, University of Gdansk, Poland

Rhodopsin (RD) is the only representative of G-protein coupled receptors (GPCRs) whose structure has been described with high resolution. Thus, it has become the structural prototype for other GPCR. These receptors are involved in transduction of various signals into the cell and actions of many hormones and neurotransmitters. About 50% of all drugs act through GPCR.

Growing evidence that RD and related GPCRs form functional dimers/oligomers, followed by direct proof (using atomic force microscopy - AFM) that in the retina RD associates into a paracristalline network of rows of dimers, need models of RD-transducin (G□□□t - heterotrimeric protein) complex that would envision an optimal RD dimer/oligomer amenable to satisfy all well documented interactions with Gt.

Current model includes tetramer built of two activated (MetaII) and two inactive RD molecules, ligands stabilising MetaII: Gtα(Ile338-Phe350) and Gtγ(Asp60-Cys71)farnezy, lipid bilayer built of 36 PC (phosphatidylocholin head groups) , 6 PS (phosphatidyloserine) and 30 PE (phosphatidyloetanolamine) (all three types of phospholipids contain the polyunsaturated docosahexaenoyl chain - DHA) and water. Experimental data concerning shape of oligomer, conformational changes in MetaII, proper interactions and distances among residues have been looked upon.

The poster shows results of the molecular dynamic carried in Amber force field for ~6000ps in the periodic box. Conformational changes which took place during simulation caused proper adaptation one another monomers in tetramer and ligands to activated receptors.

Supported by KBN grant DS 8372-4-0138-6 and BW 1132/t09/2005/28.

Tu350

CONFORMATIONAL STUDIES OF THE BETA2-L1-BETA3 HUMAN CYSTATIN FRAGMENT AND ITS MUTANTS

J. Łągiewka, R. Sosnowska, S. Rodziewicz-Motowidło, K. Stachowiak,
S. Ołdziej, Z. Grzonka

Faculty of Chemistry, University of Gdansk, Poland

The human cystatin C (hCC) is a one of known domain swapping proteins. During this process, one of the hCC β-hairpins (β2-L1-β3) changes its conformation forming long β-strand. This conformational transition destabilizes the monomer structure and leads to domain-swapped dimer. The causative force for changing the β-hairpin conformation is assumed to be the alleviation of distortions of the L1-loop Val57 amino acid residue's backbone. Following the above assumption and our previous conformational studies of the hCC β-hairpin peptide we investigated the influence of the point mutations, V57D, V57P and V57N of the Val57 residue, on the β-hairpin peptide structure. The conformational studies by means of CD spectroscopy and molecular dynamics studies were performed. The study revealed that the hCC peptide with the wild-type sequence has the strongest tendency from all studied peptides to form a β-hairpin structure. On the basis of these results we conclude that the presence of distortions in the Val residue of L1-loop is unlikely to cause the 3D domain swapping of the human cystatin C.

Acknowledgments:
This work was financially supported by the Ministry of Scientific Research and Information Technology of Poland under grant 1T09A10430.

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CONFORMATIONAL BEHAVIOUR OF TEMPORIN A AND TEMPORIN L BY NMR SPECTROSCOPY IN DIFFERENT ENVIRONMENTAL CONDITIONS

L. Auriemma[1], A. Carotenuto[1], A. Mazzella di Bosco[1], M.L. Mangoni[2], P. Campiglia[3], P. Grieco[1]

[1]Dipartimento di Chimica Farmaceutica E Toss., Università di Napoli Federico II, Napoli [2]Dipartimento di Scienze Biomediche, Università la Sapienza, Roma [3]Dipartimento di Scienze Farmaceutiche, Università di Salerno, Italy

Temporin A (TA) (FLPLIGRVLSGIL-NH2) and Temporin L (TL) (FVQWFSKFLGRIL-NH2) are small, basic, hydrophobic, linear antimicrobial peptides amide found in the skin of the European red frog, Rana temporaria. These peptides have variable antibiotic activities against a broad spectrum of microorganisms, including clinically important methicillin-sensitive and resistant Staphylococcus aureus as well as vancomycin-resistant Enterococcus faecium strains. To gain further insight into the mechanism of action of these small antimicrobial peptides, we have investigated their conformational behaviour in different environmental conditions. More specifically, we deeply investigated by solution NMR spectroscopy in water and water/DMSO (8:2) solutions as isotropic solutions and 200 mM aqueous solution of DPC (dodecylphosphocholine) was used as membrane mimetic environment.

Understanding the basis of the interactions of temporins with membranes could be crucial for the design and synthesis of potent antimicrobial agents.

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SDF GAMMA : SYNTHESIS AND HEPARIN BINDING SITES IDENTIFICATION

F. Baleux[1], F. Arenzana-Seisdedos[2], H. Lortat-Jacob[3]

[1]Unité de Chimie Organique [2]Laboratoire de Pathogénie Virale Moléculaire, Institut Pasteur, Paris [3]Institut de Biologie Structurale, Grenoble, France

Chemokines are small proteins involved in numerous biological processes (inflammation, immunity, morphogenesis, tissue repair, and tumour development). Chemokines activity is also thought to be regulated through binding to heparan sulfate (HS), a complex polysaccharide found on almost cell surface and within extracellular matrix. The general goal of our project is to elucidate the role that HS plays in vivo in the physiologic and pathologic activities of the chemokine CXCL12/Stromal Derived Factor-1, and to characterise the molecular and structural determinants accounting for the interaction. Three SDF isoforms, alpha (68 aa), beta (72 aa) and gamma (98 aa) have been identified. We previously identified the major heparin binding site on SDF alpha and demonstrated the importance of HS/SDF interaction in HIV entry cell inhibition (1,2). SDF gamma amino acids sequence corresponds to the SDF alpha sequence extended by a C-ter 30 amino acids sequence containing putative heparin binding sites. In order to determine SDF gamma heparin binding sites, wild type and mutants proteins were synthesised by stepwise solid-phase peptide synthesis using Fmoc chemistry. Synthesis and comparative elution profiles on heparin column will be presented.

1- A. Amara, & all JBC, 1999, 274(34), 23916-23925
2- A. Valenzuela-Fernandez,& all. JBC, 2001,276, 26550-26558

Tu353

THE CHEMICAL SYNTHESIS OF CRIPTO CFC VARIANTS

D. Marasco[1], A. Saporito[1], S. Ponticelli[2], A. Chambery[3], S. De Falco[2], C. Pedone[1], G. Minchiotti[2], **E. Benedetti**[1], M. Ruvo[1]

[1]Istituto di Biostrutture E Bioimmagini del CNR, Sezione Biostrutture [2]Istituto di Genetica E Biofisica Adriano B. Traverso" Del CNR, Napoli [3]Facoltà di Scienze MMFFNN, Seconda Università di Napoli, Caserta, Italy

Cripto is the founding member of a family of soluble and cell bound growth factors known as EGF-CFC [1] distinguished by the presence of an N-terminal signal peptide, two distinct cysteine-rich domains (CRD) and a C-terminal hydrophobic region involved in cell surface attachment by a post-translational GPI modification. The characteristic CRDs, known as EGF-like and CFC domains (from the first members Cripto, FRL1 and Cryptic), both span about 40 residues with 3 disulfide bridges [2] each, which, presumably, beside a possible functional modularity, confer them also a structural independence.
In this work we have focused our attention on the CFC domain of mouse Cripto. The domain has been produced by SSPS, along with variants bearing mutation on H104 and W107, that have been described as crucial for Alk4 receptor recognition. The two variants have been purified and refolded, achieving the correct disulfide bridges, and then comparatively analyzed by CD spectroscopy under different pH conditions; thus obtaining experimental insights on the structural arrangements of this new class of protein domains. Furthermore, the binding properties of wild type and mutants CFC domains to Alk4 receptor have been determined by using an ELISA-based assay. Our results demonstrated that the CFC domain alone can directly bind Alk4 in the absence of additional ligands and, furthermore, confirmed a role of H104/W107 in Cripto/Alk4 interaction.

References:
1.Minchiotti G. Oncogene 2005; 24, 5668−5675.
2.Foley Sf.et al Eur. J. Biochem. 2003; 270 (17), 3610-3618.

CONFORMATIONALLY CONSTRAINED CCK8 ANALOGUES AS LIGANDS FOR CHOLECYSTOKININ TYPE-B RECEPTOR

E. Benedetti[1], S. De Luca[1], R. Della Moglie[1], M. Saviano[1], G. Digilio[2], L. Aloj[3], G. Morelli[1]

[1]Department of Biological Science & CIRPeB, Univerity of Naples "Federico II", Naples [2]Bioindustry Park Canavese, Colleretto Giacosa (TO) [3]Department of Nuclear Medicine, National Institute of Tumors, "Fondazione Pascale", Napoli, Italy

There is considerable interest in the pharmacology of the two cholecystokinin (CCK) receptors CCKA-R (or CCK-1) and CCKB-R (or CCK-2) that mediate the biological action of the CCK hormone. They are membrane receptors belonging to the superfamily of G-protein coupled receptors (GCPR) and are predominantly located in the gastrointestinal tract and in the central nervous system, respectively.

A library of 14 cyclic peptide analogues derived from the octapeptide C-terminus sequence of the human cholecystokinin hormone [CCK(26-33), or CCK8] has been designed, synthesised and characterised. The 14 peptide analogues have been rationally designed to specifically interact with the CCK type B receptor (CCKB-R) on the basis of the structure [2] of the bimolecular complex between CCK8 and the third extracellular loop of CCKB-R [namely, CCKB-R(352-379)].

The new ligands showed binding affinities generally lower than that of parent CCK8. Anyway, structure activity relationship data underline that preservation of the Trp30-Met31 motif is essential, and that the Phe33 side chain and a carboxylic group close to the C-terminal end must both be present. The NMR conformational study in DPC micelles of the compound endowed with maximal binding affinity (cyclo-B11, IC50=11 M) shows that this compound presents the turn-like conformation, centred at the Trp30-Met31 segment, as planned by rational design, and that such conformation is stabilised both by the cyclic constrain and interaction with the micelle.

References:

[1] D. Fourmy, et al. Pharmacology & Toxicology 2002, 91, 313-320.

[2] C. Giragossian, D.F. Mierke, Biochemistry 2001, 40, 3804-3809

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NMR STRUCTURAL STUDY OF CFC CRIPTO DOMAIN

A. Saporito[1], L. Calvanese[2], D. Marasco[1], S. Monti[1], L. Falcigno[2], G. D'Auria[2],
C. Pedone[1], **E. Benedetti**[1], M. Ruvo[1]

[1]Istituto di Biostrutture E Bioimmagini del CNR, Sezione Biostrutture [2]Dipartimento di Chimica, Università Federico II, Complesso Universitario MSA, Napoli, Italy

Cripto is the founding member of a family of extracellular growth factors called EGF-CFC found in mouse, human, chicken, xenopous and zebrafish [1]. These proteins are characterized by the presence of an N-terminal signal peptide, a C-terminal hydrophobic region and two highly conserved cysteine-rich domains, the EGF-like (Epidermal Growth Factor) and the CFC (CRIPTO/FRL1/Cryptic). Cripto is strictly required in the early embryonic development and contributes to deregulated growth of cancer cells in adults, since it is highly over-expressed in many solid carcinomas. It has been proposed that each single domain of Cripto could bind different protein partners, playing different functional roles [2]. On this grounds, investigation of the single domains 3D-structures can have also strong functional implications. We present here an extensive conformational analysis of the mouse CFC domain (96-134 sequence) and of the W107A mutant based on NMR data. Sequences have been synthesized by SPPS and refolded reconstituting the correct disulfide bridges [3]. The molecular models have been built by computational methods using the NMR data collected under both acidic (pH 3) and nearly physiological (pH 6) conditions. Both domains show a globally extended folding with three strands linked by the three disulfide bridges and two connecting loops, in which H104 and W107, key residues in receptor binding, are exposed to the solvent

References:
1.Adamson, E. D., et al., 2002, J. Cell. Phys. 190, 267-278.
2.Schiffer, S. G. et al, 2001, J. Biol. Chem. 276, 37769-37778.
3.Marasco D., et al., Proteins Structure and Function, submitted.

CHARACTERIZATION OF UROTENSIN-II RECEPTOR STRUCTURAL DOMAINS INVOLVED IN THE RECOGNITION OF U-II, URP AND URANTIDE

S. Boivin[1], L. Guilhaudis[2], I. Milazzo[2], H. Oulyadi[2], D. Davoust[2], A. Fournier[1]

[1]LEMPP, INRS - Institut Armand-Frappier, Université du Québec, Pointe-Claire, Montréal, QC, Canada [2]IFRMP 23, UMR 6014 CNRS, Université de Rouen, Mont-Saint-Aignan, France

Urotensin-II (U-II) and urotensin-II-related peptide (URP) are potent vasoconstrictors and this action is mediated through a G protein-coupled receptor identified as UT. This receptor is expressed abundantly in the mammalian cardiovasculature and the effects of U-II and URP can be blocked with Urantide, a selective antagonist. Thus, we carried out a study aiming at the characterization of conformational arrangement and affinity properties of UT extracellular segments.We measured by surface plasmon resonance (SPR) technology the binding affinities of the three ligands, U-II, URP and Urantide towards the three extracellular loops of UT. Furthermore, the secondary structures of the synthetic receptor fragments in presence of dodecylphosphocholine micelles and interaction with UT ligands were analysed using NMR spectroscopy. SPR data showed that the EC loop II was able to recognize the ligands U-II, URP and Urantide with similar affinities while none of these two ligands were able to interact with the extracellular loop I. Furthermore, the absence of binding of Urantide, a peptide antagonist, suggested strongly that loop III would be involved in the signal transduction process and implies that U-II and URP, but not Urantide, would bind to UT according to a common pattern. Moreover, the results indicate that potent UT antagonists could be designed by producing high-affinity ligand targeting the extracellular loop II. Also, the SPR and NMR studies revealed that the synthetic structural UT domains contained some of the conformational and chemical features essential for the binding of hU-II, URP and Urantide to hUT.

UNEXPECTED DIFFERENT DISULFIDE CONNECTIVITIES OF CYSTEINE-RICH PEPTIDES COMPRISING IDENTICAL CYSTEINE PATTERNS

C. Boulègue[1], A.G. Milbradt[1], C. Renner[2], L. Moroder[1]

[1]Max-Planck-Institute of Biochemistry, Martinsried, Germany [2]School of Biomedical and Natural Sciences, Nottingham Trent University, Nottingham, UK

Synthetic cysteine-rich replicates of naturally occurring peptides such as hormones, neurotransmitters, enzyme inhibitors, defensins and toxins often can be oxidatively folded in high yields to their native structure. The presence of identical cysteine patterns in the sequence were found to lead to identical disulfide connectivities and homologous spatial structures despite significant variability in the non-cysteine positions. Therefore, it is generally accepted to attribute the disulfide connectivities based on the homology of their cysteine pattern.
Minicollagen-1 from the nematocysts of Hydra is a trimeric protein containing N- and C- terminal cysteine-rich domains involved in the assembly of an intermolecular disulfide network. Examination of three-dimensional structures of peptides corresponding to these folded domains by NMR spectroscopy revealed a remarkable exception from the general admitted rule [1].

Despite an identical cysteine pattern, they form different disulfide bridges and exhibit distinctly different folds. Additionally, comparative analysis of the oxidative folding revealed for the C-terminal domain a fast and highly cooperative formation of a single disulfide isomer, the N-terminal domain proceeding mainly via an intermediate that results from the fast quasi-stochastic disulfide formation according to the proximity rule. To our knowledge, this is the first case where two short peptides with identical cysteine pattern fold uniquely and with high yields into defined, but differing, structures. Therefore, these cysteine-rich domains may well represent ideal targets for structure calculations to learn more about the elementary information encoded in such primordial molecules.

[1]Milbradt, A. G., Boulègue, C., Moroder, L., Renner, C. (2005) J. Mol. Biol. 354, 591-600.

M358

FOLDING NUCLEUS OF SHEEP PRION PROTEINS

K.C. Chen, W.J. Wedemeyer

Biochemistry Department, Michigan State University, East Lansing, MI, USA

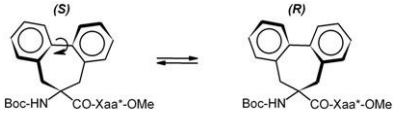
The conformational change of the cellular prion protein, PrPC, to its virulent "scrapie" form, PrPSc, is believed to be responsible for prion infectivity. Recently, stopped-flow kinetic studies detected an intermediate in the refolding pathway of a prion protein, which may be a precursor of the PrPSc isoform. It has been hypothesized that this folding intermediate corresponds to local structure in the vicinity of the lone disulfide bond found in prion proteins. Such local structure has been observed in other disulfide-bonded peptides and several studies suggest that the prion disulfide bond is important for the stability, structure, and propagation of prion oligomers. To test this hypothesis, we selected two conserved peptides flanking the disulfide bond in the sheep prion protein, and measured the secondary structure of these peptides with circular dichroism, hydrogen/deuterium exchange, and molecular dynamics simulations. Our preliminary data suggests that the two peptides do not adopt stable secondary structure, native or otherwise. Thus, the folding intermediate of a prion protein seems unlikely to comprise local structure around the disulfide bond.

THE "BIP METHOD" FOR ASSIGNMENT OF THE ABSOLUTE CONFIGURATION OF β-AMINO ACIDS: ICD IN THE BIPHENYL CORE OF BIP-β-XAA* DIPEPTIDES

L. Dutot[1], A. Gaucher[1], K. Wright[1], M. Wakselman[1], J.-P. Mazaleyrat[1],
C. Peggion[2], F. Formaggio[2], C. Toniolo[2]

[1]*SIRCOB, UMR CNRS 8086, University of Versailles, Versailles, France* [2]*Department of Chemistry, University of Padova, Italy*

The conformationally labile Cα-tetrasubstituted α-amino acid residue Bip possesses non isolable (R) and (S) atropoisomers. We have previously reported that in the linear dipeptides Boc-Bip-α-Xaa*-OMe with α-Xaa* = Ala, Val, Leu, Phe, (αMe)Val and (αMe)Leu residues at the C-terminal position of Bip, the onset of an equilibrium between diastereomeric conformers with unequal populations could be observed by CD and 1H NMR. The phenomenon of induced circular dichroism (ICD) represents the basis for the "Bip method", an easy and fast configurational assignment for chiral α-amino acids. In search for an extension of the Bip method, we investigated the Boc-Bip-β-Xaa*-OMe dipeptide series with β-Xaa* = β3-HAla, β3-HVal, β3-HLeu, β3-HPro, β3-HPhe, or the cyclic β2,3-amino acids (1S,2S)/(1R,2R)-ACHC and (1S,2S)/(1R,2R)-ACPC.
Low-temperature (233 K) 1H NMR spectra in CD3OD revealed the presence of two conformers. Significant d.r. (diastereomeric ratio) values were observed for all combinations of Bip with both β3- and cyclic β2,3-amino acids. CD analysis in MeOH solution of the Boc-Bip-β-Xaa*-OMe dipeptides allowed us to conclude that the CD resulting from the induced axial chirality in the biphenyl core of the Bip residue gives clear information on the β-Xaa* configuration for both β3- and cyclic β2,3-amino acids (except the aromatic β3-HPhe), with a P torsion of the biphenyl axial bond of Bip being preferentially induced by (L)-β3-Xaa* as well as cyclic (1S,2S)-β2,3-Xaa* C-terminal residues.



Boc-Bip-Xaa*-OMe

DESIGNED 314-HELICAL β-PEPTIDES BASED ON CROWNED (S)-β3-H-DOPA COMBINED WITH (1S,2S)-ACHC RESIDUES

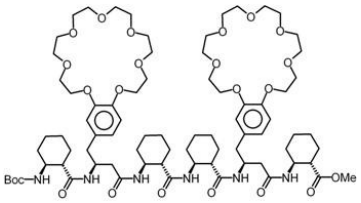
L. Dutot[1], A. Gaucher[1], K. Elkassimi[1], J. Drapeau[1], M. Wakselman[1],
J.-P. Mazaleyrat[1], C. Peggion[2], F. Formaggio[2], C. Toniolo[2]

[1]SIRCOB, UMR CNRS 8086, University of Versailles, France [2]Department of Chemistry, University of Padova, Italy

According to the well-documented ability of β-peptides to adopt a variety of helical secondary structures, different in nature and even more stable than those formed by α-peptides, we found it interesting to introduce crown-ether receptors on the side chains of β-amino acids in view of their assembly in structurally well-defined β-peptide architectures of bis-crown compounds. In connection with the pioneering studies of Voyer and coworkers,1 who explored the use of α-peptide scaffolds based on crowned (L)-DOPA residues for the construction of molecular receptors and devices, we synthesized the (S)-β3-H-DOPA analogue and exploited its catechol function to prepare a new series of β-amino acids carrying various crown-ether receptors on their side-chains: (S)-β3-H-DOPA[CROWN] with [CROWN] = [15-C-5], [18-C-6], [21-C-7], [Benzo-24-C-8] and [(R)-Binol-20-C-6].

Peptides based on these novel crowned β-amino acids, combined with the cyclic β-amino acid (1S,2S)-ACHC (2-aminocyclohexanecarboxylic acid) known as a potent 314-helix inducer, to the hexamer level, with two crowned residues at the i and i+3 positions of the main chain (a representative example is shown), were synthesized by solution methods. Their conformational analysis was performed using FT-IR absorption, NMR and CD techniques.

Reference
1. Biron, E., Otis, F., Meillon, J.-C., Robitaille, M., Lamothe, J., Van Hove, P., Cormier, M.-E., Voyer, N., Bioorg. Med. Chem. 2004, 12, 1279-1290.



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INDUCED AXIAL CHIRALITY IN THE BIPHENYL CORE OF 6, 7-DIHYDRO-5H-DIBENZ[C, E]AZEPINE DERIVATIVES OF α- AND β-AMINO ESTERS

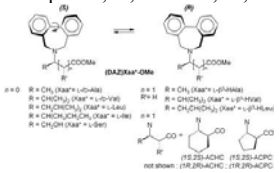
L. Dutot[1], K. Wright[1], M. Wakselman[1], J.-P. Mazaleyrat[1], C. Peggion[2], F. Formaggio[2], C. Toniolo[2]

[1]*SIRCOB, UMR CNRS 8086, University of Versailles, Versailles, France*
[2]*Department of Chemistry, University of Padova, Italy*

We have recently reported that the induced circular dichroism (ICD) of the biphenyl core of Boc-Bip-Xaa*-OMe dipeptides based on the conformationally labile Cα-tetrasubstituted α-amino acid residue Bip could allow an easy and fast configurational assignment for both α- and β-Xaa* amino acid residues. In search for other biphenyl/Xaa* architectures in which a transfer of central to axial chirality could result in a potentially useful ICD, we considered N-substituted 6,7-dihydro-5H-dibenz[c,e]azepine (DAZ) derivatives from α- and β-amino acids as interesting candidates. In the present communication, we report the syntheses, and the 1H NMR and CD analyses of a series of (DAZ)Xaa*-OMe amino esters derived from α-, β3-, and cyclic β2,3-Xaa* residues, namely D-/L-Ala, D-/L-Val, L-Leu, L-Ile, L-Ser, L-β3-HAla, L-β3-HVal, L-β3-HLeu, (1S,2S)/(1R,2R)-ACHC and (1S,2S)/(1R,2R)-ACPC. The parent amide derivatives Xaa*-DAZ have been recently investigated by Rosini and coworkers.1

Reference:

1. Superchi, S., Bisaccia, R., Casarini, D., Rosini, C., 10th International Conference on Circular Dichroism, Book of Abstracts (2005), p. 5.



Tu362

STRUCTURAL CHARACTERIZATION OF AMYLOID BETA-PEPTIDE (25-35) IN PRESENCE OF NICOTINE AND ITS SYNTHETIC ANALOGUES.

C. Esposito, M.R. Armenante, M. Scrima, P. Campiglia, A.M. D'Ursi

Dipartimento di Scienze Farmaceutiche, Università Di salerno, Fisciano, Italy

The major components of neuritic plaques found in Alzheimer Disease (AD) are peptides known as amyloid β-peptides (Aβ-peptides). Aβ-(25-35) sequence (GSNKGAIIGLM) is a synthetic derivative of amyloid β-peptide, that is highly toxic and forms fibrillar aggregates typical of β-amyloid. Like the Aβ-(1-42), Aβ-(25-35) undergoes a conformational transition from a soluble, alpha-helical form to neurotoxic aggregated fibrillary β-sheet structures. Thus, it can be used as a suitable model of full-length peptides, for testing inhibitors of aggregation and toxicity. A largely employed approach, in the research of anti Alzheimer molecules, involves the research of disaggregating and/or aggregation preventing substances. Thousand of compounds have been screened in the attempt to find molecules with a powerful anti-aggregating or disaggregating property. Many studies show the ability of nicotine to inhibit the conversion of several synthetic fragments of Aβ-peptide (1-40) from its soluble into insoluble form, nevertheless the modality of interaction of Aβ peptides with nicotine is still an opened question. Here, we present a NMR and CD structural investigation of Aβ-(25-35) in the presence of nicotine and a series of nicotine related compounds. The analysis of NMR and CD data lead to formulate hypotheses on the mechanism of Aβ-(25-35) anti-aggregating process mediated by nicotine and its related compounds.

EPR DISTANCE MEASUREMENT IN A DOUBLY NITROXIDE-LABELLED HELICAL β-PEPTIDE

F. Formaggio[1], C. Toniolo[1], J.P. Mazaleyrat[2], K. Wright[2], F. Dal Porto[1], C. Corvaja[1], A. Toffoletti[1]

[1]Department of Chemistry, University of Padova, Italy [2]SIRCOB, UMR CNRS 8086, University of Versailles, France

β-Peptide molecules possess interesting conformational characteristics and biological properties. They may represent a new class of rigid foldamers potentially useful as templates or spacers. 3D-structures of β-peptides have been experimentally investigated using X-ray diffraction and various spectroscopic techniques, but they have never been doubly spin labelled and studied by EPR.

A terminally protected β-hexapeptide, based on trans-(3R,4S)-β-TOAC and trans-(1S,2S)-ACHC, synthesized using classical solution methods, was found by FT-IR absorption and CD techniques to adopt the 3-14-helical conformation.
EPR measurements of 1 in MeOH and HFIP (at 1.0 and 0.1 mM concentrations) were performed in the temperature range 120 K - 318 K. The spectra show three sharp lines with separations of about 1.5 mT (the same at all temperatures) superimposed on two broad signals, the separation of which increases as the temperature is lowered. The solvent and concentration effects are of minor significance. The spectra of the polycrystalline solid samples at low temperature extend over about 30 mT and their shape is mainly governed by the electron dipolar interaction. The intramolecular distance (6.1 ± 0.1 Å) between the nitroxide labels of the two β-TOAC residues at positions i and i+3, obtained from the analysis of the low temperature spectra, allowed us to confirm the expected ternary helical structure.

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ILE/ALLO-ILE DYAD: LACK OF SCREW-SENSE CONTROL OF THE 3-10-HELICAL STRUCTURE BY β-CARBON CONFIGURATION

F. Formaggio, E. Andreetto, M. Crisma, C. Toniolo

Department of Chemistry, University of Padova, Italy

A set of four, terminally blocked, hexapeptide sequences, each characterized by four strongly helicogenic Aib residues and all combinations of the two isomeric Ile/allo-Ile residues at positions 2 and 5 was synthesized by solution methods and fully characterized. A detailed solution (by FT-IR absorption, NMR, and CD) and solid (crystalline)-state (by CD and X-ray diffraction) conformational investigation allowed us to validate our assumption that all four peptides are folded in well developed 3-10-helical structures.

However, the most relevant conformational conclusion extracted from this 3D-analysis is that the handedness of the 3-10-helical structures formed does not seem to be sensitive to the configurational change at the β-carbon atom of the constituent Ile versus the diastereomeric allo-Ile residues (in other words, the dominant control on this important structural parameter appears to be exerted by the chirality of the amino acid α-carbon atom). These results complement published findings on the diverging relative stabilities of the intermolecularly H-bonded β-sheet structures generated by Ile versus allo-Ile homo-oligopeptides.

Taken together, these data represent an experimental proof for the intuitive view that potentially different conformational properties are magnified in a strongly self-aggregated homo-peptide system (as compared to weakly self-aggregated, helical, host-guest peptides such as those investigated in this work).

DESIGN AND CHARACTERIZATION OF SYNTHETIC BETA-SANDWICH PROTEINS

K. Fritzemeier[1], G. Gemmecker[2], H. Kessler[2], W. Haehnel[1]

[1]*Institute of Biology / Biochemistry, University of Freiburg, Freiburg* [2]*Institute of Organic Chemistry II, Technical University of Munich, Germany*

In a first approach to β-sandwich proteins the hydrophobic core between two symmetrical sheets each with four antiparallel β-strands was computationally designed by packing of amino acid side chain conformations (rotamers) in an initially given backbone structure. The proteins were synthesized by coupling four peptides with β-hairpin structure to a cyclic decapeptide template (TASP). An aggregation observed by equilibrium ultracentrifugation with the first designed proteins was decreased to a dimer by increasing the surface charge in two further variants of this protein from -1 to +3 and +5. Replacement of L-Pro by D-Pro in the loops and the template proved to stabilize the β-structure. These results led us to an improved design of an asymmetric core with algorithms for selection of proteins with a minimal number of atom clashes and cavities in the core, and a maximum number of hydrogen bonds after energy minimization. This protein termed beta-MOP (Modular Organized Protein) was synthesized in amounts to allow a characterization by CD, FTIR, tryptophan fluorescence during reversible unfolding, and by high resolution NMR. NMR measurements of diffusion indicate a dimeric structure. The β-structure is stable up to 80 °C (353 K) as determined by 1D 1H NMR showing sharp resonance lines. The 2D 1H,1H DQF-COSY spectrum at 750 MHz shows a typical β-sheet distribution extending well into the characteristic regions >8.5 ppm (for amide protons) and >5.0 ppm (for Hα signals). All data indicate a well folded protein with β-structure.

NMR STRUCTURAL ANALYSIS OF THE HIV-1 GP120 V3 – CCR5 CO-RECEPTOR N-TERMINAL INTERACTION

P.A. Galanakis[1], N. Kandias[1], G.A. Spyroulias[1], M. Sioumpara[2], D. Morikis[3], A. Rizos[4], E. Krambovitis[2]

[1]Department of Pharmacy, University Of patras [2]Department of Applied Immunology & Biochemistry, Institute of Molecular Biology And Biotechnology, FORTH, Heraklion, Crete, Greece [3]Department of Chemical and Environmental Engineering, University of California at Riverside, Riverside, CA, USA [4]Department of Chemistry, University of Crete, Heraklion, Crete, Greece

Standard multidimensional and multinuclear NMR spectroscopy was applied to probe the structural and physicochemical determinants of three representative peptides from V3 domain of the HIV-1 and a 22-residue peptide, representing the amino terminal of the chemokine receptor CCR5, in their free or interacting state. Titration of CCR5 peptide with V3-peptides was performed in NMR tube, at 286K. 1D 1H NMR spectra and 1H-15N HSQC were recorded after each addition of V3 peptides. Data analysis of HSQC and NOESY spectra verifies the interaction of the V3-CCR5 peptide constructs and according to it three sites of interaction can be noticed at the N terminal of CCR5 with different, most likely, binding affinity for the different V3 sequences. The participation of the three interacting sites seems to be enhanced with the increase of the positively charged amino acids at the 7/9-residue V3 N-terminal peptide fragment, which especially in V3 LAI peptide is rich in basic residues on either side the highly conserved GPGR motif, for which a β turn conformation is observed. SF2 and LAI V3 peptides adopt a β-hairpin like conformation, while MN V3 peptide preserves the loop that is being formed in the GPGR region but the C terminal and the N terminal are located in opposite directions. The analysis of the V3 peptides calculated structures reveals important similarities and differences which provide valuable information about the extent and the amino acids involved in their interaction with CCR5 co-receptor.

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AN NMR STUDY OF THE INTERACTION BETWEEN BRADYKININ AND ANGIOTENSIN-I AND SYNTHETIC PEPTIDE-BASED ANGIOTENSIN-I CONVERTING ENZYME CATALYTIC SITE MAQUETTES (CSM)

A.S. Galanis[1], Z. Spyranti[1], N. Tsami[1], G.A. Spyroulias[1], E. Manessi-Zoupa[2], G. Pairas[1], I.P. Gerothanassis[3], P. Cordopatis[1]

[1]Department of Pharmacy [2]Department of Chemistry, University of Patras [3]Department of Chemistry, University of Ioannina, Greece

Angiotensin-I converting enzyme (ACE) belongs to the M2 family of the MA clan of zinc metallopeptidases and can act either as a dipeptidyl carboxypeptidase, or as an endopeptidase. Among the ACE peptide substrates, the most distinguished are angiotensin I (AngI) and bradykinin (BK) due to their role in blood pressure regulation.

Despite the fact that biological data strongly suggest that the two active sites exhibit different selectivity and activity towards physiological and exogeneous substrates none experimental evidence for the interaction of AngI and BK with ACE catalytic sites, is available so far. A dual approach for studying the structure and physicochemical determinants of ACE-AngI/BK interaction has been performed. The first involves the application of molecular dynamics simulations (presented elsewhere in this book) and the second is making use of the solid-phase synthesized 36-46 aa ACE catalytic site maquettes (CSM) bearing the native sequence and the application of the NMR spectroscopy, and presented herein.

Therefore, high-resolution multinuclear NMR spectroscopy was applied to analyze the conformational features of ACE substrates AngI and BK in DMSO or aqueous mixtures. Then titration experiments were conducted and ACE CSMs were titrated by AngI/BK peptides, while monitored by NMR. 2D 1H-1H TOCSY and NOESY experiments were used in order to map the interaction site of both substrates and CSM through chemical shift perturbation and comparison of NOE signal differentiation. Competitive binding studies were also carried out through titration studies of CSM-AngI/BK and known ACE inhibitors.

EXPLORING THE CONFORMATIONAL BEHAVIOR OF MELANOCORTIN RECEPTORS LIGANDS IN DIFFERENT ENVIRONMENTAL CONDITIONS

A. Carotenuto[1], **P. Grieco**[1], L. Auriemma[1], E. Novellino[1], V.J. Hruby[2]

[1]Department of Pharmaceutical and Toxycological Chemistry, University of Naples, Italy [2]Department of Chemistry, University of Arizona, Tucson, AZ, USA

The melanocortine receptors are involved in many physiological functions, including pigmentation, sexual function, feeding behavior, and energy homeostasis, making them potential targets to treat obesity, sexual dysfunction, etc. Understanding the conformational basis of the receptor-ligand interactions is crucial for the design of potent and selective ligands for these receptors. The conformational preferences of the cyclic melanocortin agonists and antagonists MTII, SHU9119, [Pro6]MTII, and PG911 (Table 1) were comprehensively investigated by solution NMR spectroscopy under different environmental situations. In particular, water and water/DMSO (8:2) solutions were used as isotropic solutions, and a 200 mM aqueous solution of DPC (dodecylphosphocholine) was used as a membrane mimetic environment. Finally, we calculated the complexes of the obtained ligand structures with the hMC4 receptor, which is involved in feeding and sexual behavior.

Table 1.

Peptide	Sequence[a]
MTII	Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]-NH_2
SHU9119	Ac-Nle-c[Asp-His-DNal-Arg-Trp-Lys]-NH_2
[Pro6]MTII	Ac-Nle-c[Asp-Pro-DPhe-Arg-Trp-Lys]-NH_2
PG911	Ac-Nle-c[Asp-Hyp-DNal-Arg-Trp-Lys]-NH_2

[a] Nle = Norleucine; Nal = 2'-Naphtylalanine; Hyp = Hydroxyproline.

EXCITON COUPLED CIRCULAR DICHROISM OF INTRAMOLECULARLY INTERACTING BIS-PORPHYRIN CHROMOPHORES IN PEPTIDE CONJUGATES: DISTANCE AND ORIENTATION EFFECTS

I. Guryanov[1], A. Moretto[1], S. Campestrini[1], C. Peggion[1], F. Formaggio[1],
Q.B. Broxterman[2], B. Kaptein[2], C. Toniolo[1]

[1]Department of Chemistry, University Of Padova, Italy [2]DSM Research, Life Sciences, Advanced Synthesis and Catalysis, Geleen, The Netherlands

When two chromophores are chirally oriented and close enough to one another in space, their excited states couple and become non-degenerate. This phenomenon, termed exciton coupling, produces a typical bisignate CD curve. The intensity of the CD couplet is dependent on the molar extinction coefficient and the distance between the interacting chromophoric moieties, while the sign is governed by the angle between the effective electron transition moments. In particular, exciton coupling over a long distance can be observed only with strongly absorbing chromophores, e. g. porphyrin derivatives, characterized by their extremely intense and sharp Soret band near 415 nm.

In this work we examined by the exciton coupled CD method the combined distance and angular dependencies, generated by the seven conformationally restricted β-turn and 3-10-helical spacer peptides -L-Ala-[L-(αMe)Val]n- (n = 1-7) on a system formed by two intramolecularly interacting 5-carbamido-5,10,15,20-tetraphenylporphyrin chromophores. These porphyrin derivatives are confirmed to be excellent reporter groups. We find that not only the center-to-center separation (from 19 to 34 Å) between the two chromophores, but the orientation (roughly parallel or perpendicular) between the directions of their effective transition moments as well, are responsible for the onset or even for the modulation of the intensity of the exciton coupling phenomenon. In particular, the porphyrin…porphyrin interaction is still clearly detectable over the long distance of ca. 30 Å when the two chromophores are about perpendicularly oriented.

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SECONDARY STRUCTURE OF HYDRAZINO-PEPTIDES WITH CYCLIC SIDE-CHAIN

A. Hetényi[1], G.K. Tóth[2], C. Somlai[2], T.A. Martinek[1], F. Fülöp[1]

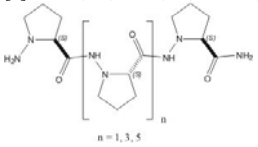
[1]Institute of Pharmaceutical Chemistry [2]Department of Medical Chemistry, University of Szeged, Hungary

β-peptides are probably the most thoroughly investigated peptidomimetic oligomers. To extend the field of β-peptides towards the construction of possible new secondary structures, the replacement of the Cα and Cβ atoms of the β-amino acid with heteroatoms could be an attractive modification, for example Cβ-atom of β-peptides by an NR moiety, leading to hydrazine peptides. In the literature, there are only a few studies [1-3] about hydrazine peptides, and hydrazine peptides with cyclic side-chain have not been studied yet.

In order to determine the secondary structure preference of 1-amino-pyrrolidine-2S-carboxylic acid homo-oligomers (Figure 1), their potential energy hypersurface were probed at the ab initio B3LYP/6-311G** level. The calculations predicted the 8-strand to be the most stable structure.
The hydrazino-peptides in question were synthetized on solid support, and their structures were characterized by NMR and CD methods. The results were found to be in good accordance with the 8-strand structure.

Figure 1. The studied homo-oligomers

[1] Günter, R.; Hofmann H-J. J. Am. Chem. Soc. 2001, 123, 247-255.
[2] Lelais, G.; Seebach, D. Helv. Chim. Acta 2003, 86, 4152-4168.
[3] Salaün, A.; Favre, A.; Le Grel, B.; Potel, M.; Le Grel, P. J. Org. Chem. 2006, 71, 150-158.



Tu371

STRUCTURAL AND CONFORMATIONAL INVESTIGATION OF PEPTIDES CONTAINING TWO DEHYDROPHENYLALANINE RESIDUES, AND THEIRS INFLUENCE ON ACTIVITY OF CATHEPSIN C.

M. Jewginski[1], R. Latajka[2], M. Pawelczak[1], M. Makowski[1], P. Kafarski[1,2]

[1]Institute of Chemistry, University of Opole [2]Faculty of Chemistr, Wroclaw University of Technology, Wroclaw, Poland

Cathepsin C [EC 3.4.14.1](1), which belongs to family of cysteine proteases, catalyzes hydrolysis of N-terminal peptide, preferential GlyPhe. This enzyme may play a part in chronic airway diseases(2). Also increaser level of enzyme was found in case of cancer, rheumatism and muscle's distrophy(3-5). For this reason we have undertook investigations of peptides containing two dehydroamino acid residues, which could act as alkylating inhibitors of this enzyme.
To define structure and conformation of investigated peptides we were used different methods of NMR spectroscopy, including standard 1D experiments, proton-proton correlations, proton-carbon correlations, and 2D NOE experiments. To complete structural research computational chemistry methods had been used. In order to predict the biological activity of investigated peptides, the simulation of docking process of these peptides to enzyme active site had been made and after that correlated with results of enzymatic test..
The obtained results suggest, that investigated peptides containing two ΔPhe residues (Z and E isomers respectively) in solution have bent conformation, which is stabilized by intermolecular hydrogen bonds. These results are confirmed by the results of theoretical calculations. Also simulation of docking process have showed two possible peptide's orientation in active site of cathepsin C and allowed the rational interpretation of biological test's results.

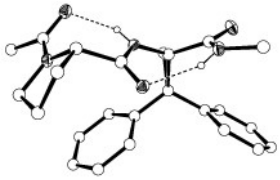
(1) Turk D, et all EMBO Jour. 2001, 20, 6570
(2) Wolters PJ, et all, Biol. Chem. 2000, 379, 137
(3) Schlagenhauff B, et all, Cancer 1992, 70, 1133
(4) Liotta LA, Cancer Res. 1986, 46, 1
(5) Sloane BF, Semin. Cancer Biol. 1990, 1, 137

A CYCLOPROPANE AMINO ACID INDUCES A DOUBLE GAMMA-TURN

G. Ballano[1], **A.I. Jimenez**[1], C. Aleman[2], J. Casanovas[3], C. Cativiela[1]

[1]Departamento de Quimica Organica, ICMA, Universidad de Zaragoza-CSIC, Zaragoza [2]Departament D'Enginyeria Quimica, ETSEIB, Universitat Politecnica de Catalunya, Barcelona [3]Departament de Quimica, EPS, Universitat de Lleida, Lleida, Spain

Turns are important elements of secondary structure in peptides and proteins. Different types of turns are distinguished according to the number of residues involved. The most abundant is the β-turn, which involves four consecutive amino acids with the CO at position i hydrogen-bonded to the i+3 NH. The γ-turn is centred at a single residue and is generally stabilized by a hydrogen bond between the i CO and the i+2 NH. Model dipeptides RCO-L-Pro-Xaa-NHR' are the smallest systems able to adopt the β-turn conformation, which is favoured by the presence of proline at i+1. A peptide of this series, incorporating a cyclopropane amino acid (Xaa), has been shown to accommodate two consecutive γ-turns in the solid state [1], instead of the expected β-turn conformation. The double γ-turn encountered is unique among crystalline short linear peptides. In fact, the γ-turn is observed almost exclusively in low-polarity solvents, and only a few oligopeptides of cyclic structure exhibit a γ-turn in the crystal. This is the first time that the strong tendency of Pro-Xaa dipeptides to adopt a β-turn in the solid state has been switched to the γ-turn. Theoretical calculations [2] also show the high preference of this cyclopropane amino acid for the γ-turn conformation. [1] Angew. Chem. Int. Ed. 44, 396 (2005); [2] J. Phys. Chem. B 110, in press (2006).



EFFECT OF HYDRATION ON THE THERMAL STABILITY OF THE COLLAGEN TRIPLE HELIX

K. Kawahara[1], Y. Nishi[1,2], S. Nakamura[1], S. Uchiyama[3], M. Doi[4], Y. Nishiuchi[5], T. Nakazawa[6], T. Ohkubo[1], Y. kobayashi[1,2]

[1]Graduate School of Pharmaceutical Sciences, Osaka University, Suita [2]Osaka University of Pharmaceutical Sciences, Takatsuki [3]Graduate School of Engineering, Osaka University, Suita, Osaka [4]Department of Materials Science, Wakayama National College of Technology, Gobo, Wakayama [5]Peptide Institute Inc., Minoh, Osaka [6]Department of Chemistry, Nara Women's University, Nara, Japan

The sequence of the collagen triple helix has a characteristic repeat of X-Y-Gly, where X and Y are often Pro or HypR (4(R)-hydroxyproline). Although it is known that the sequence position of HypR largely affects the thermal stability of the triple helix, the stabilizing mechanism has not been resolved. To solve this problem, we synthesized (HypR-HypR-Gly)10 and investigated its physico-chemical properties and structure. From CD and DSC analyses, it was demonstrated that the transition temperature from the triple-helix to the single-coil of (HypR-HypR-Gly)10 is comparable to that of (Pro-HypR-Gly)10 which is higher than that of (Pro-Pro-Gly)10. The X-ray analysis of the crystal of (HypR-HypR-Gly)10 revealed that the helical parameter and Rich & Crick II hydrogen bond geometry of (HypR-HypR-Gly)10 are similar to those of (Pro-Pro-Gly)10 and (Pro-HypR-Gly)10, whereas the pattern of hydrogen bond network mediated by water molecules is different among these three model peptides. We considered that hydration would influence the thermal stability of collagen triple helix. Therefore, we compared the hydration volumes in solution of these model peptides by volumetric measurement. It was shown that the degree of hydration of (HypR-HypR-Gly)10 is comparable to that of (Pro-HypR-Gly)10 in the triple-helix state which is higher than that of (Pro-Pro-Gly)10, but the former was more highly hydrated than (Pro-HypR-Gly)10 in the single-coil state. Combining these results, we concluded that the high thermal stability of (Pro-HypR-Gly)10 is ascribed to its high hydration in the triple-helix state and that of (HypR-HypR-Gly)10 is described by its high hydration in the single-coil state.

Tu374

BIOCHEMICAL ANALYSIS OF HAPTOGHLOBIN POLYMORPHISM IN IRANIAN PATIENTS WITH CARDIOVASCULAR DISEASE

E. Khalkhal[1], B. Kazemi[2], A. Shabani[1], Z. Afraz[3], M. Asgari Mehr[3]

[1]Department of Biochemistry, Faculty Of Science, Azzahra University [2]Department of Molecular Biology, Shaheed Beheshti University [3]Faculty of Medicine, Tehran University, Tehran, Iran

Oxidative stress plays an important part in the development of Cardiovascular Disease (CVD). Haptoglobin is a hemoglobin-binding protein that has a major role in providing protection against heme-driven oxidative stress. There are two common alleles for haptoglobin (1 and 2), and the three phenotypes, haptoglobin 1-1, haptoglobin 2-1, and haptoglobin 2-2, differ in their ability to function as antioxidants. We determined whether there was a relation between the haptoglobin phenotype and the development of Coronary Artery Diseases.

Haptoglobin (HP) phenotypes were determined in Iranian patients with Coronary Artery Diseases. We performed haptoglobin (Hp) genotyping by polymerase chain reaction (PCR) using allele-specific primer-pairs.

In multivariate analyses controlling for conventional CVD risk factors, haptoglobin phenotype was a highly statistically significant, independent predictor of CVD. The odds ratio of having CVD in patients with the haptoglobin 2-2 phenotype was 5.0 times greater than in patients with the haptoglobin 1-1 phenotype. An intermediate risk of CVD was associated with the haptoglobin 2-1 phenotype.

These results suggest that haptoglobin phenotype is an important risk factor in determining susceptibility to cardiovascular disease which may be mediated by the decreased antioxidant and antiinflammatory actions of the haptoglobin 2 allelic protein product.

KEY WORD: Haptoglobin,C ardiovascular Disease, Polymorphism

Th375

DIRECTING POLYPEPTIDE'S SECONDARY STRUCTURE AT WILL - FROM ALPHA-HELIX TO AMYLOIDS AND REVERSE?

B. Koksch, K. Pagel, S.C. Wagner, J. Scheike

Institute of Chemistry and Biochemistry, Free Universiy Berlin, Germany

The special feature of proteins involved in Alzheimer's or prion diseases is their ability to adopt at least two different stable conformations. The conformational transition that shifts the equilibrium from the functional to the pathological isoform can happen sporadically. It can also be triggered by mutations in the primary structure, changes of different environmental conditions, or the action of chaperones. Elucidation of the molecular interactions that occur during the transformation from α-helix to β-sheet and the consecutive formation of amyloids on a molecular level is still a challenge. Therefore, the development of small peptide models that can serve as tools for such studies is of paramount importance.

We succeeded in generating model peptides that, without changes in their primary structure, predictably react on changes of diverse environmental parameters by adopting different defined secondary structures. These de novo designed peptides strictly follow the characteristic heptad repeat of the α-helical coiled coil structural motif. Furthermore, domains that favour β-sheet formation and aggregation can be generated.1 Alternatively, those peptides can be equipped with functionalities that allow either the binding of metal ions or the interaction with membranes. As proof of our concept we showed that the resulting secondary structure of such peptides will strongly depend on environmental parameters. Thus, this system allows to systematically study the interplay between peptide / protein primary structure and environmental factors for peptide and protein folding on a molecular level.

1) K. Pagel, S.C. Wagner, B. Koksch. J. Am. Chem. Soc. 2006, 128, 2196-2197.

M376

FTIR SPECTROSCOPIC STUDIES ON HELICAL INTERMEDIATE STAGE DURING AGGREGATION PROCESS OF THE BETA-AMYLOID 11-28 FRAGMENT AND ITS VARIANTS

P. Juszczyk, **A.S. Kolodziejczyk**, Z. Grzonka

Faculty of Chemistry, University of GdańSk, Poland

The pathogenesis of Alzheimer's disease (AD) is strongly linked to neurotoxic assemblies of the amyloid β protein (Aβ). Aβ is a soluble component of human plasma which by an unknown mechanism becomes aggregated and neurotoxic. Some genetic mutations within the Aβ sequence cause very early onset of AD-like diseases, probably by facilitation of Aβ assembly into neurotoxic species. Recently, it was found that not amyloid fibrils, but smaller Aβ assemblies initiate a pathogenic cascade resulting in AD. Therefore, preventing the folding of nascent Aβ monomers would have therapeutic benefit.

To uncover details of structural changes accompanying the aggregation process, especially its initial stage, we have decided to study the Aβ(11-28) fragment and its mutation-related variants. Our recent studies on this Aβ fragment using the CD method and the aggregation test have proved it a good model for structural studies. The obtained results confirmed that the aggregation process follows the scheme with an α-helical intermediate and pointed out differences in the behaviour of Aβ variants. To further confirm the scheme of the structural changes accompanying aggregation we have applied FTIR spectroscopy and analysed aggregation-induced changes of the amide I band which is directly related to peptide backbone conformations. The FTIR spectra analysis indicate that water addition provoked conformational changes are strongly dependent on the Aβ(11-28) variant and in some cases the formation of α-helical intermediate seems to be preceded by 310 helix formation. To verify this hypothesis the temperature dependent ATR FTIR spectra will be analysed.

Supported by UG BW grant.

PEPTIDE/PROTEIN WASTE MATERIALS LIKE BINDERS OF FOUNDRY SANDS

D. Kramarova[1], F. Bunka[1], J. Brandstetr[2], K. Rusin[3], I. Hoza[1]

[1]Department of Food Engineering, Faculty of Technology, Thomas Bata University In Zlín [2]Faculty of Chemistry [3]Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

The aim of this study was to develop a new foundry moulding mixture with new binder systems based on peptide/protein. To evaluate the quality and performance of molds with a new binder experimental casting has been performed. As a peptide/protein binder waste materials from pharmaceutical industry were used. Character and behaviour and of peptides/proteins are derived from the content of individual amino acids, the determination of amino acids was made by automatic analyser. Pre-treatment of the foundry sand before curing of the mould were proved. Round grains facilitate good migration of the binder to the contact points. The coating process was analyzed using SEM. Good adhesion of the binder systems on a surface of the grains was obtained. After blowing the sand into the core box, a drying cycle gives rise to the core strength due to the dehydration of the binder. Excessive heat in the sand mixture changes its flowability, as the coated sand becomes sticky and begins to gain strength. The dehydration occurs between amido and hydroxy groups of the protein heated. Casting trials were performed using a core made with the new binder in the low-pressure hot box to evaluate shakeout and dimensional accuracy. Dimensional accuracy was verified and did not show any thermoplastic deformation.

Acknowledgements: This work was supported by The Grant Agency of The Czech Republic (No.106/01/1277).

Th378

SELF-ASSEMBLY IN A GLYCINE-RICH TRUNCATED PRION OCTAREPEAT CONSTRUCT

K.P. Krovi[1], M. Chandra[2], S. Verma[1]

[1]Department of Chemistry, IIT Kanpur, Kanpur, India [2]Department of Chemistry, Konstanz University, Konstanz, Germany

Amino acid octarepeats present in the prion protein bind to Cu2+ and are considered as a potential periplasmic copper ion transporters. This octarepeat is located in the unstructured region of the prion protein, which is supposedly not intricately involved in prion aggregation. Our group is involved in exploring the function of octarepeats with a special emphasis on their possible role in amyloid fibril formation and aggregation.1 In this context, we have prepared truncated peptide constructs derived from the prion protein octarepeat PHGGGWGQ and have reported their fibrillation activity.

We will present aggregative behavior of a truncated bis-pentapeptide, containing GGGWG segment, when tethered with a flexible linker diaminobutane. Fibrillar architectures were observed by this bis conjugate after incubation in water which was probed by different microscopic and steady state fluorescence techniques. Further investigations with KI revealed a homogeneous environment of the two tryptophan moieties in the conjugate. In the absence of other side-chains, it is likely that fibril formation involves hydrophobic interaction between tryptophan indole moieties and main chain backbone interactions. Interestingly, a facilitator role for aromatic-glycine motifs for amyloid aggregation has been proposed based on bioinformatics search of the Swiss-Prot and TrEMBL databases.

1. (a) C. Madhavaiah and Sandeep Verma, Chem. Commun. 2004, 638; (b) C. Madhavaiah, K. Krishna Prasad, Sandeep Verma, Tetrahedron Lett. 2005, 46, 3745.

M379

PHOTO-CONTROL OF THE COLLAGEN TRIPLE HELIX

U. Kusebauch[1], S.A. Cadamuro[1], H.J. Musiol[1], L. Moroder[1], C. Renner[2]

[1]Max-Planck-Institute of Biochemistry, Martinsried [2]School of Biomedical and Natural Sciences, Nottingham Trent University, Nottingham, Germany

Collagens are known to fold into a highly ordered rode-shaped triple helix with stretches of lower and higher suprastructural stability and even disruptions to modulate recognition by other proteins that interact with the extracellular matrix [1]. To increase understanding of folding and stability of the collagen triple helix, we have adressed the design of photocontrolled collagenous peptides. Our aim was to crosslink two side chains of the repetitive (Xaa-Yaa-Gly) sequence motifs of collagen model compounds via an azobenzene chromophore in analogy to our previous studies on photomodulation of the conformational preferences of cyclic peptides and more recently of hairpin-peptide model systems [2]. Molecular modeling experiments suggested appropriate sequence positions that could result in triple-helical peptides with conformational stabilities that can be modulated by cis/trans isomerization of the azobenzene moieties. As light switchable crosslinker azobenzene-4,4'-N-(4-iodo-2-butynenyl)carboxyamide was synthesized for reaction with two (4S)-mercaptoproline residues placed in suitable Xaa and Yaa positions, respectively. By this approach a fully folded triple helix was obtained upon thermal relaxation, and unfolding was induced by irradiation at 350 nm. The favorable optical properties of the azobenzene derivative together with the regular suprastructure resulted in a valuable model system that allows for ultrafast time-resolved studies of collagen folding and unfolding.

[1] Renner, C.; Kusebauch, U.; Löweneck, M.; Milbradt, A.G.; Moroder, L. (2005) J. Pept. Res, 65, 4-14.
[2] Renner, C.; Moroder, L. (2006) ChemBioChem, in press.

Tu380

MOLECULAR DYNAMICS STUDY OF TWO ABL-SH3 DOMAIN PEPTIDES TOWARDS AMYLOID FORMATION

I. Liepina[1], S. Ventura[2], C. Czaplewski[3], A. Liwo[3]

[1]Latvian Institute of Organic Synthesis, Riga, Latvia [2]Institut de Biotecnologia I de Biomedicina, Universitat Autonoma de Barcelona, Bellaterra, Spain [3]Faculty of Chemistry, University of Gdansk, Poland

Amyloid formation is connected with Alzheimer's disease, Parkinson's disease, Finnish familial amyloidosis. After protein misfolding short peptide sequences act as "hot spots" providing the driving force for protein aggregation in amyloid fibrils. We have identified one of these sequence stretches in the Abl-SH3 domain of Drosophila (DLSFMKGE) whereas the human homologous region (DLSFKKGE) is predicted to be less amyloidogenic. The possible reason for the difference of amyloid formation propensities of the two peptides was investigated by molecular dynamics (MD) of β-sheet structures. The antiparallel alanine β-sheets consisting of two and ten strands were constructed, minimized, and mutated to the sequences DLSFMKGE and DLSFKKGE. All four systems: 1) DLSFMKGE – two strands, 2) DLSFKKGE – two strands, 3) DLSFMKGE – ten strands, 4) DLSFKKGE – ten strands, were surrounded by 10 Å layer of water molecules over the solute and subjected to MD, Amber 8.0 force field, NTP protocol. The MD runs were started at the temperature of 10 K and the temperature was elevated stepwise by 10 degrees till 300 K. The results show considerably higher hydrogen bond percentage for DLSFMKGE than that one for DLSFKKGE during the course of the simulation, thus suggesting that DLSFMKGE is a potential fibril- maker, but DLSFKKGE is not. Two strand β-sheet systems were stable until 170 K. The ten strand β-sheets are more stable.

The work supported by NATO Collaborative Linkage Grant LST.CLG.97980, stipend for I.L. of Jozefa Mianowski Fund, Poland, Latvian Science Council Grant 05.1768, Gdansk Academic Computer Center TASK.

Th381

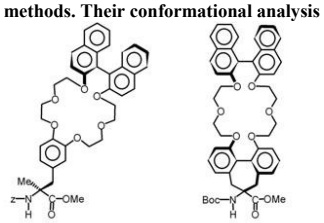
SYNTHESIS AND 3D-STRUCTURAL ANALYSIS OF PEPTIDES BASED ON Cα-TETRASUBSTITUTED α-AMINO ACIDS WITH BINAPHTHYL-CROWNED SIDE CHAINS

J.-F. Lohier[1], K. Wright[1], R. Anddad[1], M. Wakselman[1], J.-P. Mazaleyrat[1], F. Formaggio[2], C. Peggion[2], M. Crisma[2], C. Toniolo[2]

[1]*SIRCOB, UMR CNRS 8086, University of Versailles, Versailles, France*
[2]*Department of Chemistry, University of Padova, Italy*

Crowned Cα-tetrasubstituted α-amino acids with well-defined stereochemical properties are interesting tools, allowing control of the spatial organization of the crown-ether receptors in short-chain peptide structures. These latter compounds are potentially useful for the construction of new molecular receptors and supramolecular devices. We have previously shown that peptides based on L-Mdp[CROWN] residues derived from Cα-methyl-L-DOPA (Mdp) with [CROWN]= [15-C-5], [18-C-6] and [Benzo-24-C-8], have a strong propensity for folded/helical secondary structures. To the same end, peptides based on the more rigid, cyclic, Bip[20-C-6] residue have been investigated in our groups. We recently envisioned that introduction of a binaphthyl unit as a part of the crown moiety would impart supplementary structural and chiral recognition properties to these receptors.

We report here the synthesis of the terminally protected amino acids Z-L-Mdp[(S)-Binol-20-C-6]-OMe and Boc-(R)-Bip[(S)-Binol-22-C-6]-OMe. Peptides based on these new amino acids, combined with Aib and/or L-Ala residues to the hexamer level, with two crowned residues at the i and i+3 positions of the main chain, were also prepared by solution methods. Their conformational analysis was performed using FT-IR absorption and 1H NMR techniques.



Z-*(L)*-Mdp[*(S)*-Binol-20-C-6]-OMe Boc-*(R)*-Bip[*(S)*-Binol-22-C-6]-OMe

M382

SECONDARY STRUCTURE ANALYSIS OF TWO RECOMBINANT PEPTIDES FROM ACTIVE SITES OF HUMAN ANGIOTENSIN CONVERTING ENZYME (ACE)

S.-S. Vamvakas[1], L. Leondiadis[1], G.A. Spyroulias[2], G. Pairas[2],
E. Manessi-Zoupa[3], P. Cordopatis[2]

[1]Institute of Radioisotopes-Radiodiagnostic Products, NCSR "Demokritos", Athens [2]Department of Pharmacy [3]Department of Chemistry, University of Patras, Patras, Greece

Angiotensin-I converting enzyme (ACE) has a critical role in cardiovascular function, which consists of cleaving the carboxy terminal His-Leu dipeptide from Angiotensin-I producing a potent vasopressor octapeptide, Angiotensin-II. There are two isoforms of ACE. The somatic isoform is present in all human cells except the testis cells, where the testicular isoform is produced. The major difference between these two types is that, the somatic form has two active sites, at the N- and C-end respectively while the testicular has only one, which is almost identical to the somatic C-terminal active site. Here we report the structural study of a 108aa peptide (previously expressed in bacteria), which corresponds to an extended domain of the human somatic N-terminal active site of ACE (Ala361-Gly468) by circular dichroism experiments, and the overexpression in bacteria, purification and structural study, using circular dichroism techniques, of a 108aa peptide which corresponds to an extended domain of the human somatic C-terminal active site of ACE (Ala958-Gly1065). Following the subclonning into an appropriate expression vector and the expression, the peptide was isolated from the inclusion bodies using chromatography techniques. The recombinant protein fragment had a molecular weight, measured by ESI-MS, of 12102 kDa which was in consistence with the theoretical calculation based on the DNA sequence. The recombinant peptides acquired their theoretically calculated secondary structure only when 1,1,1-trifluoroethanol is present at a concentration of ~70%. In order to elucidate their structures, solutions of these peptides, labeled with 15N and/or 13C, will be studied by NMR spectroscopy.

Tu383

A FLUORESCENCE STUDY OF PEPTIDE AGGREGATION IN WATER: INFLUENCE OF PH, CONCENTRATION AND AMINO ACID COMPOSITION

M. Martari, R.D. Sanderson

UNESCO Associated Centre for Macromolecules And Materials, Chemistry and Polymer Science, Stellenbosch University, Stellenbosch, South Africa

Aggregation of peptides is believed to trigger various degenerative diseases but it also plays an important role for the preparation of peptide fibres and PEPTIDE-BASED BIOMATERIALS. It is therefore extremely important to understand the mechanisms involved in peptide aggregation and be able to control them. Studies were performed on a library of amphiphilic peptides, designed around the sequence of a model antimicrobial peptide rich in leucine and lysine. The library also included peptide hybrids in which natural amino acids were replaced by non-proteinogenic omega-amino acids, such as 6-aminohexanoic acid and 9-aminononanoic acid. The aim was to estimate the aggregation and its correlation with the biological activity by using a fluorescence technique commonly employed to calculate the CMC (critical micelle concentration) of surfactants.

Peptides and peptide hybrids were synthesized on solid support using the Fmoc polyamide protocol. They were purified by semi-preparative RP-HPLC and characterized by ESI-MS and analytical RP-HPLC.

The aggregation behaviour of the synthesized molecules was investigated in water by steady-state fluorescence measurements using pyrene as fluorescent probe. Peptides were dissolved in water/pyrene or water/pyrene/0.1% triethylamine at different concentrations, excited at 334 nm and fluorescence detected at 373 (I1) and 384 (I3) nm.

This technique has been successfully employed for the determination of critical aggregation concentration (CAC) of amphiphilic peptides and peptide hybrids. Plotting the ratio I1/I3 against the concentration, CAC values were extrapolated.

Important elements were established: presence of hydrophobic amino acids and non-natural amino acids increased the CAC, proline and charged amino acids reduced the CAC.

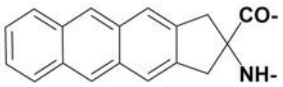
SYNTHESIS AND 3D-STRUCTURAL ANALYSIS OF PEPTIDES BASED ON ANTAIB, AN ANTHRACENE-FUSED 1-AMINOCYCLOPENTANECARBOXYLIC ACID

J.-P. Mazaleyrat[1], K. Wright[1], J.-F. Lohier[1], M. Wakselman[1], C. Peggion[2], F. Formaggio[2], C. Toniolo[2]

[1]*SIRCOB, UMR CNRS 8086, University of Versailles, Versailles, France* [2]*Department of Chemistry, University of Padova, Italy*

Fluorescence spectroscopy has become an extremely valuable technique for conformational studies of biopolymers, the development of peptide-based chemosensors, and biochemical research in general. In this connection, synthetic amino acids as fluorescent probes to be incorporated into a peptide chain may exhibit significant advantages over the related protein (Trp and Tyr) residues in terms of potentially different and ameliorated properties. We recently designed and prepared a new fluorescent amino acid, antAib, based on a planar anthracene core and belonging to the class of achiral, Ciα↔Ciα cyclized, Cα-tetrasubstituted α-amino acids (strong β-turn and helix inducers in peptides).

Peptides based on antAib combined with (L)-Ala residues were synthesized and subjected to a conformational analysis. More specifically, the protected derivatives Boc-antAib-OEt (OH) and Fmoc-antAib-OtBu (OH) were prepared in seven steps from 1,2,4-trimethylbenzene. The peptides Boc-antAib-(L)-Ala-OMe 2a, X-(L)-Ala-antAib-(L)-Ala-OMe 3a (X = Boc) and 3b (X = Fmoc), Boc-antAib-[(L)-Ala]2-OMe 3'a, X-[(L)-Ala]2-antAib-(L)-Ala-OMe 4a (X = Boc) and 4b (X = Fmoc), Boc-antAib-[(L)-Ala]2-antAib-(L)-Ala-OMe 5a, Boc-[(L)-Ala]3-antAib-(L)-Ala-OMe 5'a, and Boc-antAib-[(L)-Ala]3-antAib-(L)-Ala-OMe 6a were synthesized by solution methods. Their secondary structures were analyzed by use of FT-IR absorption, NMR, and CD techniques.



antAib

M385

TWO-DIMENSIONAL INFRARED SPECTROSCOPY DISCRIMINATES BETWEEN 3-10- AND α-HELICAL PEPTIDES

A. Moretto[1], C. Toniolo[1], Q.B. Broxterman[2], H. Maekawa[3], N.H. Ge[3]

[1]Department of Chemistry, University of Padova, Italy [2]DSM Research, Life Sciences, Advanced Synthesis and Catalysis, Geleen, The Netherlands
[3]Department of Chemistry, University of California, Irvine, CA, USA

We believe that two-dimensional infrared (2D IR) spectroscopy is the most promising vibrational technique for studying peptide 3-10/α-helix transitions and equilibria because it can be used to obtain a cross-peak pattern of the amide I modes. Cross-peaks appear in these spectra only if the vibrational modes are coupled, the coupling strength acutely depending on the peptide secondary structure. In this work we applied femtosecond 2D IR spectroscopy to the homo-octapeptide Z-[L-(αMe)Val]8-OtBu in CDCl3, TFE, and HFIP solutions to acquire spectral signatures that distinguish between 3-10- and α-helix structures [1]. Suppression of diagonal peaks by controlling polarizations of IR pulses clearly reveals cross-peak patterns that are crucial for secondary structure determination.
A doublet feature is observed when the peptide ester forms a 3-10-helix in CDCl3 and TFE, and when it is at the initial stage of 3-10- to α-helix transition in HFIP. By contrast, the 2D IR spectrum shows a multiple peak pattern after the peptide ester has acidolyzed and become an α-helix in HFIP. Electronic CD spectra accompanying the acidolysis-driven conformational change were also recorded. This is the first report on the experimental 2D IR signature of a 3-10-helical peptide. These results, using a model octapeptide, demonstrate the powerful capability of 2D IR spectroscopy to discriminate between different helical structures. Further developments will open avenues for elucidating the role of the 3-10-helix in early protein folding events.

[1] Maekawa H., Toniolo C., Moretto A., Broxterman Q. B. and Ge N.-H., J. Phys Chem. B, 2006, in press.

HOW METAL IONS AFFECT AMYLOID FORMATION - MODEL PEPTIDES ADOPTING DIFFERENT CONFORMATIONS IN THE PRESENCE OF COPPER(II) AND ZINC(II)

K. Pagel[1], T. Seri[1], H.V. Berlepsch[2], C. Böttcher[2], B. Koksch[1]

[1]Institute of Chemistry and Biochemistry [2]Research Center for Electron Microscopy, Free University Berlin, Germany

Conformational transitions in peptides and proteins emerge to play the major role in the genesis and evolution of prion related diseases and Alzheimer's disease (AD).1 In this context, conditions influencing this transition and the following aggregation process are of paramount interest. Peptides and proteins that are involved in aggregation processes contain potential metal binding sites. The concentration of metal ions in the brain tissue is naturally high and Zn in the mM range has been found in AD amyloid plaques. Thus, it is widely accepted that metal complexation is one of the key incidents that lead to conformational transitions and aggregation.
We present here a coiled coil based model peptide system with an intrinsic amyloid forming tendency which can be used to study the impact of different metal ions on secondary structure and aggregation. Metal complexing Histidine residues were incorporated to create potential binding sites which, depending on their position and the nature of the metal ion, dictate folding and aggregation. The time dependent conformational transition was monitored by CD-spectroscopy. Aggregates were characterized by cryo TEM. High resolution FTICR-MS experiments revealed information on the stoichiometry of the peptide-metal complexes. In the absence of metal ions the presented peptides formed amyloids in a time range of weeks. Depending on the His positions and milieu conditions, the nature of the metal ion determines folding and aggregate morphology. Furthermore, metal binding was shown to inhibit the amyloid formation.

1) K. Pagel, T. Vagt, B. Koksch. Org. Biomol. Chem. 2005, 3, 3843-3850.

Th387

DESIGN AND SYNTHESIS OF BETA-BARREL PROTEINS FROM FIRST PRINCIPLE

P. Pollet, K. Fritzemeier, W. Haehnel

Institute of Biology II / Biochemistry, University of Freiburg, Germany

A challenge to our understanding of protein folding is the design of a protein from first principle, i.e. starting from geometric restraints and applying properties of amino acids expected to be essential for folding to a defined structure.

We developed a program to calculate the backbone coordinates of antiparallel strands to match the surface of an elliptical cylinder. Various parameters like the number and shearing of the strands and the ellipticity of the structure can be varied. The relative orientation of the β-strands and the geometrical features of the hydrogen-bonds were derived from statistical analyzes of natural β-sheet structures.

Iterative cycles of core-packing with amino acid rotamers, molecular dynamics simulation and energy minimization with the CHARMM forcefield are used to include backbone movements and to minimize the risk of trapping an energetically unfavorable structure. The quality of residue packing is assessed with the help of criteria which proved to be successful in our design of β-sandwiches. At the protein surface, a network of salt-bridges with an excess of positive charges has been designed to increase the stability and the solubility of the protein.

The final sequence is synthesized by standard solid phase Fmoc-chemistry. Insights gained from the analysis of the synthesized structure with FTIR and CD spectrometry should help us to refine the parameters for subsequent designs. With this strategy, we hope to contribute to a better understanding of protein folding.

M388

CONFORMATIONAL STUDIES OF A SHORT PEPTIDE CORRESPONDING TO THE C-TERMINAL BETA-HAIRPIN STRUCTURE OF PROTEIN G B1 DOMAIN

S. Rodziewicz-Motowidło[1], A. Skwierawska[1], S. Ołdziej[1], W. Wiczk[1], A. Liwo[1], H.A. Scheraga[2]

[1]Faculty of Chemistry, University of GdańSk, Poland [2]Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA

The immunoglobulin binding protein G (1IGD) from Streptococcus species consists of 61 amino acids residues, which form two antiparalell-packed beta-hairpins and an alpha-helix in the middle of the sequence packed to the beta-sheet. The second hairpin was found to be stable in isolation. This fragment is therefore likely to be the first folding initiation site of the protein which could provide an adequate nucleation center on which the rest of the polypeptide chain would find a favorable environment to fold. Thus, among the two beta-hairpins, the 48-59 fragment of 1IGD corresponding to the C-terminal beta-hairpin was synthesized. In our studies, we investigated different environmental and temperature conditions for formation of the 48-59 beta-hairpin structure. Its structure was examined by means of CD spectroscopy in water, buffer solutions (pH = 3 to 9) and in aqueous solutions of trifluoroethanol. Additionally, its structure was investigated in the solid state by FTIR spectroscopy. The CD studies revealed that the 48-59 fragment of 1IGD in water forms mainly a statistical-coil structure, whereas the FTIR technique shows formation of a regular beta-sheet structure. NMR spectroscopy and calorimetric measurements were carried out at various temperatures. Our studies show that the 48-59 fragment at low temperature exists in an equilibrium between two conformations – a regular beta-hairpin and a statistical-coil. Although increasing temperature resulted in shifting the equilibrium in the direction of the statistical-coil structure, the overall beta-hairpin shape of the 48-59 fragment was maintained.

Acknowledgments:
This work was financially supported by KBN under grant 3T09A03226.

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FLUORIMETRIC INVESTIGATION AND MOLECULAR DYNAMICS SIMULATION ON INTERACTION BETWEEN PRION PROTEIN HELIX 2 AND TETRACYCLINE

L. Ronga[1], E. Langella[2], R. Improta[2], B. Tizzano[1], P. Palladino[1], D. Marasco[2], M. Ruvo[2], C. Pedone[1,2], E. Benedetti[1]

[1]*Dipartimento Delle Scienze Biologiche and C.I.R.Pe.B., Università Federico II di Napoli* [2]*Istituto di Biostrutture E Bioimmagini-CNR, Naples, Italy*

Prion diseases are characterized by the conversion of the physiological cellular form of the prion protein (PrPC) into an insoluble, protease-resistant abnormal scrapie form (PrPSc) with an highly beta-sheet content [1]. The analyses of intrinsic structural propensities of the PrP C-terminal domain showed an high conformational flexibility for the α-helix 2 fragment which indicates that this region may be particularly important in the PrPC→PrPSc transition [2]. Therefore conformation-based approaches focalized on helix 2 region appear to be the most promising for the study of prion protein misfolding. Recent studies on tetracycline properties showed that this molecule binds and disrupts PrP peptide aggregates and inactivates the pathogenic forms of PrP [3,4]. A fluorometric titration of the fluoresceinated peptide corresponding to prion protein helix-2 with tetracycline has been carried out to determine the value of the apparent dissociation constant of this interaction (estimated to be 189 ± 7 nM). Remarkably, the fluoresceinated peptide exhibits in water a canonical α-helical CD spectrum, that is maintained even in presence of tetracycline.
Accordingly, docking calculations and molecular dynamics simulations suggest that tetracycline interacts preferentially with the C-terminal end (residues 183-195) of helix-2 with a significant involvement of the treonine rich region.

References

1. S.B. Prusiner Proc. Natl. Acad. Sci. USA (1998) 95, 13363-13383 Nobel Lecture.
2. B. Tizzano et al. Proteins: Structure, Function, and Bioinformatics (2005) 59, 72-79.
3. F. Tagliavini et al. J. Mol. Biol. (2000) 300, 1309-1322.
4. G. Forloni et al. Proc. Natl. Acad. Sci. USA (2002) 99, 10849-10854.

Th390

PRION PROTEIN HELIX 2 CONFORMATIONAL PROPERTIES: IMPLICATIONS FOR FULL LENGTH PROTEIN FOLDING AND STABILITY

L. Ronga[1], P. Palladino[1], B. Tizzano[1], D. Marasco[1], R. Ragone[2], M. Ruvo[1], G. Saviano[3], T. Tancredi[4], E. Benedetti[1], C. Pedone[1], F. Rossi[1]

[1]Dipartimento delle Scienze Biologiche, C.I.R.Pe.B., Università Federico II di Napoli and Istituto Di Bioimmagini E Biostrutture, CNR [2]Dipartimento di Biochimica E Biofisica and CRISCEB, Seconda Università di Napoli [3]Dipartimento di Scienze E Tecnologie per L'Ambiente E Il Territorio, Università Degli Studi del Molise, Isernia [4]Istituto di Chimica Biomolecolare, CNR, Pozzuoli, Italy

Aetiology of the neurodegenerative prion diseases is currently associated to the unfolding of the cellular prion protein, PrPC, and to its conversion, by a yet unknow mechanism, into an oligomeric, β-pleated, but still structurally mysterious "scrapie" variant (PrPSc) [1].
Knowledge of the molecular basis of such a mechanism can provide key clues for the rational structure-based drug design of compounds able to block or prevent the diseases. We have previously reported on the ambivalent structural behavior of the 173-195 protein fragment, corresponding to the helix 2, that could play a role in the nucleation process of protein misfolding and oligomerization [2,3]. We have now futher investigated the conformational properties of this protein sub-domain by CD, MD and NMR and assessed that the helix 2 C-terminal region 180-195 adopts a β-structure that is invariably retained under a large variety of conditions. The results so far obtained are presented and discussed in the context of the full length protein folding and stability.

References:
[1] Carrel, R.W. and Lomas, D.A. (1997) Lancet, 350, 134-138.
[2] Tizzano, B., Palladino, P., De Capua, A., Marasco, D., Rossi, F., Benedetti, E., Pedone, C., Ragone, R. and Ruvo, M., (2005) Proteins, 59, 72-79.
[3] Haire, L.F., Whyte, S.M., Vasisht, N., Gill, A.C., Verma, C., Dodson, E.J., Dodson, G.G., and Bayley, P.M. (2004) J. Mol. Biol., 336, 1175-1183.

M391

CARBOHYDRATE-INDUCED MODIFICATIONS OF OPIOID PEPTIDES – COMPARATIVE CD AND FTIR SPECTROSCOPIC STUDIES

M. Roscic[1], E. Vass[2], S. Horvat[1], M. Hollósi[2]

[1]Division of Organic Chemistry and Biochemistry, Rudjer Boskovic Institute, Zagreb, Croatia [2]Department of Organic Chemistry, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary

In the last decades, a series of discoveries have shed light on the role played by the carbohydrate moiety in glycoproteins. It has been shown that covalently linked sugar moieties influence peptide/protein properties such as hydrophobicity, conformation, biostability and bioactivity. The design of carbohydrate-peptide analogs with increased, retained or modified biological activity requires an understanding of their conformational preferences both in solution and in the receptor-bound state.
In our recent work we have created two classes of well-structured linear and cyclic carbohydrate-modified analogs of opioid peptides, Leu-enkephalin and Leu-enkephalin amide. The first class represents a group of compounds in which the linear peptide is alkylated at the N-terminal position by 1-deoxy-D-fructose unit, while its cyclic analog possesses an ester bond between the C-6 hydroxy group of the sugar moiety and the C-terminal carboxy group of peptide, 1-deoxy-D-fructofuranose acting as a bridge between the Leu-enkephalin terminal parts. The rigid 5-membered imidazolidinone ring is characteristic for the second class of compounds. In these adducts an imidazolidinone moiety connects the acyclic sugar residue with the linear peptide chain. In the corresponding bicyclic imidazolidinone analogs 19-membered ring is formed through an ester bond between the primary hydroxyl group of the D-gluco-pentitolyl residue and the C-terminus of peptide.
This work reports the comparative CD and FTIR spectroscopic properties of the prepared glycopeptides in comparison with data on the non-modified flexible parent peptides performed in different solvents in order to expose the structural and conformational differences caused by a keto-sugar, rigid 5-membered imidazolidinone ring and/or cyclization.

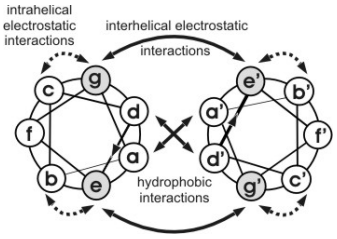
Tu392

TWO EFFICIENT WAYS TO MASK DESTABILIZING INTERHELICAL INTERACTIONS IN ALPHA-HELICAL COILED COIL-PEPTIDES

J.A. Scheike, B. Koksch

Department of Chemistry and Biochemistry - Organic Chemistry, Freie Universität Berlin, Germany

The α-helical coiled coil structural motif consists of two to five α-helices which are wrapped around each other with a slight superhelical twist. The simplicity and regularity of this motif have made it an attractive system to study the role of complementary interactions for protein folding.
Here we present a systematic study showing that intermolecular electrostatic interactions between positions e and g of the helices are in competition with the intramolecular interactions between positions e/b and g/c. Those competitive interactions affect folding and stability of the motif which were monitored by temperature dependent CD-spectroscopy. Incorporation of oppositely charged amino acids in positions e/b and g/c reduced considerably electrostatic repulsion between equally charged amino acids in positions e and g.
In addition coiled coil stability can be increased by the alkyl part of the amino acid side chains in positions e and g. Studies with natural and unnatural amino acids showed that the longer this alkyl part the better is the hydrophobic core protected from solvent.
Therefore the repulsion of equally charged amino acids in positions e and g can be overruled by involving them either into attractive intrahelical electrostatic interactions or into hydrophobic core formation.



Th393

NMR-BASED CONFORMATIONAL STUDIES OF VASOPRESSIN ANALOGUES MODIFIED AT POSITION 2 WITH 1-NAPHTHYLALANINE ENANTIOMERS

E. Sikorska, B. Lammek

Department of Chemistry, University of GdańSk, Poland

The structures of two vasopressin analogues: [Cpa1,L-1-Nal2]AVP and [Cpa1,D-1-Nal2]AVP in DMSO solution has been determined using 2D NMR spectroscopy and theoretical calculations. NMR spectra of both analogues displayed two distinct sets of residual proton resonance, which is a result of cis/trans isomerization of Cys6-Pro7 peptide bond. Concentration of the cis isomer is 7% and 6% for [Cpa1,L-1-Nal2]AVP and [Cpa1,D-1-Nal2]AVP analogues, respectively. The three-dimensional structures were generated by molecular dynamics calculations with simulated annealing algorithm using the AMBER program with time-averaged distances and dihedral angle restraints obtained from nuclear Overhauser effects (NOE) volumes and vicinal coupling constants JNHHa, respectively. Finally 300 energy-minimized conformations were obtained for each peptide. The results of calculations show that the main structural element of both peptides is b-turn at position 3,4. In the case of analogue [Cpa1,D-1-Nal2]AVP we observed also b-turn of type III at position 2,3.

Both analogues turned out to be potent V1a antagonists. The inversion of configuration of residue at position 2 converted a weak antidiuretic agonist, [Cpa1,L-1-Nal2]AVP, into a highly potent antidiuretic antagonist, [Cpa1,D-1-Nal2]AVP.

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EFFECTS OF BRANCHED BETA - CARBON RESIDUES ON CONFORMATIONS OF PEPTIDES CONTAINING DEHYDRO - RESIDUES

R.K. Somvanshi, S. Dey, T.P. Singh

Department of Biophysics, All India Institute of Medical Sciences,
New Delhi, India

Dehydro-amino acid residues have been shown to be strong inducers of folded conformations in peptides and thus can be used for the design of specific structures. The systematic studies with various dehydro–residues have been carried out and a set of design rules have been prepared. These investigations have indicated that the branched β–carbon dehydro–residues also introduce steric constraints in peptides. The structure of a pseudotripeptide N-Ac-Pro-D Val-NHCH3 has shown a nice formation of a type III β-turn conformation in contrast to a type II structure induced by D Phe at (i+2) position. In order to define the peptide conformation fully, systematic studies have been carried out with D Val and D Ile by placing them at various positions in peptides using different combinations with other dehydro residues. It shows that D Val and D Ile at (i+1) position favoured a type II β- turn conformation. Furthermore, the structure of pseudopentapeptide Cbz-D Val-Val-D Phe-Ile-OCH3 with D Val at (i+1) and D Phe at (i+3) positions resulted in a highly distorted 3 10 –helical conformation indicating a specific behaviour of the combined sequence of D Val and D Phe residues. It has been further observed that the single branched β-carbon amino acid at (i+1) position in a peptide forms type II β-turn and at (i+2) position favours a type III β-turn structure while in combination with D Phe it gives rise to a 3 10- helical conformation.

“ D = Dehydro”

Tu395

CD STUDIES OF THE CONFORMATIONAL STABILITY OF THE HCC α-HELICAL FRAGMENT

R. Sosnowska, K. Stachowiak, A. StĘPieŃ-Pade, S. OŁDziej, Z. Grzonka

Faculty of Chemistry, University of GdańSk, Poland

Human cystatin C (hCC) is a 120 amino acid residues protein that reversibly inhibits papain-like cysteine proteases. This inhibitor belongs to the amyloidogenic proteins shown to oligomerize through 3D domain swapping mechanism. The crystal structure of hCC reveals the way the protein refolds to produce symmetric dimer while retaining the secondary structure of the monomer. The monomeric form of hCC consists of a core with a five-stranded antiparallel β-sheet wrapped around a central α-helix. The hCC dimerization is preceded by an opening movement of L1 loop from β2-L1-β3 hairpin and separation of the β1-helix-β2 fragment from the remaining part of the molecule. The amino acid sequence of β1-helix region suggests additional possible partial unfolding in the N-terminal part of helix. In order to investigate the structural stability of β1-helix region the peptide corresponding to the helix and its N-terminal truncated analogs was synthesized along with the peptide analogs of helix containing point mutations that could stabilize helical structure of the N-terminus. The peptides were synthesized by the solid-phase method using Fmoc/tBu tactics. Purified products were identified by MALDI-TOF. The secondary structure content was calculated from the CD spectra using SELCON3. The random coil was the predominant structure of the peptide corresponding to the α-helical fragment of hCC and its N-truncated analogs. However, an increase of α-helix content was observed in some of the peptides corresponding to the helix containing point mutations. We expect that these mutations could stabilize the hCC monomer and suppress dimerization.

Supported by UG-DS/8350-5-0131-6 and EFS ZPORR/2.22/II/2.6/ARP/U/2/05.

CRYSTAL STRUCTURE ANALYSIS ON P53 TETRAMERIZATION DOMAIN WITH A SUBSTITUTION OF CYCLOHEXYLALANINE FOR 341PHENYLALANINE

R. Takahashi[1], T. Nomura[2], S. Nakamura[1], Y. Kobayashi[1,3], K. Sakaguchi[2], T. Ohkubo[1]

[1]Graduate School of Pharmaceutical Sciences, Osaka University, Suita [2]Graduate School of Science, Hokkaido University, Sapporo [3]Osaka University of Pharmaceutical Sciences, Takatsuki, Japan

The tumor suppressor protein p53 is a trarnscription factor that triggers cell-cycle arrest and apoptosis in response to genotoxic stress signals. The tetramereric structure of the p53, which is essential for its activity as a transcription factor, is formed as a dimer of dimers. While the primary dimer is constructed from inter molecular formation of a two-stranded anti-parallel b-sheet and a two anti-parallel a-helix bundle, the secondary dimer is stabilized through interactions between residues on the surface of the primary dimer. From various substitution experiments on p53, it has been shown that hydrophobicity of Phe341 is critical for the tetramer formation of p53. Also we have substituted three phenyl groups of p53 with cyclohexylalanine (Cha) and showed that Phe341Cha is dramatically stabilized against temperature, chemical denaturant, and organic solvent by CD measurements.

Here, to clarify the mechanism of the stabilization of Phe341Cha, we analyzed its three dimensional structure using X-ray crystallography. We obtained two kinds of crystals, one is a hexagonal bipyramid crystal in the space group of P<I>6₄22</I> with <I>a</I>=50.12 Å, <I>b</I>=50.12 Å, <I>c</I>=48.18 Å diffracted to about 2.0 Å, and another is tabular crystal in the space group C<I>2</I> with <I>a</I>=77.25 Å, <I>b</I>=50.04Å, <I>c</I>=55.10 Å diffracted to about 2.1 Å. In these crystals, the peptides formed tetramers which are very similar to those observed in the wild-type. The structure of the pocket where the side chain of Cha341 is incorporated was defined to elucidate the hydrophobic interaction to determine the stability.

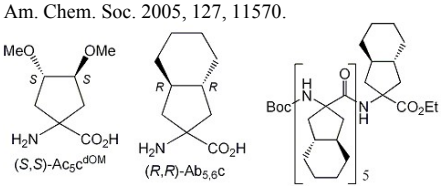
SIDE-CHAIN CHIRAL CENTERS OF AMINO ACIDS AND HELICAL-SCREW HANDEDNESS OF THEIR PEPTIDES

M. Tanaka[1], M. Nagano[1], Y. Demizu[1], K. Anan[1], M. Kurihara[2], M. Doi[3], H. Suemune[1]

[1]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka [2]Division of Organic Chemistry, National Institute of Health Sciences, Tokyo [3]Osaka University of Pharmaceutical Sciences, Osaka, Japan

Helices shown in proteins, as a secondary structure, almost always form right-handed screw sense. This right-handedness is believed to result from the chiral center at the α-position of proteinogenic L-α-amino acids. Among proteinogenic amino acids, L-isoleucine and L-threonine possess an additional chiral center at the side-chain β-carbon besides the α-carbon. However, no attention has be paid how the side-chain chiral centers affect the secondary structures of their peptides. Recently, we have reported that side-chain chiral centers of chiral cyclic α,α-disubstituted amino acid (S,S)-Ac(5)c(dOM) affected the helical secondary structure of its peptides, and the helical-screw direction could be controlled without a chiral center at the α-carbon atom. Herein, we synthesized a chiral bicyclic α,α-disubstituted amino acid (R,R)-Ab(5,6)c and its homopeptides, and studied the relationship between the chiral centers and the helical-screw handedness of peptides. Contrary to the left-handed helices of (S,S)-Ac(5)c(dOM) peptides, the homopeptides composed of bicyclic amino acid (R,R)-Ab(5,6)c formed both diastereomeric right-handed and left-handed helices.

Ref. M. Tanaka, Y. Demizu, M. Doi, M. Kurihara, H. Suemune, Angew. Chem. Int. Ed. 2004, 43, 5360; M. Tanaka, K. Anan, Y. Demizu, M. Kurihara, M. Doi., H. Suemune, J. Am. Chem. Soc. 2005, 127, 11570.



Tu398

COMPARATIVE PROTEOMICS AND FUNCTIONAL STUDIES OF THREE-FINGER TOXINS IN DIFFERENT GEOGRAPHIC VENOM SAMPLES OF BUNGARUS FASCIATUS

I.H. Tsai, S.Y. Tsai

Institute of Biol. Chem., Academia Sinica, Taipei, Taiwan-R.O.C.

Four threefinger-toxins (TFs) have been purified from the pooled venom of golden krait (Bungarus fasciatus, i.e. Bf) from Thailand and studied previously. These peptide toxins contain 60-65 residues and 4 or 5 pairs of disulfide bonds, and are rich in β-structure. We herein analyzed the TF-isoforms in Bf venoms from Kolkata (eastern India), Hunan province (eastern China) and Indonesia to study the geographic variations and structure-function relationships of the venom polypeptide family. A total of five or six TFs of low lethality were purified from each of the geographic venom samples, the N-terminal sequences and accurate masses of the peptides were determined. The cDNAs encoding some of these TFs were also cloned and sequenced. Full peptide sequences were deduced and match with those of the TFs purified from crude venom. Intra-species variations of the venom TFs were found to be surprisingly high since sequence-identities between the majorities of orthologous toxins in different geographic samples are only 75-80 %. Most of the Bf proteins were not neurotoxic by electrophysiological assays using chick biventer-cervicis and mouse diaphragm neuromuscular tissues. The toxins appear to be associated with weak toxins or non-conventional snake venom TFs as analyzed by a phylogenetic tree. The reason behind their lack of neurotoxicity would be discussed.

THE ROLE OF THE ARGININE SIDE CHAIN TO THE STRUCTURE STABILIZATION OF LHRH

V. Moussis, E. Panou-Pomonis, C. Sakarellos, **V. Tsikaris**

Department of Chemistry, University of Ioannina, Greece

Luteinizing hormone-releasing hormone (LHRH) is a linear hypothalamic decapeptide that is secreted from the hypothalamus in a pulsatile pattern and regulates the reproductive system by controlling the secrection of the gonadotropic hormones, luteinizing hormone (LH) and follicle hormone (FSH). Like many hormones LHRH is a highly flexible molecule that can adopt many interconverting conformations in solution and the assignment of a predominant bioactive conformation is very difficult. Previous conformational studies have greatly contributed in understanding the structural basis of the activities of LHRH and its analogues. Two type II β-turns placed in the central part of the molecule (-S4-Y-G-L7- and –Y5-G-L-R8-) have been proposed to stabilize the bioactive conformation. In this work, using various LHRH analogues and peptide models derived from the C-terminal sequence of the LHRH, we investigated the contribution of the Arg side chain to the structure stabilization of LHRH. 1H-NMR results in DMSO-d6 solution are favored toward a side chain-backbone interaction involving the guanidinium and the C-terminal Gly carbonyl groups.

M400

VIBRATIONAL AND CHIROPTICAL SPECTROSCOPIC INVESTIGATIONS ON CIS-2-AMINOCYCLOPENTANECARBOXYLIC ACID OLIGOMERS

E. Vass[1], M. Hollósi[1], T.A. Martinek[2], F. Fülöp[2]

[1]Department of Organic Chemistry, Eötvös Loránd University, Budapest [2]Institute of Pharmaceutical Chemistry, University of szeged, Hungary

Incorporation of conformationally restricted cyclic β-amino acid residues in β-peptides permits a rational control over helix and strand secondary structures. It was shown earlier that homo-oligomers composed of (1R,2S)-2-aminocyclopentanecarboxylic acid residues [(1R,2S)-cis-ACPC] form a self-stabilizing six-strand secondary structure in the solution phase [1], while those composed of (1R,2R)-trans-ACPC residues are known to adopt a highly stable H12 helix conformation [2]. Thus, inversion of the relative configuration (from trans to cis) of the ACPC residues enabled the preferred periodic structure to be switched from a helix to a non-polar strand. Herein we report a circular dichroism (CD), Fourier-transform infrared (FTIR) and vibrational circular dichroism (VCD) spectroscopic study of recently synthesized tetra- and hexamers consisting of alternating enantiomers of cis-ACPC residues, H-[(1S,2R)-cis-ACPC-(1R,2S)-cis-ACPC]n-NH2 (n=2, 3). VCD, combined with quantum chemical calculations performed at the B3LYP/6-31G level of theory was particularly useful for identifying the type of preferred secondary structure. The VCD spectra indicate that these oligomers adopt a well-defined alternating H10-H12 right-handed helix in dichloromethane solution, stabilized by C10 and C12 intramolecular H-bonds. The formed helical structure is very stable, being mostly preserved even in dimethyl sulfoxide. [1] Martinek, T.A., Tóth, G.K., Vass, E., Hollósi, M., Fülöp, F., Angew. Chem. Int. Ed. 41, 1718-1721 (2002). [2] Appella, D.H., Christianson, L.A., Klein, D.A., Richards, M.R., Powell, D.R., Gellmann, S.H., J. Am. Chem. Soc., 121, 7574-7581 (1999).** **This work was supported by the Hungarian Research Fund OTKA (grants T 047186 to E.V. and T 049792 to M.H.).**

Tu401

RANDOM COILS, BETA-SHEET RIBBONS AND ALPHA-HELICAL FIBERS-ONE PEPTIDE ADOPTING THREE DIFFERENT SECONDARY STRUCTURES AT WILL

S.C. Wagner[1], K. Pagel[1], H. von Berlepsch[2], C. Böttcher[2], B. Koksch[1]

[1]Department of Chemistry and Biochemistry-Organic Chemistry [2]Research Center for Electron Microscopy, Freie Universität Berlin, Germany

Peptides involved in neurodegenerative diseases can adopt at least two different stable secondary structures. Amyloid-forming proteins can experience a conformational transition from the native, mostly α-helical structure, into a ß-sheet rich isoform. The latter conformation is probably present in intermediates for the formation of amyloids. The conformational change can be triggered by protein concentration or environmental changes.

Therefore, our aim was to generate a de novo designed peptide that contains structural elements for both, stable α-helical as well as ß-sheet formation. This model peptide can be used to elucidate the conformational changes dependent on concentration and pH.1 The design is based on the well studied α-helical coiled coil folding motif. The conformation and structure of the resulting aggregates were characterized by CD-spectroscopy and cryo transmission electron microscopy. As a result, three distinct secondary structures can be induced at will by adjustment of pH or peptide concentration. Low concentrations at pH 4.0 yield globular particles of the unfolded peptide whereas, at the same pH but higher concentration, defined ß-sheet ribbons are formed. In contrast, at high concentrations and pH 7.4, the peptide prefers highly ordered α-helical fibers.

In conclusion, we successfully generated a model peptide that, without changes in its primary structure, predictably reacts on environmental changes by adopting different defined secondary structures. Thus, this system allows to systematically study now the consequences of the interplay between peptide primary structure and environmental factors for conformation on a molecular level.

1 B. Koksch et al. J. Am. Chem. Soc., 2006, 128, 2196-2197

INFLUENCE OF LEU-5 CONFIGURATION ON EQUILIBRIUM CONSTANTS OF COMPLEXES OF [LEU]-ENKEPHALIN WITH BETA-CYCLODEXTRIN

J. Mrozek[1], J. Malicka[1], A. Skwierawska[1], E. Sikorska[1], B. Banecki[2], **W. Wiczk**[1]

[1]Faculty of Chemistry, University of GdańSk [2]Intercollegiate Faculty of Biotechnology, University of GdańSk And Medical University of GdańSk, Poland

Natural enkephalins and their analogues are very important as potential therapeutic agents (analgetics). However, their action time is very short (about 2 minutes) what is connected with enzymatic degradation. Hydrolysis of their peptide bonds can be avoided when the peptide chain is chemically modified or specific molecules such as cyclodextrins (CD) which can form inclusion complexes with enkephalins are used. In our work we studied the interactions of beta-cyclodextrin with cyclic Leu-enkephalins: H-c[D-Dab2-Gly3-Trp4-(L- or D)-Leu5] (L-EN or D-EN) using isothermal titration microcalorimetry and 2D 1H NMR (ROESY) spectroscopy. The equilibrium constants obtained from the calorimetry measurements depend on leucine configuration and are equal 269±27 and 162±4 for D-EN and L-EN, respectively. The analysis of NMR spectra reveals that Trp residue of D-EN is deeper located in cyclodextrin cavity than that of L-EN. Additionally, the orientation of Leu residue towards Trp aromatic ring as well as cyclodextrin differs for both analogues.

This work was financially supported by KBN under grant 0192/T09/2004/26.

M403

PARALLEL DETECTION OF CHANGES OF MOLECULAR INTERACTIONS IN CELLULAR SIGNAL TRANSDUCTION USING PEPTIDE MICROARRAYS

O. Stoevesandt, K. Köhler, S. Wolf, T. André, M. Fotin-Mleczek, **R. Brock**

Department of Molecular Biology, Interfaculty Institute for Cell Biology, University of Tuebingen, Germany

Cells respond simultaneously to a multitude of different signals. Inside a cell signals from activated receptors are integrated by networks of enzymatic reactions and molecular interactions, leading to a spectrum of cellular responses. In order to understand the relationship between a specific cellular stimulus and a cellular response, methods are required to detect in parallel the pattern of molecular interactions.

A large number of molecular interactions is mediated by protein domains, binding to linear peptide motifs. Lysates of activated and resting cells were incubated on peptide microarrays carrying peptides corresponding to such binding motifs of signalling domains. Binding of proteins to a spot of the array was probed by immunofluorescence. Jurkat T cells were stimulated either with the phosphatase inhibitor pervanadate or with antibodies directed against cell surface receptors.

Upon activation of T cells, numerous changes in the pattern of molecular interactions were detected for a total of 10 proteins and 30 peptides. These changes were caused either (i) by masking or unmasking of a binding domain which resulted in a reduced or increased binding of a protein to the microarray or (ii) by recruitment of a protein into a complex that in turn bound to the microarray. The changes were dependent on the nature of the stimulus.

In contrast to established techniques for the analysis of molecular interactions, such as coimmunoprecipitation, with the peptide microarrays information on thousands of molecular interactions can be obtained in one experiment.

Tu404

THE PEPTAIBIOME AND PEPTAIBIOMICS. STRUCTURAL CHARACTERIZATION OF THE ENTIRETY OF PEPTAIBIOTICS EXPRESSED BY FUNGI

H. Brückner, C. Krause, J. Kirschbaum

Interdisciplinary Research Center, Department of Food Sciences, University of Giessen, Germany

In analogy to the proteome and proteomics we define the peptaibiome as the entirety of fungal peptides containing the characteristic non-protein amino acid Aib. Accordingly, peptaibiomics is the analytical methodology for the structural characterization of all peptaibiotics expressed in cells of filamentous fungi. A peptaibiotic is defined as fungal peptide containing Aib and exhibiting antibiotic or other bioactivities. Peptides of this group consist of mixtures of 5 to 20-residue peptides. We present a rapid and sensitive method for the detection and structural characterization of the peptaibiome expressed by fungal multienzyme complexes. The method comprises growth of fungi on Petri agar dishes and solid-phase extraction of mycelia followed by HPLC-ES-MS. Characteristic fragment ions and search in data bases enable detection of new and matching with known peptaibiotics. The peptaibiome of recently described species and strains of Trichoderma and teleomorphs Hypocrea are presented.

CELL SIGNALING OF MELANOCORTIN RECEPTOR I BY MASS SPECTROMETRIC ANALYSIS

M. Cai[1], G. Tsaprailis[2], S. Bordelon[1], H. Qu[1], D. Trivedi[1], V.J. Hruby[1]

[1]Chemistry Department [2]Proteomics & Toxicology Center, University of Arizona, Tucson, AZ, USA

The human melanocortin receptor 1 (hMC1R) was constructed to contain a FLAG epitope and a hexahistidine tag at the amino-terminus as well as at the carboxyl terminus to facilitate purification. Stably transfected hMC1R in human embryonic kidney (HEK293) cell lines that expressed the receptor resulted in a KD value of 0.1 and 0.2 nM respectively in each case when the super potent agonist MTII was competed with [125I]NDP-α-MSH. Treatment of the tagged receptors in the HEK293 cells with agonist resulted in down-regulation which indicates that these tagged receptors retain their biological functions. The hMC1R was solubilized from cell membranes with n-dodecyl-β-D-maltoside and purified at a Nickel chelating resin and a newly constructed affinity column. The purified hMC1R was a glycoprotein that migrated on SDS/PAGE with a molecular mass of 58 kDa. The results from Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was used to identify and characterize peptides derived from the hMC1R following in-gel digestion with chymotrypsin. The phosphorylation sites were identified on the purified human melanocortin receptor 1 with agonists (peptide vs small molecule) treatment.

Supported by Grants from the USPHS DK 17420 and DA 06284

M406

IDENTIFICATION AND CHARACTERIZATION OF ANTIMICROBIAL COMPOUNDS FROM LARVAE OF FLESHFLY NEOBELLIERIA BULLATA

A Ciencialova[1], T. Neubauerova[2], R. Sindelka[3], M. Sanda[1], J. Cvacka[1], V. Kasicka[1], B. Koutek[1], J. Jiracek[1]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic [2]Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology [3]Department of Gene Expression, Institute of Molecular Genetics, Academy of Sciences of The Czech Republic, Praha, Czech Republic

The discovery of antibiotics in the 1930s has been one of the most revolutionary events in the history of medicine. However, during last decades, the increase of antibiotic resistance has significantly hampered the application of antibiotics. Therefore, further scientific effort to find new antibiotics with novel mechanisms of action is of high importance.
Insects are the largest and the most diverse group of living animals on earth. They have potentially been confronting high variety of microorganisms. As a result, they have evolved powerful defense system, thus representing vast source of novel potential therapeutics.

We chose larvae of fleshfly Neobellieria bullata for identification and characterization of new promising molecules, peptides or proteins, which participate in immunity response against microbial infections. The hemolymph of the third-instar larvae of Neobellieria bullata was used for isolation. The larvae were injected with bacterial suspension of Escherichia coli or Staphylococcus aureus to induce antimicrobial response. The hemolymph was separated into crude fractions, which were subdivided by RP-HPLC. Isolated fractions were characterized by UV-VIS spectroscopy, amino-acid analysis, mass spectroscopy, 1-D and 2-D SDS electrophoresis, capillary zone electrophoresis, ion-exchange HPLC, tryptic digests and N-terminal sequencing. We found out significant antimicrobial activities against Escherichia coli, Staphylococus aureus or Pseudomonas aeruginosa in several fractions. Using real-time PCR, we followed and compared levels of mRNA of different proteins and peptides in induced and non-induced larvae.

Tu407

NEW SYNTHETIC PROBES FOR THE ANALYSIS OF THE CELL MEMBRANE PROTEOME AND INTERACTOME

M. Collet, J. Lenger, N. Sewald

Department of Chemistry, University of Bielefeld, Germany

Despite the fact that many technological advances are currently involved in proteome analysis, like two-dimensional gel electrophoresis and mass spectrometry, there is still a great need for the development of novel engineered chemical probes for proteomics and interactomics. Here, we describe our approach concerning the study of proteome and interactome of proteins involved in cell-matrix interactions. It relies on the use of a small synthetic inhibitor chemically modified to allow for its immobilisation to magnetic beads or affinity chromatography materials. Proteins will be detected together with their native interaction partners because of non-denaturing conditions. This general procedure is applied for the enrichment of metalloproteinases, especially matrix metalloproteinases, which are potential target in tumour therapy.

Hydroxamic acids are known to be potent inhibitors of metalloproteinases. Marimastat is a reversible inhibitor with a good potency and shows activity towards a wide range of metalloproteinases. The synthesis of new marimastat derivatives will be reported. The parent compound is modified with a linker to allow immobilisation on a solid surface. Binding studies were performed using surface plasmon resonance. This approach is not only appropriate for the generation of metalloproteinase proteome subsets by affinity column or using magnetic beads, but also to enrich and isolate interaction partners of the target proteins.

Th408

ROLE OF THE C-TERMINAL CONSERVED SEQUENCE MOTIF IN THE FUNCTION OF DUTPASE FROM MASON-PFIZER MONKEY RETROVIRUS

O. Barabás[1,2], V. Németh-Pongrácz[1], **A. Magyar**[3], F. Hudecz[3,4], B.G. Vértessy[1]

[1]Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences [2]Department of Theoretical Chemistry, Eötvös Loránd University [3]Hungarian Academy of Sciences, Research Group of Peptide Chemistry at Eötvös Loránd University of Sciences [4]Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary

The fusion protein nucleocapsid-dUTPase is present in virions of Mason-Pfizer monkey betaretrovirus and in virus-infected cells where it potentially contributes to RNA/DNA folding and reverse transcription (Barabas, et al., 2003; Bergman et al., 1994; Berkowitz, et al., 1995). In addition to trimeric dUTPase core, the protein possesses flexible N- and C-termini consisting of the nucleocapsid segment, and a peptide motif conserved in dUTPases. To analyze the function of the flexible C-terminal peptide segment, reconstitution experiments were designed with truncated enzyme lacking the C-terminal 14mer oligopeptide and the synthetic oligopeptide prepared on Rink-amid resin by solid-phase peptide synthesis, using Fmoc strategy. The truncated enzyme proved to be practically inactive. Addition of the synthetic 14mer (PYRGQGSFGSSDIY) at 100fold molar excess resulted in partial complamentation of the catalytic activity (to 10% of original). A mixture of the truncated enzyme and the 14mer oligopeptide (this latter at 100fold excess) was put to crystallization trials. We conclude that the C-terminal 14mer is essential for catalytic activity.

This work was supported by grant from National Office for Research and Technology, Hungary (GVOP–3.1.1.-2004-05-0412/3.0.)

Barabas, O., Rumlova, M., Erdei, A., Pongracz, V., Pichova, I. and Vertessy, B.G. (2003) J. Biol. Chem., 278, 38803-38812.
Bergman, A.C., Bjornberg, O., Nord, J., Nyman, P.O. and Rosengren, A.M. (1994) Virology, 204, 420-424.
Berkowitz, R.D., Ohagen, A., Hoglund, S. and Goff, S.P. (1995) J. Virol., 69, 6445-6456.

SYTHESIS OF MODIFIED N-ACYLHOMOSERINELACTONES

H.P. Plattner[1], C. Metzendorf[2], M. McIntosh[2], F.W. Bartels[3], D. Anselmetti[3], R. Ross[3], A. Becker[2], N. Sewald[1]

[1]Department of Chemistry [2]Department of Biology [3]Department of Physics, Bielefeld University, Bielefeld, Germany

Quorum sensing (QS) is a cell-density dependent phenomenon of bacterial colonies. This system facilitates them to sense the excess of a critical cell concentration. Small amounts of permanently produced signal molcules get enriched and initiate a specific gene expression. In gram-negative bacteria N-acyl-homoserinelactones (AHLs) are utilized, for gram-positive bacteria post-translationally modified peptides were observed as signal molecules. Synthesis and modification of those molecules, especially of AHLs was done to investigate the function of their receptor proteins and the following protein-DNA interaction. For this purpose the modifications should not harm the biological activity but offer the opportunity to introduce reporter groups. Starting with L-methionine as a chiral substrate cis and trans-3-hydroxy-L-homoserine lactone was synthesized. After acylation of the amino function with fatty acids full activity was maintained, which could be proofed by electrophoretic mobility shift assays (EMSA). Further modifications of the hydroxyl group are done to introduce a spacer unit. If the bioactivity is still conserved a functionalized AHL can be designed. The new probe facilitates investigations of QS systems (SPR, AFM, fluorescence studies) concerning Proteomics.

Tu410

SOLID-STATE ISOTOPIC EXCHANGE WITH SPILLOVER-HYDROGEN IN PROTEINS AND PEPTIDES

Yu.A. Zolotarev[1], **B.V. Vaskovsky**[2], A.K. Dadayan[1], V.S. Kozik[1],
R.H. Ziganshin[2], N.F. Myasoedov[1]

[1]Institute of Molecular Genetics [2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

The present report summarizes the latest data devoted to theoretical and experimental investigation of the high temperature solid state catalytic isotope exchange reaction (HSCIE) that takes place in peptides and proteins by the action of deuterium and tritium [1]. The available MS-procedures, designed to estimate the amount of protein, are aimed at derivatization at different stages of sample preparation, and as the best result, it is only possible to achieve quality comparison of the objects involved. The HSCIE reaction allows the production of evenly deuterium labelled proteins and peptides, and their application makes it possible to create a qualitative mass spectrometry method for protein analysis. Introduction of definite amounts of these deuterium-labeled proteins into biological objects, prior to isolation, separation and trypsinolysis, will generate quantitative information concerning the composition of the proteins under study. Tritium labelled proteins produced at a temperature of 100-120oC carry the isotopic label in all the peptide fragments and completely retain their enzymatic activity. The proteins' reactivity is dependent on their three-dimensional structure. The HSCIE reaction has been shown to be used both in the production of tritium labelled proteins and in the investigation of spatial interactions in protein complexes. In addition, evenly deuterium and tritium labelled peptides can be used in studies of the kinetics and transformation paths of peptides in the organism's tissues.

[1 | Zolotarev YuA, Dadayan AK, Borisov YuA (2005) Solid-state isotopic exchange with spillover-tritium in amino acids, peptides and proteins. Russian J Bioorg Chem 31:3-21

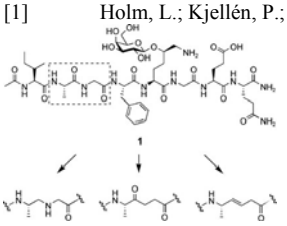
AMIDE BOND ISOSTERES INTRODUCED IN A GLYCOPEPTIDE THAT IS RECOGNIZED BY T CELLS IN A MOUSE MODEL FOR RHEUMATOID ARTHRITIS

I. Andersson[1], A. Linusson[1], J. Kihlberg[1,2]

[1]*Organic Chemistry, Department Of Chemistry, Umeå University, Umeå, Sweden*
[2]*AstraZeneca R&D Mölndal, Mölndal, Sweden*
Sweden

Immunization of mice with type II collagen (CII) from rat leads to development of collagen-induced arthritis (CIA). Susceptibility to CIA is associated with the major histacompatibility class II protein H-2Aq that binds the glycopeptide epitope CII260-267 (1) and presents it to helper T cells.[1] To explore the interactions in the system and to stabilize 1 towards in vivo degradation, amide bond isosteres have been introduced in its backbone.Glycopeptide 1 was virtually docked into the binding site of a comparative model of H-2Aq. Based on the hydrogen bonding network between the peptide backbone and H-2Aq, the amide bond between Ala261-Gly262 was chosen for isosteric replacement. To vary the geometric and hydrogen bonding properties, mimetics of the dipeptide were synthesized with the amide bond replaced by ψ[CH2NH], ψ[COCH2] and ψ[(E)-CH=CH], respectively. These were introduced in 1 using solid-phase synthesis to give glycopeptidomimetics that were biologically tested for their ability to bind to the H-2Aq protein and for recognition by T cells.

[1] Holm, L.; Kjellén, P.; Holmdahl, R.; Kihlberg, J. Bioorg. Med. Chem. 2005, 13, 473-482.



SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF NOVEL ALPHA-CONOTOXINS WITH SUBSTITUTED PROLINES

C.J. Armishaw, A.A. Jensen, T. Liljefors, K. STRØMGAARD

Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Copenhagen Ø, Denmark

alpha-Conotoxins isolated from the venom of carnivorous marine snails are highly potent and selective inhibitors for different subtypes of nicotinic acetylcholine receptors (nAChR). They contain two disulfide bonds which gives rise to two loops of intervening amino acids, with a single conserved proline residue in loop 1 that contributes to a very rigid and well defined 3D-structure. Acetylcholine binding protein (AChBP) shares sequence homolgy with the ligand binding domain of nAChRs and provides a valuable tool for the rational design and synthesis of novel alpha-conotoxin analogues. Recent crystal structures of alpha-conotoxins ImI and PnIA complexed with AChBP shows that upon ligand binding the conserved proline in loop 1 is brought into close proximity to a hydrophobic binding pocket consisting of three aromatic residues. Therefore substituting the 4-position of the conserved proline with various substituents may lead to the development of potent new nAChR ligands with novel pharmacological properties, without compromising the native conformation. We have synthesised a series of analogues of alpha-conotoxins ImI, AuIB and GID, with hydroxyl, phenyl, guanidino, amino, tertiary amino and quaternary-amino substituents on the 4-cis and -trans positions of proline. The peptides were assembled in parallel using Fmoc-solid phase peptide synthesis and the correct disulfide bond isomers formed by air mediated oxidation in aqueous buffer following cleavage from the resin with TFA. The compounds were investigated at various subtypes of nAChRs and were found to possess unique pharmacologies.

Tu413

ZINC-LEUPROLIDE COMPLEX:SYNTHESIS AND ANALYTICAL EVALUSIONS

R. Astaneh[1], N. Nafissi[2]

[1]School of Pharmacy, Shaheeb Beheshti Universityof Medical Sciences [2]Shaheeb Beheshti University of Medical Sciences, Tehran, Iran

Background and aims: Various attempts have been made to produce peptides with prolonged action. One approch is a complex formation. Leuprolide acetate (LA) is currently used for the treatment of prostatic cancer and endometriosis. In this study we used LA to form a low solubility product, Zinc-Leuprolide, which should be sutiable for sustained parenteral delivery.

Methods: Preparation of complex: LA was dissolved in water. An appropriate amount of zncl2 solution was added into peptide solution. After mixing, the pH was adjusted by adding 3N sodium hydroxide and then incubated for 1h at room temperature to form zinc-leuprolide complex prior to lyophilization.

Instrumental analysis: Fourier transforms infrared (FTIR) were recorded on a Bruker EQUINOX55 FRA 10615. Diffrential scanning calorimetry (DSC) thermogram of leuprolide acetate and complex was conducted on a DSC- 60 Shimadzu instrument. The amorphous or crystallinity of complex was observed using X-ray diffraction patterns.

Results: The IR spectra of complex revealed a definite shift in absorption of carboxyl OH stretching. DSC curves of leuprolide acetate and complex showed the appearance of a new endothermic peak at 113.50°C, also the drug peak at 166.89°C disappeared completely in the complex, confirming the formation of a new compound. X-ray pattern for complex showed preseance of crystallinity as well.

Conclusions: The addition of divalent cations to proteins formulations is known to decrease solubility and dissolution. So we prepare the zinc-leuprolide complex for improving the sustained release activity of this valuable drug in controlled release systems such as microsphere or injectable implant.

Th414

RETROINVERSO PEPTIDES INHIBIT AMYLOID NEUROTOXIOCITY AND ARE POTENTIAL THERAPEUTICS AGAINST ALZHEIMER'S DISEASE

B.M. Austen, O.M. El-Agnaf, B. Maltharu, N. Gunasekera

Department of Basic Medical Sciences, St. George's University, London, UK

We have synthesised retroinverso (RI) forms of sequences from the central portion (residues 16-21) of beta-amyloid, the prime causative agent in Alzheimer's disease. The RI peptides are more active than the native sequences at inhibiting aggregation and fibrilisation of beta-amyloid 40 and 42. The peptides are highly active in preventing aggregation of beta-amyloid, and preventing neurotoxicity in human neuronal cell lines. and in view of their in vivo stability, have good prospect as therapeutic agents.

M415

CHEMOTACTIC PEPTIDE BASED DRUG TARGETING OF METHOTREXATE

K.B. Bai[1], O. Láng[2], J. Birinyi[2], J. Láng[2], G. Schlosser[1], L. Kőhidai[2], F. Hudecz[1,3], G. Mező[1]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eotvos L. University [2]Department of Genetics, Cell- and Immunobiology, Semmelweis University [3]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

Water soluble drug conjugates, which consist of a targeting sequence and drug molecule attached with or without a carrier molecule, offer specific cellular uptake pathway. by receptormediated endocytosis. Targeting sequence can be e.g. hormone or chemotactic peptides recognized by receptors that are over expressed on tumour cells. In this study, the synthesis, the chemotactic activity as well as the cytotoxic effect of bioconjugates developed for chemotactic drug targeting (CDT) will be presented. In these conjugates, a tetratuftsin dervative ([TKPKG]4) was used as carrier and formyl-tripeptides or tuftsin derivatives (For-MLF, For-NleLF, TKPR, For-TKPR, TKPKG, Ac-TKPKG) as chemoattractant target sequences. Methotrexate (Mtx) was applied as anticancer agent and was attached to the carrier via an enzyme (e.g. Chatepsin B) labile pentapeptide (GFLGC) spacer. The drug-spacer conjugate was synthesised first and than conjugated with the chloroacetylated carrier via thioether bond.Seven conjugates containing Mtx were prepared; (Mtx-GFLGC{CH2CO}-NH2)-K(Mtx-GFLGC{CH2CO}-NH2)-[TKPK(X)G]4-NH2, where X=chemotactic peptides and For-NleLF-K(For-NleLF)-[TKPK(Mtx-GFLGC{CH2CO}-NH2)G]4-NH2. The conjugates and their componenst as controls were studied in chemotaxis and cytotoxicity assays on Tetrahymena pyriformis cells and THP-1 monocytes. Cellular uptake of the compounds were studied by using fluorescent labelled analogues. The results of the biological tests confirmed the feasibility of this new chemotactic drug targeting strategy for increasing the efficacy and specificity of cancer chemotherapy.

Acknowledgement: This work was supported by grants from the Hungarian National Science Fund (OTKA T 032533, T 043576 and T 049814) and "Medichem 2" 1/A/005/2004.

DIPEPTIDYL-PEPTIDASE IV-RESISTANT ANALOGUES OF PACAP WITH IMPROVED IN VITRO PLASMA STABILITY

S. Bourgault[1], D. Vaudry[2], B.J. Gonzalez[2], H. Vaudry[2], A. Fournier[1]

[1]LEMPP, INRS - Institut Armand-Frappier, Université du Québec, Pointe-Claire, Montréal, QC, Canada [2]INSERM U413, Université de Rouen, Mont-Saint-Aignan, France

PACAP (pituitary adenylate cyclase-activating polypeptide) is a 38 amino acid peptide initially isolated from hypothalamic extracts that has recently been shown to be a potent neuroprotective factor in various pathophysiological models. Despite its therapeutic potential in diverse neurodegenerative diseases, its short in vivo half-life limits its utility as a useful clinical agent. Moreover, the development of a peptidomimetic that reproduces the pharmacological activity of PACAP is unlikely since the pharmacophores are spreaded throughout the entire peptide chain. Therefore, the development of PACAP analogues with lower susceptibility to proteolysis represents a first step toward clinical applications. In the present study, derivatives of both PACAP27 and PACAP38 with particular chemical modifications were developed targeting specific peptidase sites of action. Results indicate that the incorporation of an acetyl or a hexanoyl group at the N-terminus and modifications at the Ser2 residue contributed to increase stability against dipeptidyl peptidase IV, the major enzyme involved in PACAP degradation. Moreover, after determination of PACAP metabolites in human plasma, the amide bond between residues 21 and 22 was substituted by a CH2NH surrogate and this derivative showed increased plasma stability. All modified peptides were tested for their ability to induce PC12 differentiation. The effects of PACAP analogs on PC12 cells are mediated through the PAC1 receptor which is the major receptor involved in the neuroprotective effects of PACAP. This study exposes interesting data concerning PACAP metabolism in isolated human plasma and demonstrates the possibility of increasing the metabolic stability of PACAP without significantly reducing its biological activity.

TRICHOBREVINS, TRICHOCOMPACTINS, TRICHOCRYPTINS AND TRICHOFERINS – NEW PEPTAIBIOTICS FROM PLANT-PROTECTIVE STRAINS OF THE TRICHODERMA BREVICOMPACTUM COMPLEX

T. Degenkolb[1], W. Gams[2], T. Gräfenhan[3], H.I. Nirenberg[3], **H. Brückner**[1]

[1]Interdisciplinary Research Center, Department of Food Sciences, University of Giessen, Germany [2]Centraalbureau Voor Schimmelcultures (CBS), Utrecht, The Netherlands [3]Federal Biological Research Center for Agriculture and Forestry (BBA), Institute for Plant Virology, Microbiology and Biological Safety, Berlin, Germany

Species of the fungal genus Trichoderma are commercially used as bioprotective agents against fungal plant diseases. More than 400 strains were collected from their natural habitats and evaluated for biocontrol properties. Seven of the most active isolates exhibiting strong biological activity towards Eutypa dieback and Esca diesease of grapevine were classified as Trichoderma brevicompactum, or shown to be closely related to that species.
These strains were screened for production of peptaibiotics. The formation and synergistic action of hydrolytic enzymes and peptaibiotics were to play an important role in mycoparasitism.
After six days of cultivation, plate cultures were extracted with CH2Cl2/MeOH, 1:1. Clean-up was performed using a rapid and selective adsorption on Sep-Pak-C18 cartridges. The methanolic eluates were used for HPLC/Ion-Trap-ESI-LC-MS measurements.
The T. brevicompactum strains investigated produced 68 novel peptaibiotics (number of peptides in parantheses): 12-residue trichocryptins B (14), 11-residue trichocryptins A (12), 11-residue trichobrevins A and B (19), 10-residue trichoferins (6) and 8-residue trichocompactins (17). Notably, all isolates also produced alamethicins F-30.
The data support the hypothesis that peptaibiotics may partly be responsible for the established plant-protective activity of the Trichoderma strains tested.

M418

CONOPEPTIDE TO DRUG: THE DEVELOPMENT, STRUCTURE AND ACTIVITY CORRELATION OF XEN2174

A. Brust[1], D. Alewood[1], B. Colless[1], E. Palant[1], B. Patterson[1], D. Wilson[1], F. Watson[1], C.I. Schroeder[2], P.F. Alewood[2], R.J Lewis[1,2]

[1]Xenome Ltd., Indooroopilly [2]Institute for Molecular Bioscience, University of Queensland, St.Lucia, QLD, Australia

Venomous animals have evolved a vast array of peptide toxins for prey capture and defence. These peptides are highly prospective for rapid drug discovery as they are active towards a wide variety of pharmacological targets such as ion channels or G-protein coupled receptors (GPCRs) in the nervous system.

Here we describe Xenome's drug development process for the chi family, conopeptide □-MrIA[1] of the predatory marine snail Conus marmoreus, leading to a suitable drug candidate (Xen2174). Xen2174 is highly selective for the norepinephrine transporter (NET) compared to other transporters, such as dopamine and serotonin, and inhibits NET via an allosteric mechanism. Xen2174 is currently in a phase I/IIa clinical trial for the treatment of severe pain.

An intensive synthetic analogue and screening program around □-MrIA, incorporating early stage animal data, resulted in the identification of Xen2174, a drug with improved plasma stability, linear pharmacokinetics and wide therapeutic window.

Xen2174 isomers were synthesized via selective disulfide bond formation to identify the active connectivity. Data from alanine- scans, single amino acid mutations and probing of backbone interactions combined with the full 3D NMR structure, led to the development of a pharmacophore for Xen2174. This model is refined from further studies where STRUCTURE-ACTIVITY RELATIONSHIPs were developed utilising binding and functional assay data for a range of peptides.

[1] I.A. Sharpe, E. Palant, C.I. Schroeder, D.M. Kaye, D.J .Adams, P.F. Alewood, R.J. Lewis, J.Biol.Chem., 2003, 278, 40317-40323.

Tu419

EXAMPLES OF CONOTOXIN ENGINEERING: INTRODUCTION OF NON-PEPTIDIC BACKBONE SPACERS INTO CONOTOXINS AND PEPTIDE-PEPTOID CHIMERAS (CONOPEPTOIDS)

B.R. Green[1], W. Bayudan[1], M.E. Ellison[1], M.M. Zhang[1], D. Yoshikami[1],
A. Legowska[2], K. Rolka[2], B.M. Olivera[1], **G. Bulaj**[1]

[1]Department of Biology, University of Utah, Salt Lake City, UT, USA [2]Faculty of Chemistry, University of Gdansk, Poland

Background and aims: Conotoxins are short, disulfide-rich neurotoxins that target various ion channels and receptors. These peptides have desirable pharmacological properties to become therapeutics for neurological disorders; several conotoxins have already reached clinical development stage. Our long-term goal is to improve bioavailability, metabolic stability and pharmacokinetics of conotoxins using a variety of chemical modifications.
Methods: We designed and chemically synthesized conotoxin analogs containing two distinct types of backbone modifications: (1) peptide-peptoid chimeras (conopeptoids) of alpha-conotoxin ImI and (2) peptide chimeras of mu-conotoxin KIIIA containing non-peptidic "backbone spacers".
Results: Conopeptoid-ImI, containing Ala9 replaced by N-methyl glycine potently blocked activity of nicotinic acetylcholine receptors. In mu-Conotoxin KIIIA, aminohexanoic acid or amino-3-oxapemtanoic acid were inserted to be a part of the peptide backbone. The two oxidized analogs containing "backbone prosthesis" differed in their hydrophibicity profile, but they both potently inhibited neuronal sodium channels.
Conclusion: Our results suggest that backbone engineering may become an effective method of producing conotoxin analogs with modified bioavailability.

BIOENGINEERED CYCLIC PEPTIDES FOR TREATMENT OF MULTIPLE SCLEROSIS

M. Cemazar[1], C.C. Bernard[2], D.J. Craik[1]

[1]Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD [2]Immunology and Stem Cell Laboratories, Monash University, Melbourne, Vic., Australia

To increase the stability and the therapeutic efficacy of peptide sequences from myelin oligodendrocyte protein (MOG) that act as multiple sclerosis (MS) antigens, we grafted them onto a framework of a particularly stable class of peptides, the cyclotides. They are a recently discovered family of cyclic plant peptides with superb intrinsic stability. The limitations of linear peptides as drugs due to their instability and poor bio-availability can be overcome by using the cyclotide scaffold as a framework for novel drug design.

Peptide epitopes from MOG protein were incorporated onto the framework of the model cyclotide kalata B1 by means of Boc-SPPS approach. After successful backbone cyclisation and oxidation of the cysteine residues, the peptides were purified to high purity with RP-HPLC. NMR chemical shift analysis was used to assess whether the grafted analogues have a stable scaffold, similar to that of kalata B1. A structure of a representative peptide was determined and it shows remarkable resemblance to the native scaffold of kalata B1.

The activity of the bioengineered peptides has been tested in vivo. A group of mice injected with one of the peptides have shown a depression in the clinical score and have not fallen ill. This is an exciting result that shows the first active bioengineered cyclotide in an animal model of disease. The structural information from NMR studies will be used in conjunction with the results from the activity studies in a feedback loop to design second-generation lead molecules.

M421

SYNTHESIS OF LANTHIONINE ANALOGS OF CONOTOXINS

A.D. de Araujo, P.F. Alewood

Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

Conotoxins are small, disulfide-rich peptide neurotoxins produced in the venom of marine cone snails that enable these molluscs to capture their prey. These compounds exhibit a high degree of selectivity and potency for different ion channels and their respective subtypes, making them useful tools in the investigation of the nervous system. Due to the key role of ion channels in many physiological processes, conotoxins are also excellent drug lead candidates in drug discovery, with some examples currently undergoing clinical trials and one recently approved drug (Prialt®).

Like other peptide drugs, the use of conotoxins in drug development is limited by the low oral bioavailability of these compounds due to pre-systemic enzymatic degradation and poor penetration through the intestinal mucosa. Peptide analogs that mimic the native structure and incorporate rationally designed non-natural modifications in the disulfide framework or peptide backbone may exhibit increased resistance to proteolytic degradation. In biological environments, disulfide linkages are susceptible to attack by enzymes and reducing agents (such as glutathione). Therefore we carried out the synthesis of redox-stable conotoxin analogs in which intrinsic disulfide bonds were replaced by thioether linkages.

In this work, wehave explored two solid-phase synthetic routes in the preparation of thioether conotoxin mimetics: the first route based on peptide assembly using a tetra-orthogonally protected lanthionine building block, and the second route based on intramolecular on-bead cyclisation between cysteine and beta-bromoalanine residues.

Tu422

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL DIALKYL-SUBSTITUTED HISTIDINE CONTAINING THYROTROPIN-RELEASING HORMONE (TRH) ANALOGUES

V. Deep[1], S. Kumar[2], S.S. Sharma[2], R. Jain[1]

[1]Department of Medicinal Chemistry [2]Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, S.A.S. Nagar, India

Thyrotropin releasing hormone (TRH, L-pGlu-L-His-L-Pro-NH2), a tripeptide synthesized in the hypothalamus, operates in the anterior pituitary to control levels of TSH (thyroid-stimulating hormone) and prolactin. The thyrotropin-releasing hormone (TRH) receptor (TRH-R) belongs to the rhodopsin/β-adrenergic receptor subfamily of seven transmembrane (TM)-spanning, G protein-coupled receptors (GPCRs). The two G-protein-coupled receptors for TRH, TRH receptor type 1 (TRH-R1) and TRH receptor type 2 (TRH-R2), have been cloned from mammals and are distributed differently in the brain and peripheral tissues. The TRH receptor subtype-1 appears to mediate the hormonal and visceral effects, whereas TRH receptor subtype-2 has been implicated in its central stimulatory actions. Identification of critical features of the TRH, separation of its multiple activities through design of selective analogues and affinity labels have been elusive and unfulfilled goals for more then 30 years.

This presentation will highlight our studies on effect of the biological activity of TRH with the introduction of alkyl groups of varying sizes at the N-1 and C-2 position of the centrally placed histidine residue of TRH peptide. The requisite N-□-Boc-dialkyl-L-histidines as building scaffolds have been synthesized in six steps from L-histidine methyl ester dihydrochloride and used for the synthesis of various TRH analogues.

The results of receptor binding studies of synthesized analogues indicate them to show selectivity to TRH-R2 subtype. In addition, all synthesized TRH analogues were biologically evaluated for their effect on pentobarbitone induced sleeping time duration. Some of the reported analogues displayed activity superior to that of TRH in vivo

SYNTHESIS OF ANTHRAQUINONE-TUFTSIN ANALOGUES AS POTENTIAL TOPOISOMERASE INHIBITORS

K. Dzierzbicka, A. Kołodziejczyk

Department of Organic Chemistry, GdańSk University of Technology, Gdansk, Poland

Hundred anthraquinone derivatives have been synthesis to this day. Among them anthraquinone analogues containing amino acids or peptide chain as topoisomerase inhibitors. Topoisomerase inhibition represents a key therapeutic target in chemotherapy. We presented synthesis novel anthraquinone peptide analogues, 1-(tuftsin or retro-tuftsin)-(4-,5- or 8)-hydroxy-9,10-anthraquinones. During the first step of the synthesis of 1,4-, 1,5- or 1,8-bis(tosyloxy)anthraquinone from 1,4-, 1,5- or 1,8-dihyroxy-anthraquinone and p-toluene-sulfonyl chloride were obtained. These compounds with an excess of corresponding tuftsin derivatives in acetonitryl as solvent and in the presence of triethylamine gave mono-substituted analogues. The yields of compounds depended on the excess of tuftsin or retro-tuftsin derivatives: TFAxThr-Lys(Z)-Pro-Arg(NO2)-OBn, TFAxThr-Lys(Ala-Z)-Pro-Arg(NO2)-OMe, TFAxArg(NO2)-Pro-Lys(Z)-Thr-OMe, TFAxArg(NO2)-Pro-Lys(Ala-Z)-Thr-OMe, TFAxArg(NO2)-Pro-Lys(Val-Z)-Thr-OMe. When fourfold excess of tuftsin derivatives were used it was possible to obtained the pretected compounds with 40-45% yield. After the removing of the protected group with liquid HF expected products were obtained. Their identity were confirmed by mass spectra and H-NMR (500 MHz, COSY, ROESY, TOCSY) spectroscopy. Their biological activity will be tested.

This work was supported by the Polish State Committee for Scientific Research (Grant No. 3 P05F 014 25).

M424

SYNTHESIS OF CONJUGATES OF COMBRETASTATIN A-4 WITH TUFTSIN DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

K. Dzierzbicka, A. Kołodziejczyk

Department of Organic Chemistry, GdańSk University of Technology, Gdansk, Poland

Combretastatin A-4 (CA-4) is a potent antineoplastic and antiangiogenesis natural substance isolated from the South African tree Combretum caffrum [1]. However, the limited water solubility of this compound complicates the drug application. Transformation of phosphate ester into ammonium, potassium, and sodium salts increased solubility in water [2]. Currently one of them (sodium salts) is under clinical evaluation. In the literature water-soluble amino acid prodrugs of amino-combretastatin with potent antitumor activity in vivo are also described [3]. Continuing our search for anticancer drug candidates we synthesized new combretastatin A-4 analogues containing chemically bonded tuftsin and its derivatives. The combretastatin-tuftsin analogues were obtained according to standard procedures used in peptide chemistry. Structures of all products were established on the basis of NMR (500 MHz) spectra and microanalyses. We hope that the conjugation of immunomodulators like tuftsin derivatives with CA-4 would improve the therapeutical properties of combretastatin A-4.

This work was supported by the Polish State Committee for Scientific Research (Grant No. 3 P05F 014 25).

References:

1. Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garcia-Kendall D, Experientia 45 (1989), 209.
2. Pettit GR, Rhodes MR, Anti-Cancer Drug Design 13 (1998), 183.
3. Ohsumi K, Hatanaka T, Nakagawa R, et al. Anti-Cancer **Drug Design 14 (1999), 539.**

GROWTH HORMONES SECRETAGOGUES (GHSS) OR GHRELIN RECEPTOR LIGANDS

J.-A. Fehrentz[1], L. Demange[1], A. Moulin[1], D. Boeglin[1], D. Mousseaux[1], J. Ryan[1], J.-C. Galleyrand[1], D. Gagne[1], G. Bergé[1], C. Gozé[1], C. M'Kadmi[1], D. Perrissoud[2], V. Locatelli[3], J. Martinez[1]

[1]UMR 5810, LAPP, Faculté de Pharmacie, Montpellier, France [2]Zentaris GmbH, Frankfurt am Main, Germany [3]School of Medecine, University of Milano-Bicocca, Monza, Italy

Ghrelin, the natural ligand for the growth hormone secretagogue receptor (GHSR-1a), has received a great deal of attention due to its ability to stimulate growth hormone secretion and to control food intake. During these last years, ghrelin analogues or mimetics gained interest for their implication in food intake regulation. In the course of our studies concerning ghrelin analogs, we developed new ligands of the GHSR-1a based on heterocyclic structures. Interestingly, one heterocyclic family presented high affinity for the ghrelin receptor. A structure activity study was performed within this family and led to potent GHSR-1a agonists and antagonists. The binding affinities were determined by displacement of radiolabelled ghrelin and the agonist or antagonist character was measured by intracellular calcium mobilisation. The first in vivo results revealed that in vitro activities and in vivo responses were not correlated. Particularly, binding to GHSR-1a and in vivo GH release and food intake control were not fully correlated. These results strongly suggest the presence of receptor subtypes that modulate ghrelin actions. Some examples will be reported. Further investigations are going on in the laboratory.

DESIGN, SYNTHESIS AND INVESTIGATION OF PUTATIVE BETA-AMYLOID AGGREGATION INHIBITORS BASED ON THE SEQUENCE OF BETA-AMYLOID (30-34)

L. Fülöp[1], M. Zarándi[1], Z. Datki[1], A. Hetényi[2], T. Martinek[2], A. Lakatos[3], K. Soós[1], B. Penke[1]

[1]Department of Medical Chemistry [2]Institut of Pharmaceutical Chemistry [3]Bioinorganic Chemistry Research Group of The Academy of Sciences, University of Szeged, Hungary

Background and aims: Alzheimer's disease (AD) related beta-amyloid (Aβ) peptides possess high propensity towards aggregation. Nowadays one of the major directions of the drug-design against AD is the synthesis of putative amyloid aggregation inhibitor molecules (AAI) which are able to hinder the formation of these toxic amyloid aggregates. In the present work we report the design, synthesis and investigation of putative AAIs derived form the peptide sequence AIIGL identical to Aβ(30-34).

Methods: Aβ(1-42) peptide and putative aggregation inhibitors were synthesized manually using Fmoc-chemistry and DCC/HOBt activation. Studies on both the Aβ aggregation and the effect of the AAIs were performed with several instrumental techniques. The total amount of the aggregates was determined by Thioflavine-T binding assay, while their size distribution was characterized with dynamic light scattering (DLS). Aggregated Aβ forms were visualized with transmission electron microscopy (TEM). The binding affinity of the AAIs to Aβ fibrils was studied in saturation transfer NMR experiments, while in vitro viability assays were performed on cultured human SH-SY5Y neuroblastoma cells to monitor the neuroprotective effect of the amyloid aggregation inhibitors.

Results and conclusions: 32 derivatives of Aβ(30-34) were synthesized and tested concerning their neuroprotective effect against Aβ-mediated toxicity. The most promising candidates were examined with physico-chemical methods to characterize their aggregation altering ability. The pentapeptide RIIGL-amide proved to be the most powerful neuroprotective compound, and it was also able to alter considerably the aggregation kinetics of Aβ(1-42). This molecule might serve as a lead compound in the drug-design against AD in the future.

IDENTIFICATION OF E. COLI GENES CONFERRING RESISTANCE TO THE ANTIMICROBIAL PEPTIDE BAC7

M. Mattiuzzo, A. Bandiera, M. Benincasa, I. Samborska, M. Scocchi, **R. Gennaro**

Department of Biochemistry and Biophysics, University of Trieste, Italy

Antimicrobial peptides (AMPs) are ancient molecules of the innate immune defence system. Most of them kill bacteria by lysing their membranes. The proline-rich group of AMPs represents an exception, as they act via a permeabilization-independent mechanism that is likely based on recognition of molecular targets/transporters. However, specific internal targets have not yet been identified for most of these AMPs.

Bac7 is a Pro-rich AMP isolated from bovine neutrophils that is capable to translocate across membranes to target hypothetical intracellular proteins. In this study, we used a molecular approach to identify putative targets for Bac7 by selection of peptide-resistant mutants followed by identification of the genes responsible of this resistance. To this aim, an E. coli strain susceptible to the fully active fragment Bac7(1-35) was subjected to chemical mutagenesis and a number of peptide-resistant mutants was obtained. A pool of genomic DNA from these mutants was used to construct a plasmid library that was used to transform a susceptible strain. This approach allowed the identification of 14 different clones that provided a high level of resistance to Bac7. Sequence analysis revealed the presence of genes originating from five different regions of the E. coli chromosome. Among them, one clone contained ptrB, a gene coding for a serine peptidase broadly distributed among Gram - bacteria, which could be involved in resistance by degrading the peptide. Other resistance-conferring clones, which encode for membrane proteins that may be involved in peptide translocation across the memmbrane, are currently under investigation.

NEW HIGHLY POTENT PEPTIDE BRADYKININ B-1 RECEPTOR ANTAGONISTS

L. Gera[1], J.M. Stewart[1], J-P. Fortin[2], F. Marceau[2]

[1]Department of Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, Denver, CO, USA [2]Rheumatology Research Department, Universtiy Laval, Quebec, QC, Canada

Bradykinin (BK) has two major classes of receptors, B-1 and B-2. There is growing evidence that the B-1 receptor, in addition to the B-2 receptor, plays an important role in various pathophysiological states including acute and chronic inflammation, pain, trauma and cancer. Therefore, there is current interest in the development of highly potent bradykinin B-1 receptor antagonists. The first full-chain bradykinin antagonist which showed high BK antagonism both on B-1 and B-2 receptors, B9430 (DR-R-P-Hyp-G-Igl-S-DIgl-Oic-R, Hyp: trans-4-hydroxyproline; Igl: α-(2-indanyl)glycine; Oic: octahydroindole-2-carboxylic acid), was developed by Gera at the Stewart laboratory a decade ago. The des-Arg9-analog of B9430, B9858 (K-K-R-P-Hyp-G-Igl-S-DIgl-Oic) had a remarkably high B-1 antagonist activity. Aminopeptidase degradation limits the potency and lifetime in vivo of BK peptide B-1 receptor antagonists which typically have N-terminal Lys or Arg residues. Protection by acylation seriously degrades potency. To overcome this limitation several new B-1 antagonists have been synthesized with N-terminal basic residues in the D-configuration. Analogs of B9958 (K-K-R-P-Hyp-G-CpG-S-DTic-CpG, CpG: α-cyclopentylglycine; Tic: tetrahydroisoquinoline-3-carboxylic acid) having DLys (B10352) or DArg (B10356) replacements of the N-terminal residue were slightly less potent than B9958 in receptor binding, but were more potent in the contractility assay. Their action on rabbit aorta was not potentiated by amastatin. Remarkably, B10356 was found to be a competitive inhibitor of aminopeptidase N for hydrolysis of LAla-p-nitroanilide, a standard aminopeptidase N chromogenic substrate; it potentiates vasoactive peptides susceptible to this peptidase, such as angiotensin III. Dual inhibitors of the B-1 receptor and aminopeptidase N are of potential interest for specific applications.

LEISHMANICIDAL ACTIVITY OF ANTIMICROBIAL PEPTIDES FROM THE WOLF SPIDER LYCOSA CAROLINENSIS

J.R. Luque-Ortega[1], **P. Gomes**[2], R. Seixas[2], L. Rivas[1], D. Andreu[3]

[1]Centro de Investigaciones Biologicas (CSIC), Madrid, Spain [2]CIQ(UP), Department of Chemistry, Faculty of Sciences, University of Porto, Portugal
[3]Facultat de Ciències de la Salut I de la Vida, Universitat Pompeu Fabra, Barcelona, Spain

Lycotoxins are peptides from the venom of the wolf spider that were predicted to have an amphipatic alpha-helical structure and confirmed to possess significant antimicrobial and pore-forming activities. [1] We became interested in these peptides as potential leishmanicidal agents against Leishmania donovani promastigotes and Leishmania pifanoi amastigotes. Thus, lycotoxin I (LyI) and lycotoxin II (LyII), [1] and shortened analogues LyI1-19, LyI1-15, LyII1-21 and LyII1-17, were synthesized as C-terminal carboxamides. Short- and long-term parasite proliferation measurements showed all peptides except LyI1-5 to be active against both promastigotes and amastigotes at the micromolar range, and suggest peptide effects on parasites to be irreversible. LyII, that showed the lower activity, became more leishmanicidal upon residue trimming, whereas the most active LyI displayed the opposite behaviour. A set of complementary techniques showed that lycotoxin peptides are membrane-disruptive to promastigotes. Electron microscopy showed that two populations of promastigotes, one with intact parasites and the other extensively damaged, are formed upon addition of the peptides at their EC50. All peptides were non-hemolytic for sheep erythrocytes below 20 micromolar.

[1] Yan, L. and Adams, M. E. J. Biol. Chem. 1998, 273, 2059-2066.

M430

STRUCTURAL STUDIES OF GLUTEN - PEPTIDE ANALOGS

P. Grieco[1], S. Albrizio[1], A. Carotenuto[1], G. Caliendo[1], D. Campiglia[1], D. Guarino[2], O. Fierro[2], L. Auriemma[1], E. Novellino[1]

[1]*Dip. Chimica Farmaceutica E Toss., University of Naples- Federico II –, Naples*
[2]*Istituto di Scienze Dell'Alimentazione CNR, Avellino, Italy*

Tissue transglutaminase (TG2) is an enzyme that plays a key role in the pathogenesis of the celiac disease. TG2 is the main autoantigen recognized by the anti-endomysium antibodies and, furthermore, catalyzes the deamidation of strategic glutamine to glutamic acid within the sequence of immunodominant gliadin epitopes. Recently, another unexpected role for surface TG2, in the innate immune response in the celiac disease, has been suggested. It follows that TG2 inhibitors might represent a potential attractive pharmacological alternative to the gluten-free diet that, nowadays, is the only possible therapy for Celiac patients.
Starting from the sequence of the heptapeptide PQPQLPY, known to be a high affinity substrate of human TG2, we have synthesized new analogs replacing Pro3 with different constrained amino acids (D-Pro, Pip, Chg, Ind, Deg, Inp, Hyp, Thz)) with the aim to develop specific inhibitors of TG2. Actually, proline residues present in the gluten epitopes are important in determining the immunogenicity of the epitopes and the specificity of TG2.
Herein, we describe the preliminary conformational studies of the synthesized analogs by CD and NMR spectroscopy and molecular modeling methods. The structural features of the peptides have analyzed in different environment.
Given the role of the domain PQPQLPY in the gliadin proteins, structural analysis on its analogs are of considerable interest. The results of our studies might be useful to clarify the role of the proline residues in the interaction of the gluten epitopes with TG2 and, consequently, to gain new insight in the molecular mechanism of celiac disease.

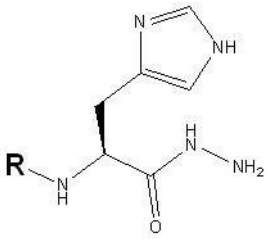
Tu431

CARNOSINE ANALOGUES: ALPHA, BETA-UNSATURATED ALDEHYDE SCAVENGERS BASED ON THE HISTIDYL HYDRAZIDE MOIETY

A. Guiotto[1], A. Calderan[1], P. Ruzza[1], B. Biondi[1], C. Rubini[1], A. Osler[1], M.P. Mattson[2]

[1]CNR Institute of Biomolecular Chemistry, Padova Unit, Padova, Italy [2]Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, Baltimore, MD, USA

Carnosine and related histidine-containing dipeptides are known to react with high efficiency with the products of lipid peroxidation, namely 4-hydroxy-trans-2,3-nonenal (HNE) and other alpha, beta-unsaturated aldehydes, preventing their reaction with nucleophilic residues in proteins and nucleic acids. Histidyl hydrazide alone or conjugated with aminoacids, long chain fatty acids, cholesterol and alpha-tocopherol synthesized in our laboratories demonstrated higher aldehyde-sequestering efficiency than carnosine, and were also efficient in protecting SH-SY5Y neuroblastoma cells and rat hippocampal neurons from HNE-mediated death. The cytoprotective efficacy of these compounds suggests their potential as therapeutic agents for disorders that involve excessive membrane lipid peroxidation.

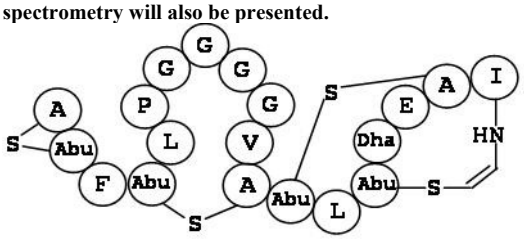


CHEMICAL MODIFICATION OF THE LANTIBIOTIC MERSACIDIN

A.K. Hagan[1], M.J. Dawson[2], N.J Turner[1], S. Wadman[2]

[1]*Manchester Interdisciplinary Biocentre, University of Manchester, Manchester*
[2]*Novacta Biosystems Ltd., UH Innovation Centre, Hatfield, UK*

Lantibiotics are antibiotic natural products that are synthesised ribosomally and undergo extensive post-translational modification, resulting in multiple thioether bridges and dehydro amino acids in the mature peptide. One such lantibiotic is mersacidin, which is produced by Bacillus sp. and exhibits extremely promising in vitro and in vivo efficacy versus a number of clinically relevant pathogens, most notably methicillin-resistant Staphylococcus aureus (MRSA). Mersacidin is believed to function by binding to the bacterial cell wall precursor lipid II, thereby preventing its incorporation into the growing peptidoglycan network. In an attempt to better understand this mode of action and develop more active analogues, we have embarked upon its chemical derivatisation. Some of these modifications resulted in an altered antibacterial spectrum, permitting some insight into tentative STRUCTURE-ACTIVITY RELATIONSHIPs. The characterisation of these structurally complex compounds via a combination of multidimensional NMR and tandem mass spectrometry will also be presented.



Abu = aminobutyric acid
Dha = 2,3-didehydroalanine

SYNTHESIS AND IN VITRO EVALUATION OF FLUORINATED P-BORONOPHENYLALANINE DERIVATIVES AS BORON CARRIER AND MRI PROBE

Y. Hattori[1], Y. Niki[2,3], H. Kondoh[2], T. Morita[4], H. Yamamoto[5], T. Asano[6], M. Kirihata[6], Y. Yamaguchi[1], T. Wakamiya[1]

[1]Faculty of Science and Technology, Kinki University, Higashi, Osaka [2]Bio-Research Inc. [3]Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Kobe [4]Department of Radiation Oncology, Kawasaki Medical School, Kurashiki-shi [5]Graduate School of Science, Osaka University, Toyonaka [6]Graduate School of Life & Environmental Sciences, University of Osaka Prefecture, Sakai, Japan

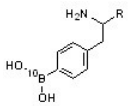
The boron neutron capture therapy (BNCT) based on the interaction between 10B isotope and thermal neutron has been highly noted in recent years as one of quite useful techniques for treatment of cancers. □-[4-(10B)-Boronophenyl] alanine (10Bpa) (1) and □-[4-(10B)boronophenyl]alaninol (10Bpa-ol) (2), in which boron atom was enriched with 10B isotope, had been developed as the 10B carrier for BNCT, and 10Bpa is now using clinically for the treatment of patients with malignant brain tumor and melanoma [1].

The magnetic resonance imaging (MRI) is noted as one of common techniques for diagnosis of cancers. In particular, MRI based on the measurement of 19F atom is becoming a remarkable one.

To develop the practical materials utilizing not only for the 10B carrier but also MRI probe, we had already reported the syntheses of compounds containing both 10B and 19F atoms in a single molecule such as DL-□-[4-(10B)borono-2,6- difluorophenyl]alanine [10Bpa(2,6F2)] (3), DL-□-[4-(10B)borono-2,6-difluorophenyl] alaninol [10Bpa(2,6F2)-ol] (4) [2], DL-□-[4-(10B)borono-2-trifluoromethylphenyl]alanine [10Bpa(2CF3)] (5) and DL-□-[4-(10B)borono-2-trifluoromethylphenyl]alaninol [10Bpa(2CF3)-ol] (6) [3].

In the present paper we report the biological activities of fluorinated p-boronophenylalanine derivatives 3 ~ 6, and 19F NMR measurements of these compounds as well.

[1] Kato, I., et al., (2004) Appl. Radiat. Isot., 1069-1073.
[2] Hattori Y., et al., (2005) Peptide Science 2004, 411-414.
[3] Hattori Y., et al., (2006) Peptide Science 2005, in press.



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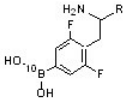
2: R=CH_2OH



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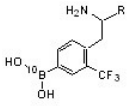


5: R=CO_2H
6: R=CH_2OH

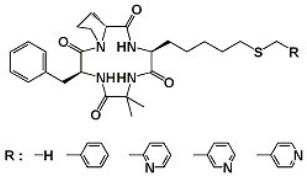
Tu434

DESIGN AND SYNTHESIS OF HISTONE DEACETYLASE INHIBITORS CONTAINING THIOETHER MOIETY AS THE FUNCTIONAL GROUP

Y. Hirashima[1], T. Kato[1], N. Nishino[1], S. Maeda[2], T.G. Nishino[2], M. Yoshida[2]

[1]Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu, Fukuoka [2]Riken, Wako, Japan

Histone deacetylase (HDAC) has metalloprotease-related mechanism in its catalytic activity. Published structural data for HDAC-like protein, a bacterial enzyme sharing high homology to the HDACs in its active site, confirmed that this protein contains a zinc in the active site. For the discovery of specific HDAC inhibitors, a number of hydroxamic acis and related compounds have been designed based on the ligating function to the zinc atom. The mechanism also involves an appropriate nucleophile in the active site.
Chlamydocin is a cyclic tetrapeptide, which contains unique (2S,9S)-2-amino-8-oxo-9,10-
epoxydecanoic acid (Aoe). Based on its cyclic tetrapeptide framework, cyclo(-L-Aoe-Aib-L-Phe-
D-Pro-), we have been focusing the cyclic tetrapeptide to develop the potent and specific inhibitors of HDACs. In the course of the design of zinc ligands, we have reported the cyclic tetrapeptides containing trifluoromethylketone moiety. On the other hand, we have been focusing the cyclic tetrapeptide to develop the potent and specific inhibitors of HDACs.
In the present report, we employed the chlamydocin scaffold and successfully introduced the series of thioether as the functional group to the cyclic tetrapeptide. It is well argued that the strong inhibition of HDAC requires the best combination of zinc ligand, capping group, and appropriate spacer between them. These compounds were profiled by the inhibition of HDAC1, HDAC4, and HDAC6. The p21 promoter assay was also employed to evaluate cell-level effects.



ENGINEERING OF TRIMERIC COILED COILS OF HIV GP41 PROTEIN HR1 REGION FOR COVALENT STABILIZATION AND FURTHER CONJUGATION

P. Ingallinella[1], M. Finotto[1], R. Geleziunas[2], M.D. Miller[2], A. Pessi[1], E. Bianchi[1]

[1]IRBM P. Angeletti, Pomezia (Rome), Italy [2]Merck Research Laboratories, West Point, PA, USA

Conjugation of peptides with different moieties, such as PEG, lipids, carrier proteins and Toll-like receptor ligands is an established approach to improve their pharmacokinetic profile for drug use and/or to enhance their immunogenicity as subunit vaccines. The development of suitable conjugation strategies for peptides of any complexity is therefore fundamental for their effective use in human therapeutic applications.
Here we describe our strategy to engineer a trimeric coiled coil to obtain a covalently linked, structurally stable construct endowed of extra functionality for further derivatization. We previously showed that covalent stabilization of the designed trimeric coiled coil IZN17, by interchain disulfide bonds, yielded an extremely potent and broad inhibitor of viral infection, (CCIZN17)3, [1]. This potent inhibitory activity makes (CCIZN17)3 not only attractive as an antiviral compound, but also as an immunogen to elicit a neutralizing antibody response [2]. We have now developed an alternative synthetic strategy to obtain the covalently-linked IZN17 trimer, which allows the presence in the molecule of a free thiol for subsequent chemoselective reactions. First, we showed that stable interchain thioether bonds can be effectively substituted for the disulfides. Second, we devised an orthogonal cysteine protection scheme which allows formation of the thioether bonds, while leaving an extra free cysteine on demand. This thiol group can be used for conjugation of the trimeric coiled coil to adjuvant/carrier proteins or, as reported more specifically here, to a PEG40kDa moiety.

[1] Bianchi E. et al., PNAS, 102 (2005), 12903.
[2] Miller M. D. et al., PNAS, 102 (2005), 14759.

M436

HUMAN IMMUNOGLOBULIN-G FC-SPECIFIC BINDING PEPTIDE MOTIFS REQUIRED FOR DETECTION AND PURIFICATION OF IGG

Y. Ito, K. Sakamoto, S. Muraoka, S. Hashiguchi, K. Sugimura

Department of Bioengineering, Faculty of Engineering, Kagoshima University, Kagoshima, Japan

Human IgG is a most abundant type of immunoglobulin in serum and most of antibody drugs applied for therapy of cancer and autoimmune diseases also belong to this group of human immunoglobulin. Specifically binding peptide to human IgG is very useful for detection and purification as an affinity ligand of human IgG. Although some previous reports described such peptides, we tried here to isolate high-specific and high affinity ligands by employing random peptide-displayed T7 phage library. Our T7 random peptide phage library possesses a total diversity of 10 powered 10th consisting of different sequence length constrained by disulfide bond. By biopanning against human IgG, we isolated several IgG specific clones from our library. The peptides displayed on these phages shared some common sequences in the limited region surrounded by Cys residues, which suggests they are essential for binding. These clones bound only to Fc region of IgG and did neither other types of human immunoglobulin nor IgG of other animals. A synthetic peptide derived from a phage clone showed a sub-nanomolar of Kd value in binding to human IgG Fc on SPR analysis.

These results indicate that the peptide motifs obtained here are a strong candidate of human IgG-specific affinity ligand for detection and purification of IgG. Therefore, we are now going on constructing detection and purification system using modified and improved peptide motifs.

SYNTHESIS OF GLP-1 ANALOGUES AS POTENTIAL AGENTS FOR BLOOD GLUCOSE CONTROL

P. Kanda, R.P. Sharma, C.P. Hodgkinson

Activotec, Ltd., Southampton, UK

Glucagon-like peptide-1(GLP-1) 7-36 amide, a 30 amino acid peptide secreted by the intestinal L cells in response to nutrient ingestion, is the most potent incretin for potentiating insulin secretion yet identified. This and its other anti-diabetic properties have generated much commercial interest in related analogues as therapeutics. The natural hormone has a short plasma half-life (1-2 min), being either degraded into an inactive form by circulating dipeptidyl peptidase IV (DPP-IV) and/or rapidly cleared through renal filtration. DPP-IV cleaves GLP-1 between Ala8 and Glu9, yielding an inactive truncated species. In this study, we synthesised a panel of GLP-1 analogues stabilised against DPP-IV degradation through either selective amino acid substitutions for Ala8, or introduction of amide bond surrogates into the peptide backbone between Ala8 and Glu9. Each was made by standard Fmoc or Boc chemistry, purified by HPLC, and characterized by electrospray mass spectroscopy. All derivatives except one bearing a hydrazine modification were stable to DPP-IV proteolysis for up to 48 hours at pH 7.5, 37o. Each was tested for its ability to augment insulin release from a glucose- sensitive, murine insulinoma-derived TC-6 cell line in culture. It was found that each compound acted as a GLP-1 agonist to varying degrees, with some exhibiting higher activity than native GLP-1 toward promoting insulin release. The most active analogues have been chosen as candidates for stabilisation against renal clearance in efforts to develop new GLP-1 analogues with therapeutic potential.

REDESIGNING HUMAN IAPP INTO A NONAMYLOIDOGENIC AND BIOACTIVE ANALOG AND A NANOMOLAR ACTIVITY INHIBITOR OF IAPP AMYLOID CYTOTOXICITY

L.M. Yan, M. Tatarek-Nossol, A. Velkova, A. Kazantzis, **A. Kapurniotu**

Laboratory of Bioorganic and Medicinal Chemistry, Institute of Biochemistry, University Hospital of The RWTH Aachen, Germany

The high propensity of the glucose regulatory hormone human islet amyloid polypeptide (IAPP) to misfold and aggregate into cytotoxic beta-sheets and fibrils is strongly associated with beta-cell degeneration in type II diabetes (T2D) and precludes its pharmacological use for the treatment of diabetes. IAPP analogs that combine solubility, lack of cytotoxicity, and bioactivity with the ability to block IAPP aggregation and cytotoxicity could thus be of high biomedical interest.
Here we apply a minimalistic conformational restriction strategy to redesign the extremely insoluble and amyloidogenic 37-residue IAPP sequence into a soluble, nonamyloidogenic, noncytotoxic, and bioactive IAPP mimic (Yan et al., PNAS (2006)). The designed mimic has nearly the same sequence as IAPP but is highly soluble, nonamyloidogenic, noncytotoxic and a full IAPP receptor agonist. In addition, the mimic binds with high affinity IAPP and blocks and reverses with nanomolar activity its cytotoxic self-assembly which makes it to the most potent known IAPP cytotoxic self-assembly inhibitor. Due to its bifunctional nature, the mimic might find therapeutic application for the treatment of diabetes both as an inhibitor of amyloid cytotoxicity and as a soluble IAPP receptor agonist.
Our findings offer a proof-of-principle of a chemical design approach for generating a novel class of highly potent inhibitors of polypeptide cytotoxic self-assembly which are nonamyloidogenic mimics of the native amyloidogenic sequence as well. Such reengineered biomolecules -the design of novel mimics is in progress- are of high biomedical significance for understanding the mechanism of protein aggregation diseases and for the development of prospective therapeutic treatments.

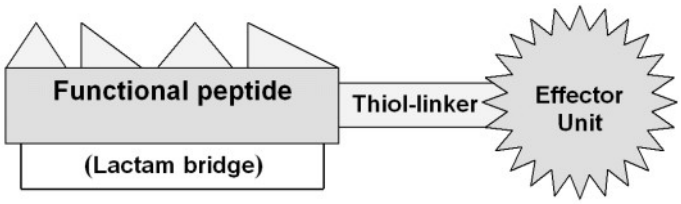
M439

MULTI-PURPOSE PEPTIDE CONJUGATES FOR TUMOR TARGETING

K. Karisalmi, M. Justander, H. Peräkylä

Karyon Ltd., Helsinki, Finland

Peptides that are capable of targeting abnormal changes of living tissue can be very useful in early detection or diagnosis of, e.g., cancer. Conjugating a functional agent, an effector unit, to such a peptide may provide the agent with improved pharmacodynamic properties.The specificity of a thiol group for reactive groups offer a unique way to attach effector units to cysteine-free linear or cyclic peptides.
Tumor targeting peptides were synthesized by Fmoc-type solid phase methods. Peptides cyclic by cystine were modified by lactam bridges. Fluorescein, metal (e.g. lanthanide) chelates and cytotoxins were coupled to tumor targeting peptides via e.g. a Peg-type spacer, or the conjugates were immobilized on plates for adhesion assays.
The method was uncomplicated and gave stable conjugates with good solubility. The approach is useful in making stable peptide-effector conjugates and sets of them for e.g. detection assays such as Delfia method and has prospective use in development of diagnosis and therapy.



RESTRAIN OF BONE GROWTH BY ESTROGEN-MIMETIC PEPTIDE-1 (EMP-1): A MICRO-COMPUTED TOMOGRAPHIC STUDY

R. Kasher[1], A. Bajayo[2], Y. Gabet[2], N. Nevo[3], M. Fridkin[4], E. Katchalski-Katzir[5], F. Kohen[3], I. Bab[2]

[1]Department of Desalination and Water Treatment, The Blaustein Institutes for Desert Research, Ben-Gurion University of The Negev, Beer-Sheva [2]Bone Laboratory, Hebrew University of Jerusalem, Jerusalem [3]Department of Biological Regulation [4]Department of Organic Chemistry [5]Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel

Estrogen has a key role in the regulation of skeletal growth and maintenance of bone mass. The use of estrogen and selective estrogen receptor (ER) modulators in treatment of osteoporosis is limited due to substantial risks for breast cancer. Recently, we developed peptides having estrogen-like activity (Kasher et al., Biopolymers 76, 404-420, 2004) as potential estrogen-based new drugs. The aim of the present study was to evaluate the influence of long-term administration of the estrogen-like peptides on bone mass and development. The estrogen-mimetic hexapeptide EMP-1 (VSWFFE) was injected daily to groups of ovariectomized (OVX) and intact young, sexually mature female mice for 10 weeks. Whole femora, including cartilage-growth plates were analyzed by micro-computed tomography (μCT). We found that peptide EMP-1 restrains bone growth in OVX mice: It inhibited dramatically bone longitudinal growth (40%), and decreased femoral diaphyseal diameter. Peptide EMP-1 had no effect on bone growth in normal mice, and did not influence the OVX-induced bone-loss. We then developed a new μCT methodology to evaluate uncalcified and calcified growth-plate parameters. In the OVX mice, peptide EMP-1 reduced volume and thickness of the uncalcified growth-plate, a possible cause for the inhibition of bone longitudinal growth. Based on a reported enhancement of ER-□ in female mice (Chagin et al., JBMR 19, 72-77, 2004), which leads to longitudinal growth inhibition, we suggest that peptide EMP-1 restrains bone growth by activating skeletal ER-□ . Peptide EMP-1 may be used as a lead compound for development of drugs to treat acromegalic patients.

ANTIBACTERIAL PROLINE-RICH PEPTIDES - SYNTHESIS AND ANTIBACTERIAL ACTIVITY

D. Knappe, R. Hoffmann

Bioanalytics, Center für Biotechnology and Biomedicine, Faculty of Chemistry and Mineralogy, University of Leipzig, Germany

The antibacterial proline-rich peptide family, originally isolated from insects, shows remarkable activity against diverse bacterial and fungal pathogens. While more and more bacterial pathogens become resistant to common drugs, this part of insect innate immunity provides a new promising approach to develop future peptide-drugs. Proline-rich peptides possess a significant sequence homology and share a common mechanism of action. In addition O-glycosylated threonine residues of drosocin and formaecin appear to be necessary for full antimicrobial activity, although the significance of the carbohydrate moiety in interaction with intracellular targets is still unknown. We synthesized analogues of different antibacterial peptides on solid phase by the Fmoc-tBu-strategy. The combination or insertion of sequence regions from different native antibacterial peptide sequences offers several advantageous effects, including further reduction of toxicity and broadening of the antimicrobial activity. Furthermore, mimicking the O-glycosylation site and changing the carbohydrate moieties, may yield new synthetic approaches to increase both the activity and the selectivity of these oligopeptides.

M442

MOLECULAR MIMIC OF RIBOSOME-RRF INTERACTION FOR RATIONAL DESIGN OF ANTIBIOTICS

T. Yoshida[1], Y. Nakamura[1], M. Nishimura[1], H. Yamauchi[1], Y. Kawasaki[2], S. Iwai[2], T. Ishizu[3], S. Isaka[3], K. Yoshizawa-Kumagaye[3], P. Fucini[4], S. Uchiyama[5], T. Ohkubo[1], **Y. Kobayashi**[1,6]

[1]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka [2]Graduate School of Engineering Science, Toyonaka [3]Peptide Institute Inc., Minoh, Osaka, Japan [4]Max-Planck-Institute for Molecular Genetics, Berlin, Germany
[5]Graduate School of Engineering, Suita [6]Osaka University of Pharmaceutical Sciences, Takatsuki, Osaka, Japan

During protein biosynthesis, after the release of the nascent polypeptide chain, ribosome recycling factor (RRF) disassembles the post-translational complex. RRF has been shown to be essential for bacterial growth. Thus, we are attempting to design suitable compounds to inhibit the RRF function as candidates for new-type antibiotics.
We have determined the structure of RRF with 185 amino acid residues by NMR and X-ray analyses and shown that RRF has two domains; Domain I with three stranded helical bundles and Domain II with β/α/β sandwich structure [1]. Furthermore, we recently determined the structures of the 70S ribosome-RRF complex by cryo-electron microscopy and the 50S ribosome-RRF Domain I complex by X-ray analysis [2].
Using the results of these experiments, we elucidated the interaction profiles between RRF and ribosome and found that the cationic center consisting of three arginine residues on the surface of the helical bundle, which we have shown to be essential for the activity of RRF, is bound to helix 69-71 of 23S ribosomal RNA.
We synthesized the RNA and peptide fragments around this interacting site and characterized them by physico-chemical analyses. The results of CD and Biacore experiments to investigate the details of the interactions between them showed that a 27 mer of RNA fragment is bound to RRF with a dissociation constant of 3 μM which is comparable to that of 70S ribosome.
[1] T. Yoshida et al., <I>Biochemistry</I> <B>42</B>, 4101 (2003).
[2] D. N. Wilson et al., <I>EMBO J.</I> <B>24</B>, 251 (2005).

CHIMERIC MULTIFUNCTIONAL LIGANDS FOR THE TREATMENT OF PAIN

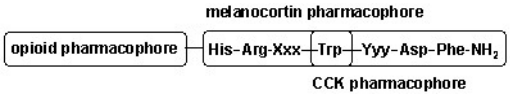
Y.S. Lee[1], V. Kulkani[1], S.W. Ma[2], H. Badghisi[2], P. Davis[2], T. Vanderah[2], J. Lai[2], F. Porreca[2], V.J. Hruvy[1]

[1]Department of Chemistry [2]Department of Pharmacology, University of Arizona, Tucson, AZ, USA

CCK is known to give an anti-opioid effect and as a result, causes an increase of pain by inhibiting the opioid response [1]. Recent research has shown further that melancortin receptors, mainly subtype MC4R, produce an increase in response to pain stimuli [2]. Based on this previous work, we are developing chimeric ligands which will be of benefit to therapeutic pain treatment with enhanced opioid efficacy by acting as agonists at opioid receptors and antagonists at both CCK and melanocortin receptors [3].

The design of the ligands was based on the hypothesis of targeting multiple receptors with overlapping pharmacophores. CCK (I) and melanocortin (II) pharmacophores were overlapped by Trp, and different profiles of opioid pharmacophores (III) were linked to the N-terminal of the melanocortin pharmacophore (Figure). The designed ligands showed moderate to high biological activity at all three receptors depending on their respective structures. Design considerations and STRUCTURE-ACTIVITY RELATIONSHIPs will be discussed in detail along with in-vivo assay results. This work was supported by grants from National Institute on Drug Abuse (DA12394 and DA06284).

[1] Faris, P. L.; Komisaruk, B. P.; Watkins, L. R.; Mayer, D. J. Science 1983, 219, 310.
[2] Starowicz, K.; Obara, I.; Przewlocki, R.; Przewlocka, B. Pain 2005, 117, 401.
[3] Lee, Y. S.; Agnes, R. S.; Badghisi, H.; Davis, P.; Ma, S.; Lai, J.; Porreca, F.; Hruby, V. J. J. Med. Chem. 2006, 49, 1773.



STABILITY OF SYNTHETIC EXENDIN-4 IN HUMAN PLASMA IN VITRO

J. Chen, L. Yu, L.P. Wang, X.X. Fang, **W. Li**

College of Life Science, Jinlin University, Changchun, China

Synthetic exendin-4 is a 39-amino acid peptide that exhibits potent anti-diabetic and dose-dependent glucose-regulatory activity. Exendin-4 is susceptible to degradation in plasma, so its activity is limited. Our aim is to find sites in exendin-4 that are susceptible to cleavage and provide information for designing new exendin-4 analogues. In this study the stability of exendin-4 in human plasma was evaluated in vitro. Exendin-4 was incubated in plasma at 37 ℃, extracted with SEP-PAK octadecyl columns and subsequently analyzed using high performance liquid chromatography (HPLC). The results showed that exendin-4 was slowly broken down in plasma with a half-life of 9.57 h. The degradation products were identified by Quadrupole time of flight mass spectrometry (Q-TOF-MS) with electrospray ionization. We deduced that the cleavage occurred between the Thr5and Phe6, Phe6 and Thr7, and Thr7 and Ser8 of the N-terminus region of the peptide.

Reference

[1] Nielsen L. Loretta, Young A. Andrew, Parkes G. David. Pharmacology of exenatide(synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. Regulatory Peptides. 2004, 117: 77-88

M445

ORAL ADMINISTRATED BACKBONE CYCLIC SELECTIVE MELANOCORTIN-4 PEPTIDE REDUCES FOOD CONSUMPTION IN MICE

Y. Linde[1], S. Hess[2], O. Ovadia[2], C. Haskel-Luevano[3], A. Hoffman[2], C. Gilon[1]

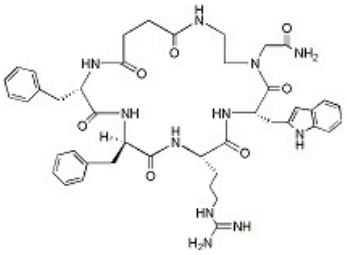
[1]Department of Organic Chemistr [2]Department of Pharmaceutics, The Hebrew University of Jerusalem, Israel [3]Department of Medicinal Chemistry, University of Florida, Gainesville, FL, USA

One of the proposed solutions for the pharmacotherapy of obesity, a major health problem in the western world, is to regulate the biochemical pathways that control the metabolic balance in the body. The melanocortin pathway regulates energy balance by binding of the catabolic endogenic neuropeptide αMSH to its MC4 receptor and thus causes a decrease in food intake.

We have synthesized a backbone cyclic peptide library, based on the minimal αMSH sequence Phe6-D-Phe-Arg-Trp9 [1], that activates the MC4 receptor. All the members of the library shared the same sequence, they differ in their ring size and ring chemistry.

Permeability experiments in Caco2 cells showed that BBC-1 (Figure 1) have the highest intestinal permeability (Papp of 52*10-5 compared to Papp of testosterone 30*10-5). Analysis using colorimetric liposomes confirmed that BBC-1 penetrate the intestinal cells by the transcellular mechanism. Moreover, BBC-1 have high metabolic stability to intestinal enzymes (100% recovery after 5 hr). EC50 analysis showed that BBC-1 selectively binds and activate the MC4 and MC5 receptors (EC50 3.97±0.63 and 7.27±0.40 respectively). Oral administration of BBC-1 in mice showed reduces food intake (~40% in 24hr) in comparison to the control group.

[1] Luevano et al. Melanocortin Tetrapeptides Modified at the N-Terminus, His, Phe,Arg and Trp Positions. Annals New York Academy of Sciens, 2003. 994: p. 36-48.



DEVELOPMENT AND IMMUNOANALYTICAL CHARACTERIZATION OF BIOCONJUGATES COMPRISING A ß-AMYLOID PLAQUE SPECIFIC EPITOPE

M. Manea[1], G. Mező[2], F. Hudecz[2,3], M. Przybylski[1]

[1]*Laboratory of Analytical Chemistry, University of Konstanz, Konstanz, Germany*
[2]*Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University* [3]*Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary*

New immunotherapeutic approaches have been developed for treatment of neurodegenerative diseases of the Alzheimer's dementia (AD) type. The identification of a ß-amyloid-plaque specific epitope, Aß(4-10) (FRHDSGY) [1], recognised by therapeutically active antibodies from transgenic AD mice, provides the basis for development of new AD vaccines and for molecular AD diagnostics. In order to produce immunogenic conjugates, the Aß4-10 epitope was attached via thioether linkage to synthetic carriers of well-defined structures, such as tetratuftsin derivative (Ac-[TKPK(ClAc)G]4-NH2) and its elongated version by a helper T-cell epitope (Ac-FFLLTRILTIPQSLD-[TKPK(ClAc)G]4-NH2); sequential oligopeptide carrier (Ac-[Lys(ClAc)-Aib-Gly]4-OH) and multiple antigenic peptide (ClAc-Lys(ClAc)-Lys(ClAc-Lys(ClAc))-Arg-Arg-ßAla-NH2). The epitope conjugates containing a cysteine residue either at the C- or N-terminus, and the chloroacetylated carriers were prepared by SPPS according to Boc/Bzl strategy. Conjugation reactions were performed in solution under slightly alkaline conditions, and monitored by HPLC and high resolution-MS. Structures and molecular homogeneities of all epitope peptides, carriers and conjugates were ascertained by HPLC, MALDI and ESI-FTICR-MS. Conformational preferences of the synthesized compounds in water and in TFE were examined by CD spectroscopy. Comparative binding studies of the conjugates with a mouse anti-amyloid protein beta-(1-17) monoclonal antibody were performed by indirect ELISA. Experimental data showed that the chemical nature of the carrier, the epitope topology and the presence of a pentaglycine spacer between the epitope peptide and the carrier, had significant effects on the antibody recognition and on the secondary structures of the conjugates.

[1] McLaurin, J. et al. (2002) Nature Med. 8: 1263-1269.

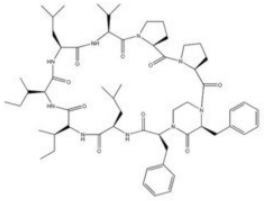
THE UNEXPECTED BIOLOGICAL ACTIVITY OF MODIFIED CYCLOLINOPEPTIDE A

A. Mazur[1], J. Katarzynska[1], S. Jankowski[1], M. Zimecki[2], E. Rudzinska[3], J. Zabrocki[1]

[1]Institute of Organic Chemistry, Department of Chemistry, Technical University of Lodz [2]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences [3]Department of Bioorganic Chemistry, Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland

The new CLA analogue 1, containing ethylene bridge between Phe nitrogen atoms, was found to exhibit unexpected stimulatory effect in the model of the in vitro humoral immune response in mice. To disclose the STRUCTURE-ACTIVITY RELATIONSHIP the NMR solution conformational analysis was carried out.
The solid-state and solution conformational analysis of native CLA indicated the existence of this cyclic system as a complex mixture of conformations [1]. The NMR spectra recorded at 600 MHz in chloroform at 214 K showed the different conformational behaviour of both cyclopeptides: CLA exists as one isomer [1], peptide 1 is in an equilibrium among at least of three conformers. The structures of major isomers of 1 were assigned on the basis of COSY, TOCSY and NOESY experiments, supported by theoretical calculations.
[1] B. Di Blasio et al., J. Am. Chem. Soc. 1989, 111, 9089.

This work was supported by the Ministry of Education and Science Grant No 2 505F 035 28.



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M448

SYNTHESIS AND IN VITRO ANTITUMOUR ACTIVITY OF NEW ANTIFOLATE DRUG - PEPTIDE CONJUGATES

Zs. Miklán[1], J. Reményi[1], G. Schlosser[1], D. Andreu[2], F. Hudecz[1,3]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Loránd University, Budapest, Hungary [2]Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain [3]Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary

Antifolate drugs are inhibitors directed to interfere with folate metabolic pathway. Methotrexate (MTX) and pemetrexed (Alimta®) are known folic acid analogues used in cancer treatment. Different peptide conjugates of MTX have been prepared for intracellular delivery. (1) In octaarginine conjugates one of the carboxylic groups of MTX was attached to the N-terminal of the peptides. (2) However, as results showed, that both carboxylic groups are required to the biological effect of MTX. Therefore we decided to synthesize peptide conjugates of folic acid analogues in which the carboxylic groups are untouched. Octaarginine, penetratin and a cyclic peptide CGNKRTRGC, which can deliver a cargo molecule in the lymphoid system, were used as delivery peptides. We introduced squaric acid or aminoxy acetic acid as linker moiety between the peptides and cargo molecules. The conjugation was monitored by RP-HPLC, the crude products were purified by RP-HPLC and were identified by mass spectrometry. The biological activity of the conjugates was evaluated in vitro on sensitive and resistant human leukemia (HL-60) cell lines.

This work was supported by Medichem 2 (1/A/005/2004) and the Spanish-Hungarian Intergovernmental Agreement.

1. Hudecz, F., Bánóczi, Z., Csík, G.: Medium-sized peptides as built in carriers for biologically active compounds. Med Res Rev. 25(6):679-736. (2005)

2. Bánóczi, Z., Miklán, Zs., Mihala, N., Szabó, R., Reményi, J., Rivas, L.,Andreu, D., Hudecz, F.: Synthesis and in vitro activity of Arg oligopeptide conjugates with antitumor and antimicrobial drugs. Journal of Peptide Science s10, (2004)

PICORNAVIRAL 3CP INHIBITORS. MULTIPLE-PEPTIDE SYNTHESIS IN PARALLEL AND BIOLOGICAL EVALUATION

I.N. Minchev[1], L.T. Vezenkov[1], L. Nikolaeva-Glomb[2], M.I. Czeuz[2],
M.Y. Penkova[1], A.S. Galabov[2]

[1]Department of Organic Chemistry, University of Chemical Technology and Metallurgy [2]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

The picornaviruses are small nonenveloped RNA viruses with a single positive strand RNA. The virus replication cycle starts after the penetration of the virus in the cytoplasm of the host cell. There are several stages of the virus life cycle used for attack. One of the most useful strategies for attacking of the virus includes inhibition of important for the virus lifecycle enzymes. The key enzymes in the replication of the picornaviruses are 3C and 2A proteases. Changes in the active center of these enzymes make them incapable to produce polyprotein in vitro, therefore the inhibition by low molecular weight molecules could stop the viral replication in vivo. 3C protease plays a major roll in the enzymatic proteolysis of the initial viral polyprotein.

The target compounds were based on structural modifications in the known as crucial for the 3CP inhibition activity dipeptides Phe-Gln by incorporation of additional amino acid and pyrrole moiety. The synthesis was cared out as multiple- peptide synthesis in parallel using stepwise SPPS, Fmoc-strategy. The obtained compounds were tested for antiviral activity by agar-diffusion plaque inhibition test against Coxsackievirus B1 replication in FL cell and on this base some structure-activity interpretations were made.

IDENTIFICATION OF AN HEXAPEPTIDE THAT BINDS TO A NEW PHARMACOPHORIC POCKET IN CYCLIN A AND INDUCES APOPTOSIS IN CANCER CELL LINES

P. Mora[1], N. Canela[2], M. Orzaez[1], O. Bachs[2], E. Perez-Paya[1,3]

[1]Department Medicinal Chemistry, Centro de Investigacion Principe Felipe, Valencia [2]Department Biologia Cel.Lular I Anatomia Patologica, Universitat de Barcelona [3]Instituto de Biomedicina de Valencia, CSIC, Valencia, Spain

The protein-protein complexes formed between different cyclins and cyclin-dependent kinases (CDKs) are central to cell cycle regulation. These complexes represent interesting points of chemical intervention for the development of antineoplastic molecules. Here we describe the identification of an all D-amino acid hexapeptide, termed NBI1, that inhibits the kinase activity of the cyclin dependent kinase 2 (cdk2)-cyclin A complex through selective binding to cyclin A. The mechanism of inhibition is non-competitive for ATP and non-competitive for protein substrates. In contrast to the existing CDKs peptide inhibitors, the hexapeptide NBI1 interferes with the formation of the cdk2-cyclin A complex. Furthermore, a cell permeable derivative of NBI1 induces apoptosis and inhibits proliferation of tumor cell lines. Thus, the NBI1-binding site on cyclin A may represent a new target site for the selective inhibition of activity cdk2-cyclin A complex.

M451

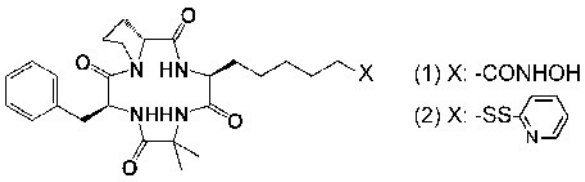
EFFECTS OF HISTONE DEACETYLASE INHIBITORS BASED ON CHLAMYDOCIN ON TYPE 2 DIABETIC MODEL MICE

N. Nishino[1], Y. Hirashima[1], T. Kato[1], J. Nakagawa[2], M. Yoshida[3]

[1]Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu [2]Taisho Pharmaceutical Co. Ltd., Saitama [3]Riken, Wako, Japan

Histone deacetylases (HDAC) play important roles in various aspects of regulation such as proliferation, differentiation, and aging by counteracting with histone acetyl transferases. The HDACs categorized in class-I and II have a metalloprotease-related mechanism in its catalytic activity. These enzymes could be inhibited by small molecules bearing various zinc ligands such as hydroxamic acid and mercaptan. Based on the structure of chlamydocin, which has a cyclic tetrapeptide framework, cyclo(-L-Aoe-Aib-L-Phe-D-Pro-), where Aoe is (2S,9S)-2-amino-8-oxo-9,10-epoxydecanoyl, we have developed potent HDAC inhibitors as shown in the figure.

In the present study, we examined the effects of the chlamydocin hydroxamic acid (1) and SS-hybrid (2) on the diabetes model mice, KK-Ay. The peptide (1) exhibited satisfactory activity to reduce both the blood glucose and blood insulin levels comparable to or even superior to those by pioglitazone, a PPARγ agonist. The SS-hybrid (2), which is expected to be reduced inside of cells to generate the corresponding thiol-containing cyclic tetrapeptide, also showed a significant effect but less than (1). The effect was dose-dependent from 3 mg/kg to 30 mg/kg. The effects of HDAC inhibitors were also confirmed by the observation of in vivo histone hyperacetylation induced in the lymphocyte cells.



SYNTHETIC PEPTIDE VACCINES; DESIGN AND DEVELOPMENT

N.M. O'Brien-Simpson, V. Tam, R.D. Pathirana, E.C. Reynolds

School of Dental Science, The Universtiy of Melbourne, Melbourne, Vic., Australia

Synthetic peptides have a number of advantages over current vaccines. However, exploitation of synthetic peptides as vaccines has been limited by the small size; copy number, inefficient delivery, poor peptide immunogenicity and MHC restriction. We have developed chemical methodologies that overcome these limitations by synthesising and polymerising vinyl-peptides. Protective B-cell and T-cell peptide epitopes from the oral pathogen Porphyromonas gingivalis were identified for three different MHC restricted mouse strains using PEPSCAN techniques. Fmoc chemistry for SPPS was used to synthesize these peptides and a vinyl moiety was incorporated at the N-terminus using acryloyl chloride. After RP-HPLC purification free radical polymerisation using ammonium persulphate and TEMED was used to polymerise the vinyl-peptides in the presence of either acrylamide or other vinyl-monomers to produce single peptide or multi-peptide polymers. Size exclusion chromatography indicated that the peptide polymers were >2 million Da. The peptide polymers were used to immunise each mouse strain (BALB/c, CBA and C57Bl6) and the T-cell response induced was evaluated using proliferation and cytokine (ELISPOT) assays. The peptide polymers were found to be highly immunogenic, the single peptide polymers were found to only induce a response in their respective mouse strain, however, the multi-peptide polymer containing all of the T-cell epitopes was found to induce a response in all three mouse strains. In conclusion, our data shows that the polymerisation method overcomes all of the limitations in developing a peptide vaccine and most importantly that of MHC restriction.

DISCOVERY OF NOVEL LOW MOLECULAR WEIGHT GPR54 AGONISTS

S. Oishi[1], K. Tomita[1], A. Niida[1], M. Akamatsu[2], T. Masui[3], R. Doi[3], J.M. Navenot[4], S.C. Peiper[4], N. Fujii[1]

[1]Graduate School of Pharmaceutical Sciences [2]Graduate School of Agriculture [3]Graduate School of Medicine, Kyoto University, Kyoto, Japan [4]Department of Pathology, Medical College of Georgia, Augusta, GA, USA

Metastin (kisspeptin-54), consisting of 54 amino acids, is a product of the metastasis suppressor gene KiSS-1. This C-terminally amidated peptide was identified as the endogenous ligand of an orphan G-protein coupled receptor, GPR54 (hOT7T175, AXOR12) [1]. Metastin-GPR54 signaling may regulate gonadotropin secretion and negatively regulate cancer metastasis. It is interesting that activation of GPR54 signaling negatively regulates the function of SDF-1-CXCR4 axis in CHO and HeLa cell transfectants [2].

The bioactive sequence of metastin as a GPR54 agonist locates at the C-terminal decapeptide amide, kisspeptin-10/metastin(45-54). We conducted the STRUCTURE-ACTIVITY RELATIONSHIP (SAR) study on kisspeptin-10 using the neuropeptide-derived RW-amide scaffold to identify five-residue peptide amides as novel GPR54 agonists equipotent to kisspeptin-10 [3]. These peptides suppressed the migration of pancreatic cancer cell lines and stimulated phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2). The quantitative STRUCTURE-ACTIVITY RELATIONSHIP study by CoMFA analysis indicated that the N-terminal functional group may be involved in the potent agonistic activity.

References:

1. Ohtaki, T., et al. Nature 411, 613-617 (2001).
2. Navenot, J-M., et al. Cancer Res., 65, 10450-10456 (2005).
3. Masui, T., et al. Biochem. Biophys. Res. Commun., 315, 85-92 (2004); Niida, A. et al. Bioorg. Med. Chem. Lett., 16, 134-137 (2006).

M454

CENTRALLY ACTIVE ANALOGUES OF THYROTROPIN-RELEASING HORMONE (TRH): STRATEGIES TO ENHANCE SELECTIVITY AND TRANSPORT

L. Prokai[1], K. Prokai-Tatrai[2], A.D. Zharikova[3], V. Nguyen[3], M. Teixido[4], X. Wang[3]

[1]Department of Molecular Biology & Immunology, University of North Texas Health Science Center [2]Department of Pharmacology & Neuroscience, University of North Texas Health Science Center, Fort Worth, TX [3]College of Pharmacy, University of Florida, Gainesville, FL, USA [4]IRB-PCB, University of Barcelona, Spain

Besides its endocrine activity, TRH (the tripeptide pGlu-His-Pro-NH₂) has also been long recognized as a modulatory neuropeptide with broad range of physiological and pharmacological activities in the central nervous system (CNS). Although numerous centrally active and metabolically stable analogues and peptidomimetics have been synthesized using TRH as a template, selectivity of their CNS action has remained an issue to be addressed. We aimed at discovering novel analogues with enhanced CNS-selectivity by incorporating pyridinium building blocks. The design also allowed for enhancing transport across the blood-brain-barrier and increasing residence time in the CNS through prodrug strategy.

Solid-phase chemistry was used to prepare the analogues and novel methods previously not used to incorporate pyridinium moieties into resin-bound peptides such as the Zincke reaction were also introduced. Comprehensive evaluation included measurement of affinity to TRH-receptor, acetylcholine-releasing, analeptic and antidepressant-like activity in animal models, as well as prediction of membrane affinity, determination of in vitro metabolic stability, and pharmacokinetics and brain uptake/retention studies that employed in vivo microdialysis sampling.

A strong connection between acetylcholine-releasing potency and analeptic effect in animals was obtained for close analogues of TRH, while pyridinium compounds designed from the structurally related pGlu-Glu-Pro-NH₂ maintained the antidepressant-like effect of the parent peptide, while showing significant decrease in analeptic action.

In conclusion, an increase in the selectivity of CNS-activity profile was obtained by the incorporation of pyridinium moieties. We have also demonstrated the benefits of the prodrug approach on the pharmacokinetics, brain uptake and retention of the analogues upon systemic administration.

Tu455

SPECIFIC AND UNSPECIFIC TRANSPORT OF ANTI-TUMOUR THERAPEUTICS INSIDE TUMOUR CELLS BY USING CARRIER PEPTIDES

R. Rennert[1], S. Jungmichel[1], I. Neundorf[1], B. Nosse[2], W.B. Jeong[2], O. Reiser[2], A.G. Beck-Sickinger[1]

[1]Institute of Biochemistry, University of Leipzig, Leipzig [2]Institute of Organic Chemistry, University of Regensburg, Germany

Cytotoxic substances are auspicious weapons in tumour therapy. This compounds inhibit cell division and proliferation, hence, they affect all cells that are able to divide. However, all these compounds act intracellularly, i.e. at first they have to enter the tumour cell efficiently. This is a serious obstacle when using highly effective cytostatica and the cause of severe adverse effects by using higher doses.

Our aim is to overcome this problem by using synthetic hybride molecules composed of the cytostatic agent, in our case derivatives of arglabin, covalently linked to shuttle peptides. In order to identify the most effective possibility we tested two different strategies.

By using the peptide hormone NPY, whose specific Y receptors are often overexpressed by tumour tissues, we intended to address the chemotherapeutic selectively to Y receptor expressing tumour cells via receptor mediated endocytosis.

On the other hand, the cytostatic agent was covalently bound to a cell penetrating peptide derived from human calcitonin (hCT). Recently, a C-terminal fragment of human calcitonin was found to internalize into excised nasal epithelium while the receptor activating N-terminal part is lacking. For the class of hCT derived carrier peptides previous studies suggested a receptor independent “lipid raft” mediated endocytotic mechanism of uptake.

Here we present comparative data investigating both strategies, the highly selective receptor mediated delivery and the highly efficient receptor independent delivery. We investigated the peptide uptake by various cell lines and examined the release of the cytostatic agents inside the cells and its toxic effects.

SYNTHESIS, CELLULAR UPTAKE AND PROTEOLYTIC STABILITY OF PEPTIDES CONJUGATED TO HPMA-POLYMERS

I.R. Ruttekolk[1], R. Fischer[1], K.-H. Wiesmüller[2], R. Brock[1]

[1]Department of Molecular Biology, Institute for Cell Biology, University of Tuebingen [2]EMC Microcolections GmbH, Tuebingen, Germany

Background and aims: Synthetic peptides provide a straight-forward access to rationally designed inhibitors of molecular interactions based on structural information of proteins. Poor membrane permeability may be overcome via cell-penetrating peptides, but low stability remains a major drawback. An increase of stability and bioactivity of peptides by coupling to polymers is intended.

Methods: For the development of peptide-functionalized cell-penetrating polymer conjugates, peptides were coupled chemoselectivly to HPMA (N-(2-hydroxypropyl)methacrylamide, average MW 28.5 kDa), as an inert backbone polymer by native chemical ligation. HPMA is water soluble and its capacity in drug delivery has been demonstrated. Peptide-functionalized polymers and free peptides were incubated with proteases or cell lysates and proteolytic break-down was determined by fluorescence correlation spectroscopy (FCS) deriving information on the number and size of fluorescent particles based on temporal fluctuations of a fluorescence signal caused by diffusion of particles through a femtoliter-size confocal detection volume. The diffusion time depends on molecular size. Therefore this technique is suited for the detection of proteolytic fragments.

Results: Efficient chemoselective conjugation of unprotected fluorescein-labelled peptides was accomplished by means of native chemical ligation. Our FCS investigations revealed that conjugation of peptides to HPMA increased their biostability. This data also indicate that this effect is peptide-dependent. A proapoptotic peptide coupled to HPMA and introduced into mammalian cells by electroporation retained its bioactivity. Cofunctionalization of HPMA with this peptide and nonaargine yielded an efficient cellular import.

Conclusions: Peptide-stability is increased by HPMA-conjugation maintaining the biological activity. Cellular uptake can be accomplished by cofunctionalisation with cell-penetrating peptides.

ANTIMICROBIAL CATIONIC PEPTIDES :DESIGN, SYNTHESIS, CONFORMATIONAL AND BIOLOGICAL STUDIES

M. Sakarellos-Daitsiotis, S. Zikou, P. Mastora, A.-I. Koukou, C. Drainas,
E. Panou-Pomonis, C. Sakarellos

Department of Chemistry, University, Ioannina, Greece

The widespread use of antibiotics led to the development of multi-resistant bacteria, resulting in an urgent need for antibiotics with a new mode of action. Cationic antimicrobial peptides are a class of positively charged peptides, with amphipathic helical conformation and a substantial portion of hydrophobicity. In the present study, amphipathic helical peptides of various chain-length incorporating Aib, an enzymatically resistant aminoacid, positively charged Arg and hydrophobic Leu, Ac-(Aib-Arg-Aib-Leu)n-NH2 (n=1-4), were synthesized in order to develop new antibiotics. Synthesis was performed by the conventional stepwise Fmoc/tBu solid-phase method and the peptides were purified by HPLC and identified by ESI-MS. The above peptides, as well as the helical sequential polypeptides (Arg-X-Gly)n, (where X represents amino acid residues Ala, Val, and Leu) were tested for their antimicrobial activity against gram(-) bacteria, E.coliDH5a, P.aeruginosa, Z.mobilis 10988, C.parapsilosis and gram(+), M.smegmatis mc2155 and B.subtilis. Experiments for hemolytic activity and proteolytic stability were performed. Conformational properties of the peptides were evaluated by CD spectroscopy.

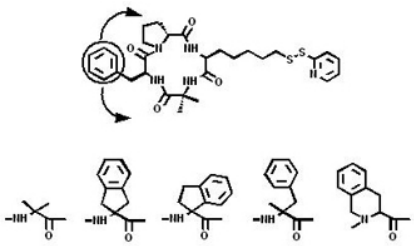
Tu458

DESIGN AND SYNTHESIS OF HISTONE DEACETYLASE INHIBITORS BY AROMATIC RING SHIFTING IN CHLAMYDOCIN FRAMEWORK

G.M. Shivashimpi[1], T. Kato[1], N. Nishino[1], S. Maeda[2], T.G. Nishino[2], M. Yoshida[2]

[1]Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu, Fukuoka [2]Riken, Wako, Japan

Chlamydocin is a cyclic tetrapeptide, cyclo(-L-Aoe-Aib-L-Phe-D-Pro-), where Aoe is (2S,9S)-2-amino-8-oxo-9,10-epoxydecanoyl. In continuation of our study to design and synthesize analogues bearing a zinc ligand to develop potent inhibitors of histone deacetylase (HDAC) inhibitors, we shifted the aromatic ring of phenylalanine at the aminoisobutric acid (Aib) position and also at the imino acid position. The aim is to explore the interaction of cyclic scaffold with the rim of HDAC paralogs. We replaced the epoxyketone moiety of Aoe with sulfhydryl group, which is protected as disulfide hybrid, as zinc ligand. Benzene ring was introduced to Aib structure to design amino-1-indane carboxylic acid and amino-2-indane carboxylic acid. Aromatic ring-containing imino acids, such as D-Tic were also employed to replace D-Pro. The cyclic tetrapeptides were profiled by the inhibition of HDAC1, HDAC4, and HDAC6. The p21 promoter assay of the peptides was also carried to evaluate cell effects.



STRUCTURE- BASED DRUG DESIGN WITH ALPHA, BETA - DEHYDRO - AMINO ACID RESIDUES

R.K. Somvanshi, N. Singh, A.S. Ethayathulla, A. Kumar, S. Sharma,
A. Srinivasan, S. Dey, T.P. Singh

Department of Biophysics, AIIMS, New Delhi, India

Conventional methods of drug development involve synthesis of a large number of compounds combined with throughput screening. This approach is lengthy, tedious, time consuming, expensive and still largely uncertain. Therefore, a more rational strategy is needed to be adopted. Structure based approach promises to eliminate various loopholes of existing methods of new drug discovery. In this effort the first step is to identify the protein associated with the disease. Then determine its three-dimensional structure and obtain the stereochemical details of the binding site of this protein. Based on this knowledge specific ligands are designed. We have been working on the drug development against inflammatory disorders such as rheumatism and arthritis. These are caused by an increase in the concentrations of pro-inflammatory compounds known as eicosanoids. The production of these compounds is catalyzed by enzymes Phospholipase A2, Cyclooxygenases (COX) and Lipoxygenase (LOX). Based on the structural and chemical knowledge of their binding sites, a series of peptides have been designed with α, β- dehydro residues. The binding affinities of these peptides have been determined. The structures of complexes of some of them with PLA2 have also been determined. Based on the analysis of the structures of complexes, new and more potent peptides have been synthesized. The peptides were designed using design rules developed in our laboratory.

OPTIMIZATION OF THE TOTAL SOLID-PHASE SYNTHESIS OF LIPODEPSIPEPTIDE ANTIBIOTIC FUSARICIDIN A

M. Stawikowski, P. Cudic

Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, FL, USA

Natural products serve as an important source of pharmacologically active compounds or lead structures for the development of novel synthetically derived drugs. It is well known that cyclic depsipeptides and their derivatives exhibit a diverse spectrum of biological activities including insecticidal, antiviral, antimicrobial, anti-tumor, anti-inflammatory and immunosuppressive actions. Depsipeptides belong to the family of nonribosomally synthesized peptides which bear unusual building blocks such as amino acids and lipid units. Due to complex character of depsipeptides their chemical synthesis presents a challenging task. In this communication we present first total solid phase synthesis of cyclic hexalipodepsipeptide fusaricidin A. During the optimization of the synthesis we have based on fusaricidin A analogue containing D-Thr instead of D-allo-Thr and 12-guanidinododecanoic acid instead of 15-guanidino-3-hydroxypentadecanoic acid. The synthetic methodologies applied included application of Fmoc/tBu chemistry together with orthogonal Aloc/Allyl protective groups and on-resin cyclization. During the optimization of the synthesis we have tested three different solid supports: MBHA Rink Amide, TentaGel S RAM, and PAL-ChemMatrix resins. In all cases depsipeptide ester bond formation was formed using DIC/DMAP coupling methodology. The best results were obtained on resins containing more PEG and DCM as a solvent. We have found that traces of DMF lower the yield of the esterification reaction considerably. For the coupling of lipid tail unit we have used several coupling reagents including DIC/DMAP, HBTU/HOBt, HCTU, and pentafluorophenyl esters. Finally, optimized conditions were successfully applied for the first total solid phase synthesis of fusaricidin A.

DESIGN AND SYNTHESIS OF PEPTIDE - MACROLIDE CONJUGATES

N.V. Sumbatyan[1], V.N. Tashlitsky[1], G.A. Korshunova[2]

[1]Chemistry Department [2]A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

Macrolides, a rather large group of natural or semi-synthetic antibiotics, are widely known translation inhibitors whose structure is based on 14-16-member lactones with carbohydrate substitutes attached. Macrolides bind to ribosomal tunnel (RT) in a way that their lactone ring is located orthogonally to the long axis of the RT, covering most of its cleft. Carbohydrate residues of the macrolides are spread along the walls of the RT. Hence, the mechanism of protein synthesis inhibition by macrolides relies on the mechanical obstruction they provide to the passage of nascent polypeptide chain through the RT.

The goal of this study was to design and obtain peptide derivatives of macrolides interesting both as antibacterial agents and potential probes for investigation of nascent peptide chain topography in the RT. Tylosin (Tyl), desmycosin (Des) and O-micaminosyltylonolid (OMT) were used as macrolides. Two types of peptide – macrolide conjugates are possible: with peptide fragments directed to peptidyl transferase center or to the exit of the RT. The formers can be obtained by the legation of peptides with C-6 sustitutes of macrolides, others - with C-14 ones. Peptides of various structure and length possessing different conformation were chosen for condensation with macrolides: (Ala)n (n = 2-5), GPGPGP, AAF, AAFAAFK. Peptide derivatives of antibiotics were obtained by reaction of aldehyde group of Tyl and Des with aminooxyacetylpeptides or by condensation of N-blocked peptides with hydroxyl moiety at 23 position of OMT and 4”-hydroxyl group of Des.

NOVEL APPROACHES TO STUDY DRUG DELIVERY TO THE BRAIN

M. Teixidó[1], T. Tarragó[1], E. Giralt[1,2]

[1]Biomedical Research Institute, Barcelona Science Park (IRB-PCB) [2]Organic Chemistry Department, University of Barcelona (UB), Barcelona, Spain

In order to reach target sites inside the CNS, neurotherapeutic candidates must overcome the blood-brain barrier (BBB). While several transport mechanisms occur at the BBB, this work has focused on the passive diffusion mechanism.

The prediction of a peptide's ability to cross the BBB is not a simple task; hence there exists the need for the rational study of the relevant factors that affect the movement across this physiological barrier. Here we present two new approaches based on in vitro non cellular assays for this type of studies. Firstly, the evaluation of compound mixtures on parallel artificial membrane permeability assays (PAMPA). This approach increases the throughput of the study and structure–activity relationships can be easily establish. Secondly, the transport and biological activity evaluation in a single assay. This second approach has been applied to the search of inhibitors for CNS proteases involved in different neurological diseases (such as Prolyl Oligopeptidase for schizophrenia) able to cross the BBB.

These two new approaches allow assaying compound's permeability in the early stages of a drug development project, and then designing novel analogues with improved BBB transport properties or using blood-brain-barrier shuttles for their delivery.

NEW PEPTIDO-MIMETIC DERIVATIVES OF L-VALIN DEMONSTRATE NEUROPHARMACOLOGICAL ACTIVITY

L. Tantcheva[1], V.V. Petkov[1], G. Karamukova[2], J. Chekalarova[1], S. Abarova[1],
D.S. Tsekova[3]

[1]Department of Experimental Psychopharmacology [2]Department of Neuropeptides, Institute of Physiology, Bulgarian Academy of Sciences, "Acad. G. Bonchev"
[3]Department of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

Two newly synthesized compounds are derivatives of L-Valin and are positional isomers of nicotinic (M-6) and isonicotinic acid (P-6). These functional groups, as well as established good lipid solubility suggest that the main target for their biological action probably will be central nervous system. The presence of aminoacid L –Valin, supposes their low toxicity, confirmed by our earlier experiments.
The aim of the present work is to study their pharmacological activity as putative drugs.
Methods: Male Albino mice (18-20g, 10 in groups) were used. Next neuropharmacological parameters were studied: Analgesic effect (acetic acid test), neuromuscular coordination (Rota-rod test), Orientation ("hole board" test) and learning and memory (passive avoidance- step down test).
Results: Significant analgesic effect of both compounds was established (more pronounced by dose 250 mg/kg i.p.). Slight depressing effect on the orientation reactions in animals was registered, but the neuromuscular coordination and locomotor activity of treated animals were not changed. Good dose-dependent effect on learning and memory was established and M-6 had stronger effect than P-6. The compounds modified the effects of some model substances with central nervous activity. Hexobarbital sleeping time was significantly prolonged by P-6, but was antagonized by M-6. Pentileneterazole threshold was increased significantly and suggests some anticonvulsant activity of both compounds.
Conclusion: As positional isomers M-6 and P-6 demonstrated some variations in their pharmacological activity probably due to the differences in their kinetics, metabolism and excretion. Registered significant neuropharmacological activity, accompanied by low toxicity motivates the new synthesis and future experimental studies.

SHORT PEPTIDE FRAGMENTS WITH ANTIULCER ACTIVITY FROM A COLLAGEN HYDROLYSATE

Yu.A. Zolotarev[1], **B.V. Vaskovsky**[2], G.E. Samonina[3], K.E. Badmaeva[3],
A.K. Dadayan[1], Yu.B. Zverkov[1], S.K. Garanin[2], I.P. Ashmarin[3], N.F. Myasoedov[1]

[1]Institute of Molecular Genetics [2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences [3]Faculty of Biology, Moscow State University, Moscow, Russia

The glyproline family of regulatory peptides includes Pro-Gly-Pro, Gly-Pro, Pro-Gly and also the simplest peptides with Hyp substituted for Pro. A distinctive feature of these peptides is that they exhibit a broad spectrum of biological activities: the antiulcer activity, the inhibition of blood clotting and thrombosis, the reduction of degranulation activity of mast cells, and the normalization of stressogenic behavioral disorders. A peptide acidic hydrolysate of collagen (PHC) was obtained under conditions (4 N HCl) ensuring the predominant formation of short peptides, glyprolines. They were separated and their antiulcer activity was studied. Thirty individual peptides with molecular masses of 174-420 Da were isolated from the PHC by HPLC. The PHC was shown to predominantly contain 2- to 4-aa peptides, including PG, GP, and PGP. Experiments on rats demonstrated that, on intragastric administration at a dose of 1 mg/kg, PHC enhances the stability of the gastric mucosa to the action of ulcerogenic factors, such as ethanol and stress and exhibits a protecting antiulcer effect. Even a lesser dose (0.1 mg/kg), which reduced ulcer area twofold, was effective in the stress model of ulcer. The intraperitoneal and intragastric administration of PHC at a dose of 1 mg/kg was found to exhibit a therapeutic effect in the acetate model of ulcer formation. Thus, we have found that PHC is effective in all the models of ulcer formation and induces the normalization of the parameters of stomach homeostasis.

Th465

DESIGN AND SYNTHESIS OF SHORT PEPTIDES FOR THE TREATMENT OF AD AND PD

M. Zarándi, L. Fülöp, Z. Bozsó, Z. Datki, V. Szegedi, K. Soós, B. Penke

Deptartment of Medical Chemistry, University of Szeged, Hungary

Alzheimer's (AD) and Parkinson's (PD) disease are progressive neurodegenerative disorders which are characterized by amyloid plaques. The main components of the plaques are β-amyloid peptides (Aβ1-40 and Aβ1-42) and α-synuclein.

We have previously shown that small peptides structurally related to the sequence of Aβ(1-42) protect against the neurotoxicity of Aβ peptides. Recent studies by other groups have shown that β-synuclein can counteract the aggregation of α-synuclein in the neurodegenerative process of PD, hereby might protect the central nervous system from the neurotoxic effects of alpha-synuclein. It was found that a tetrapeptide (KEGV) protected against the neurotoxicity of Aβ peptides in vivo.

Based on the previous findings, the following sequences of β-synuclein have been synthesized: KEGV-NH2 and KEGV-OH. After comparing the sequences of α- and β-synuclein, we found common sequences which are KEQV, REGV, KQGV, KEQA. We have synthesized these tetrapeptides in amide forms at their C-termini. The peptides have been synthesized on MBHA resin using a manual solid phase peptide synthesis equipment and Boc-chemistry.

The neuroprotective effects of peptides have been investigated in vitro in MTT test in differentiated neuroblastoma cell culture (SH-SY5Y) and in electrophysiological test on rats using multibarrel electrodes in vivo. The neuroprotective peptides might stop neuronal death and can influence AD and PD progression.

M466

NOVEL BIOLOGICAL EFFECT OF PLANT PEPTIDE HORMONE OF PHYTOSULFOKINE-ALPHA (PSK-□) AND ITS SELECTED ANALOGUES IN RATS

A. Bahyrycz[1], M. Rykaczewska-Czerwińska[2], A. Plech[2], D. Konopińska[1]

[1]Faculty of Chemistry, University of Wroclaw, Wroclaw [2]Department of Pharmacology, Medical University of Silesia, Zabrze, Poland

The present study was carried out to determine antinociceptive effect in vivo of plant
peptide hormone phytosulfokine-alpha (H-Tyr(4-SO3H)-Ile-Tyr(4-SO3H)-Thr-Gln-OH) (I)
and its selected analogues, such as H-Phe(4-NO2)-Ile-Tyr(4-SO3H)-Thr-Gln-OH (II),
H-D-Phe(4-SO3H)-Ile-Tyr(4-SO3H)-Thr-Gln-OH(III), H-Tyr-Ile-Tyr(4-SO3H)-Thr-Gln-OH(IV), H-Tyr(4-SO3H)-Ile-Tyr-Thr-Gln-OH(V) and H-Tyr-Ile-Tyr-Thr-Gln-OH (VI)
in rats.
Peptides were injected into the lateral brain ventricle at the dose of 100 nmol. In the preliminary investigation we found the PSK-□ as well analogues II and III induced a significant antinociceptive effect determined in the test of hot plate.
The probable mechanism of this effect was discussed.

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PEPTIDE CONJUGATES AS CPHV-1 VACCINES

R. Mansi[1], S. Galdiero[1], D. Tesauro[1], G. Mező[3], K. Uray[3], G. Morelli[1],
M. Galdiero[2], F. Hudecz[3], **E. Benedetti**[1]

[1]Department of Biological Sciences University Napoli "Federico II" [2]Dipartimento di Medicina Sperimentale, Facoltà di Medicina E Chirurgia, Seconda Università di Napoli, Italy [3]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eotvos L. University, Budapest, Hungary

Caprine herpes virus type 1 (CpHV-1) causes generalized disease in young kids in which the virus mainly affects the digestive tract. In adult goats, however, the infection remains unapparent and the virus may cause abortion, vulvovaginitis or balanoposthitis. The use of a vaccine could provide a powerful tool for the control of CpHV-1 infection.

Synthetic peptide-based vaccines have advantages of being selective, chemically defined and safe. In order to further localize immunogenic epitopes, glycoproteins B, C and D of CpHV-1 were analyzed with several prediction programs. Peptide conjugates incorporating T and B cell determinants in multiple copies in branched architecture are better immunogens. To this aim, epitope peptides were conjugated to tetratuftsin derivatives consisting of four repeated units of TKPKG pentapeptide [1].

For the preparation of goat vaccines, we synthesized peptide conjugates bearing T cell epitope on the N-terminal of the core. B cell epitopes were conjugated via a thioether bond on the Ne-amino group of four choroacetylated lysine residues of the core. ELISAs confirm that the B cell epitopes and the conjugates T cell-tetratuftsin induce epitope-specific and antibody responses.

References.

[1] Mezö G., Kalàszi A., Remènyi J., Majer Zs., Hilbert Á., Làng O., Kohidai L., Barna K., Gaàl D., Hudecz F. Biopolymers 2004, 73 : 645-656.

Th468

INFLUENCE OF MIF-1 AND TYR-MIF-1 ON THE DISTRIBUTION AND DENSITY OF NADPH-D AND TYROZINE HYDROXYLASE IN RAT BRAIN

A.B. Bozhilova-Pastirova[1], B.A. Landzhov[1], P.V. Yotovski[1], E.B. Dzambazova[2], **A.I. Bocheva**[2]

[1]Department of Anatomy and Histology [2]Department of Pathophysiology, Medical University of Sofia, Bulgaria

The Tyr-MIF-1 family of peptides (Tyr-MIF-1`s) includes MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1, which have been isolated from bovine hypothalamus and human brain cortex. All these peptides interact with opioid receptors and in addition bind to non-opiate sites specific for each of the peptides. Data in the literature suggest that Tyr-MIF-1`s have antiopioid and opioid - like effects.

We used Wistar rats to study distribution and density of the tyrozine hydroxylase (TH) imunoreactive fibres and NADPH-d reactive neurons in the rat ventral and dorsal striatum. Our results showed that neuropeptides MIF-1 and Tyr-MIF-1 may affect them.

M469

IN VITRO INVESTIGATION OF ANTIOXIDANT PROPERTIES OF SOME TYR-MIF-1 PEPTIDES IN ROS GENARATED SYSTEMS

V.A. Hadjimitova[1], T.T. Traykov[1], E.B. Dzambazova[2], **A.I. Bocheva**[2]

[1]Department of Medical Physics and Biophysics [2]Department of Pathophysiology, Medical University of Sofia, Bulgaria

Opioid peptides have been recognized as modulators of reactive oxygen species (ROS) in mouse macrophages and human neutrophils. Data in the literature suggest that peptides of the Tyr-MIF-1 family – MIF-1 and Tyr-MIF-1 have antiopioid and opioid - like effects. These neuropeptides are isolated from bovine hypothalamus and human brain cortex. So far no data about direct scavenger properties of Tyr-MIFs peptides were available. In this study we tested the hypothesis that they may scavenge ROS in vitro.

The antioxidant activity of these two substances was studied in the concentration range of 10-6 – 10-4 mol/l. We investigated the luminol-dependent chemiluminescence to test their ability to scavenge the biologically relevant oxygen-derived species: hydroxyl radical, superoxide radical, hypochlorous acid in vitro. We found that Tyr-MIF-1 was a powerful scavenger in all tested systems. The effects were higher for hypochlorous anion and weaker for superoxide radical. MIF-1 had no scavenge activity against the hydroxyl and superoxide radicals and showed a moderate scavenger effect on hypochlorous anion.

ANTIFUNGAL EFFECT OF THE ANTIMICROBIAL PEPTIDE HLF(1-11) ON INVASIVE INFECTIONS WITH CANDIDA ALBICANS

C.P. Brouwer[1], M. Wulferink[1], L. Jonk[1], P.H. Nibbering[2], A. Lupetti[2], M.P. Velders[1]

[1]AM-Pharma B.V., Bunnik [2]Department of Infect. Dis., LUMC, Leiden, The Netherlands

Background: Invasive fungal infections in immunocompromised patients are associated with mortality and morbidity rates. Despite the introduction of new antifungals, these infections represent a significant challenge to medicine. Here, we investigated whether human lactoferrin-derived peptide hLF(1-11) displays antifungal activity against disseminated Candida albicans infection.

Methods: Neutropenic mice were intravenously infected with C. albicans and 20 h thereafter injected with various amounts of hLF(1-11). 24 h after injection of the peptide, number of viable yeasts in the kidneys were determined microbiologically and the size and number of infectious foci was quantified through histological examination. In addition, serum cytokine levels were measured. Results: Results revealed a U-shaped dose-effect curve for the antifungal action of hLF(1-11). Effective doses of the peptide (0.04-0.4 µg/kg) were accompanied by reduced TNF-□ and IL-6 serum levels as compared to untreated infected mice whereas the poor antifungal activity of high doses of this peptide (400-40,000 µg/kg) coincided with high levels of circulating IL-10. Histological analysis of the number and size of infectious foci in kidney tissue sections confirmed our microbiological data. Effectively treated mice displayed infectious foci containing blastoconidia, whereas in untreated and sub optimally treated mice such foci contained mainly filamentous forms. In agreement with this observation, in vitro hLF(1-11) dose-dependently inhibited the transistion from blastoconidious to filamentous C. albicans. Conclusions: hLF(1-11) is effective against disseminated C. albicans infections. Clinical safety studies have been completed successfully and clinical phase II studies comprising hematopoetic stem cell transplantation patients are currently ongoing.

Th471

THYROLIBERIN IN EXPERIMENTAL FOCAL EPILEPSY REGULATION

S.A. Chepurnov[1], N.E. Chepurnova[1], O.N. Gaikova[2], K.R. Abbasova[1], A.V. Aristov[1], A.V. Suvorov[2], O.B. Goncharov[1]

[1]Human and Animal Physiology Department, Biological Faculty, Lomonosov Moscow State University, Moscow [2]Department of Neurology Military Medical Academy, Saint-Petersburg, Russia

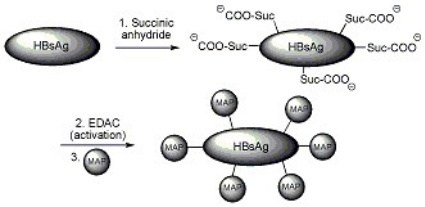
Purpose: The inhibitory effects of thyroliberin (TRH) in ultra low doses after intranasal application demonstrated before (Chepurnov et al.,1995) in generalized epilepsy in rats. The aim of study was researching of the possibility of inhibition of spontaneous epileptic discharges of local focuses in the rabbit cortex.
Methods: The metallic cobalt powder in glass pipette inserted into frontal cortex of one hemisphere (by Mutani,1967). Two recorded electrodes implanted bilaterally. EEG recorded after the mirror focus was arises. TRH applicated intranasal in ultra low doses (10-9 M and 10-12 M) or intravenously in high doses (25mg, 50mg, 100 mg). For EEG registration and analysis the computer system CONAN was used and new modification of Fractal analyze of quantized EEG.
Results: The synchronization of epileptic activity between primary and mirror focuses observed on the third day after operation. Intranasal application of TRH induced reduction of spontaneous focal epileptic activity as in primary cobalt damage focus as in the mirror focus more than 1h. The inhibition of mirror focus was more expressed. Quite the contrary intravenous TRH administration provocated the epileptic discharges in both local focus. The intense synchronized generalized activity was record during 30-40 min. In spite of the relation between doses administered intravenously and initial active dose of TRH (penetrated through Blood Brain Barrier) is 100/1, the effect could be rate as central.
Conclusion: Intranasal application TRH in ultra low doses include the protected endogenous anti-epileptic mechanisms of brain.

COMPARISON OF ANTIBODY RESPONSE IN MICE IMMUNIZED WITH DIFFERENT PRESENTATIONS OF THE JY1 PEPTIDE FROM HIV-1

L.J. Cruz[1], E. Iglesias[2], J.C. Aguilar[2], L.J. González[2], O. Reyes[2], F. Albericio[1], D. Andreu[3]

[1]Barcelona Science Park, University of Barcelona, Spain [2]Center for Genetic Engineering and Biotechnology, La Habana, Cuba [3]Pompeu Fabra University, Barcelona, Spain

We have compared different strategies to increase the immunogenicity of an antigenic HIV peptide as a vaccine candidate. Our selected B-cell epitope comprises 15 amino acids (317-331) of the V3 region of HIV-1, JY1 isolate (subtype D), and is in tandem with a T-helper epitope corresponding to the 830-844 region of tetanus toxoid. Several presentations, including oligomerization, MAP dendrimer, conjugation to dextran beads or to other macromolecular carriers, have been synthesized and evaluated. Murine sera from the different presentations of the V3 epitope have been compared with regard to antibody titers and cross-reactivity with heterologous HIV subtypes. The MAP dendrimer version of the peptide, conjugated to recombinant hepatitis B surface antigen protein, was a better immunogen than the dendrimer alone, and showed higher immunogenicity than other multimeric presentations, or than the peptide alone conjugated to dextran beads. The MAP dendrimer version, either alone or conjugated to HBsAg, enhanced cross-reactivity towards heterologous V3 sequences relative to monomeric peptide.



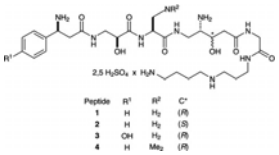
Tu473

THE IMMUNOLOGICAL PROPERTIES, CYTOTOXIC EFFECT AND ANTIMICROBIAL ACTIVITY OF NOVEL ANALOGUES OF EDEINE ANTIBIOTICS

Z. Czajgucki[1], R. Andruszkiewicz[1], M. Zimecki[2], M. Bontemps-Gracz[1], W. Kamysz[3]

[1]Department of Pharmaceutical Technology And Biochemistry, GdańSk University of Technology, Gdańsk [2]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław [3]Faculty of Pharmacy, Medical University of GdańSk, Poland

Bacillus brevis Vm4 has been shown to produce a mixture of closely related peptide antibiotics named edeines. Nowadays no biotechnological source of edeines is available. Taking into consideration their immunosuppressive activity and capacity for universal inhibition of protein biosynthesis we have synthesized four analogues of edeines A and D (1-4) with simplified structures. Their selected biological activities are presented in this communication.
New peptides were tested in several assays in the mouse model to assign their immunological properties. Cytotoxic effect of the compounds was established using HL-60 cells. The activities of edeine analogues against Gram-positive and Gram-negative bacteria and fungi were determined.
In general, new peptides possess interesting immunomodulatory properties. They are not cytotoxic compounds. All edeine analogues exhibit very weak antimicrobial activity.
In conclusion, some peptides deserve further studies, which could reveal their potential therapeutic benefit.
This work was supported by the Ministry of Education and Science (Grant No. 2 P05F 047 28).



Th474

DESIGN AND SYNTHESIS OF MYELIN BASIC PROTEIN (MBP) LINEAR AND CYCLIC PEPTIDE ANALOGUES COMPLEXED WITH MANNAN. EFFECTS IN CYTOKINE PRODUCTION

G.N. Deraos[1], M. Katsara[2], T. Tselios[1], **S.N. Deraos**[1], V. Apostolopoulos[2], J.M. Matsoukas[1]

[1]Department of Chemistry, University of Patras, Patras, Greece [2]Immunology and Vaccine Laboratory, The Austin Research Institute, Heidelberg, Vic., Australia

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system. Candidate self-antigens include constituents of the myelin sheath - Myelin Basic Protein (MBP), Proteolipid Protein (PLP). Immunotherapeutic approaches of MS involve the design and synthesis of peptide analogues of immunodominant epitopes of myelin, delivered in an appropriate manner, which could alter immune responses in patients from Th1 to Th2. A number of linear and cyclic (more stable to proteolysis), antagonist peptides have been designed based on MBP and PLP epitopes by changing principal TCR contact residues (one or two amino acid mutations), which are believed to play a crucial role in immune responses. Peptides were either emulsified in CFA or conjugated to reduced mannan via a KLH linker and injected intradermally into SJL/J mice. Previous studies demonstrated that mannan was able to generate a Th1 or Th2 response depending on the mode of conjugation, oxidized or reduced mannan respectively. In this study T cells were isolated and IFN-γ and IL-4 were measured using ELISpot and antibody responses were measured by ELISA. We note that the use of CFA for immunization induced high levels of IFN-γ and moderate levels of IL-4; however, reduced mannan-peptide complexes induced lower levels of IFN-γ and higher levels of IL-4. We found that MBP linear analogues MBP83-99(Tyr91) and MBP83-99(Phe91) did not induce IFN-γ while in contract IL-10 did. We found also that MBP83-99 (Tyr91) was not cross-recognised by MBP linear agonist. We are currently determining the effectiveness of the peptides in EAE models.

M475

A PROTEOLIPID PROTEIN CYCLIC PEPTIDE ANALOGUE REDUCES THE ENCHEPHALITOGENICITY IN EAE MODEL

S.N. Deraos[1], T.V. Tselios[1], I. Mylonas[2], G.N. Deraos[1], J.M. Matsoukas[1], N. Grigoriades[2]

[1]*Department of Chemistry, University of Patras, Rio, Patras* [2]*Ahepa University Hospital, Aristotle University of Thessaloniki, Greece*

Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated autoimmune disease model of multiple sclerosis (MS) that is useful for identifying potential strategies for modulating human inflammatory demyelinating conditions. In the present report, the cyclic analogue (c-PLP139-151) was designed and synthesized based on the Myelin Proteolipid Protein (PLP139-151) epitope (His139-Ser-Leu-Gly-Lys-Trp-Leu-Gly-His-Pro-Asp-Lys-Phe151). It is known that cyclic peptides are more stable in enzymatic degradation and conformationally restricted compared to linear. The cyclization was achieved using O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) and 1-hydroxy-7-azabenzotriazole, 2,4,6 collidine allowing fast reaction and high yield final product. The purification was achieved using Reversed Phase High Performance Liquid Chromatography (RP-HPLC) and the peptide purity was assessed by analytical HPLC and mass spectrometry (ESI-MS). The synthesized cyclic PLP analogue was found to exhibit lower agonist EAE activity compared to linear PLP139-151 epitope in SJL/J mice. This implies that the conformation of cyclic analogue does not trigger autoimmune reaction in the Central Nervous System and therefore encephalomyelitis.

MULTI-EPITOPIC LIPID CORE PEPTIDE VACCINE WITH BROAD PROTECTIVE IMMUNE RESPONSES AGAINST GROUP A STREPTOCOCCUS

Y. Fujita[1], P. Moyle[1], C. Olive[2], M. Good[2], I. Toth[1]

[1]School of Molecular & Microbial Sciences, The University of Queensland [2]The Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Brisbane, QLD, Australia

Group A Streptococcus (GAS) responsible for critical diseases (eg. acute rheumatic fever and rheumatic heart disease) are classified over 100 serotypes according to their surface virulence M proteins. Development of vaccine to prevent infection with GAS is hampered by the widespread diversity of circulating GAS strains and M protein serotypes, and multivalent vaccine strategy would contribute to prevention against various GAS infections and provide better protective immunity. We have studied the efficacy of incorporating four different epitopes derived from GAS M protein into a single synthetic lipid core peptide (LCP) construct, in inducing broadly protective immune responses against GAS following parenteral delivery to mice. Peptide vaccine was synthesized on MBHA resin by manual SPPS in situ neutralization/HBTU activation in Boc-chemistry. Immunisation with the mono- or multi-epitopic LCP vaccine led to high titers of antigen-specific systemic IgG responses, and the production of broad protective immune responses as demonstrated by the ability of immune sera to opsonise multiple GAS strains. Systemic challenge of mice after primary vaccination showed that mice were significantly protected against GAS infection in comparison with control mice demonstrating that vaccination stimulated long-lasting protective immunity. These data support to the usefulness of LCP system in the development of synthetic multiepitope vaccine to prevent GAS infection.

SYNTHESIS AND ANTIBODY RECOGNITION OF CYCLIC CONSTRUCTS OF EPITOPE PEPTIDE 9-22 OF HERPES SIMPLEX VIRUS GD

A. Jakab[1], G. Mező[1], M. Feijlbrief[2], S. Welling-Wester[2], D. Andreu[3], A. Erdei[4], Z. Majer[5], F. Hudecz[1,5]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University, Budapest, Hungary [2]Department of Medical Microbiology, University of Groningen, Groningen, The Netherlands [3]Department of Experimental and Health Sciences, University Pompeu Fabra, Barcelona, Spain [4]Department of Immunology [5]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

Glycoprotein D represents a major immunogenic component of the virion envelope of herpes simplex virus and able to induce high titres of neutralizing antibodies. One of its optimal epitopes is the 9-22 region (9LKMADPNRFRGKDL22). Several cyclic peptides possessing thioether bond and different ring size have been already prepared and some of them were conjugated with tetratuftsin derivative (Ac-[TKPKG]4-NH2) by thioether bond formation using selectively removable Cys protecting groups. Antibody recognition results suggested that the size of the cycle has considerable influence on antibody recognition, however, the replacement of Met in position 11 by Nle is permitted. Conjugation of cyclic peptide might increase the antibody recognition, but the binding depends on the structure and/or conjugation site of the cyclic peptide. Conjugate with the best binding capacity (7.2 pmol/100uL) as well as the conjugate containing the linear (9-22C) epitope (0.7 pmol/100uL) were selected for immunization. In order to increase the production of antibodies a new group of conjugates was prepared. In these constructs promiscouos T cell epitope peptide derived from tetanus toxoid (YSYFPSV) was attached to both amino groups of lysine residue coupled to the N-terminus of the carrier (Ac-YSYFPSV-K(Ac-YSYFPSV)-[TKPK(ClAc)G]4-NH2). The Cys containing linear and cyclic epitope peptides were conjugated to the carrier in solution (0.1M Tris buffer, pH 8).

This work was supported by grants of the Spanish-Hungarian Intergovernmental Program and COST Chemistry Action.

M478

EFFECTS OF SOME PROKINETICS ON PLASMA CHOLECYSTOKININE-LIKE IMMUNOREACTIVE SUBSTANCE LEVELS IN HEALTHY HUMAN

F. Katagiri, H. Itoh, M. Takeyama

Department of Clinical Pharmacy, Oita University Hospital,
Oita, Japan

Cholecystokinin (CCK) is widely distributed in mainly brain and small intestine. In gastrointestinal system, important actions of CCK are contraction of gall bladder, acceleration of bile transport to duodenum and stimulation of pancreatic exocrine function. On the other hand, in both central and peripheral nervous system, CCK acts as neurotransmitter. Recently, CCK is focused at modulation effects on feeding especially. In this study, we tried to establish a sensitive and specific enzyme immunoassay (EIA) for detecting CCK and to investigate the effect of some dopamine receptor antagonists (domperidone, metoclopramide, itopride and sulpiride).
Human CCK fragment was conjugated with b-D-galactosidase, which was used as a marker antigen, and antiserum YP030 was used as a primary antibody. We adopted delayed addition methods, and separation of bound and free antigens was performed on an anti-rabbit IgG-coated immunoplate. By using a fluorogenic substrate, we detected very small amount of plasma CCK concentrations. Using this EIA, we measured plasma CCK-like immunoreactive substance (IS) levels in five healthy human subjects after single oral administration of some prokinetics.
The minimum amount of CCK detectable by our EIA system was 2.0 pg/ml, and the IC50 of the calibration curve was 75 pg/ml. We revealed that domperidone and itopride caused significant decreases in plasma CCK-IS levels but metoclopramide and sulpiride did not.
We established a sensitive and specific EIA for CCK. Furthermore, using the EIA, we analyzed pharmacological effects of some prokinetics on plasma CCK. Our EIA may be also useful for diagnosis or analysis of diseases.

Tu479

CYCLOLINOPEPTIDE A ANALOGUES CONTAINING BETA2-ISO-PROLINE AND BETA3-HOMO-PROLINE

J. Katarzynska[1], M. Bilska[1], E. Adamek[1], M. Zimecki[2], J. Zabrocki[1]

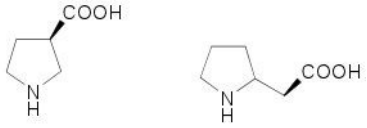
[1]Institute of Organic Chemistry, Technical University of Lodz, [2]Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wroclaw, Poland

Cyclolinopeptide A (CLA), cyclo (Leu-Ile-Ile-Leu-Val-Pro-Pro-Phe-Phe-), isolated from linseed oil [1], possesses a strong immunosuppressive activity comparable at low doses with that of cyclosporin A [2]. It has been postulated that the tetrapeptide sequence Pro-Pro-Phe-Phe is important for biological activity of CLA [3].
On the basis of this information we have synthesized a series of CLA analogues in which the alpha-proline residue was replaced by beta2-iso-proline and beta3-homo-proline residues, respectively (fig.1). The synthesis of titled beta-amino acids has been achieved according to the literature procedure [4,5]. The biological activity of all newly synthesized analogues will be evaluated.

References:
[1] H. P. Kaufmann, A. Tobschirbel, Chem. Ber. 92, 2805 (1959)
[2] Z. Wieczorek, B. Bengtsson, J. Trojnar, I. Z. Siemion, J. Peptide Res. 4, 275 (1991)
[3] I.Z. Siemion, A. Pedyczak, I. Strug, Z. Wieczorek, Arch. Immunol. Ther. Exp. 42, 459 (1994)
[4] S. I. Klein, M. Czekaj, B. F. Molino, V. Chu, Bioorg. Med. Chem. Lett. 7, 1773 (1997)
[5] G. Cardillo, L. Gentilucci, A. R. Qasem, F. Sgarzi, S. Spampinato, J. Med. Chem. 45, 2571 (2002)

This work was supported by the Ministry of Science and Information Technology,
Grant No 2 505F 035 28

Fig.1.



a) (*S*)–β²-*i*Pro b) (*S*)–β³-HPro

IMMUNOENZIMATIC ASSAY WITH PEPTIDE ANTIGENS IMMOBILIZED ON CELLULOSE: EFFECT OF THE LINKER ON ANTIBODY RECOGNITION

B. Kolesinska[1], S. Grabowski[2], W. Kaca[2,3], E. Peroni[4,5], A.M. Papini[4,5], P. Rovero[4,6], Z.J. Kaminski[1]

[1]Institute of Organic Chemistry, ŁÓdź University of Technology [2]Institute of Microbiology and Immunology, University of ŁÓdź [3]Institute of Biology, ŚWietokrzyska Academy, Kielce, Poland [4]Laboratory of Peptide & Protein Chemistry & Biology [5]Departament of Organic Chemistry "Ugo Schiff" [6]Dipartimento di Scienze Farmaceutiche, Università Di Firenze, Sesto Fiorentino (FI), Italy

Synthetic peptides are largely used as antigens in solid-phase immunoenzymatic assays (ELISA) for recognition of antibodies (Abs) in biomedical research and, most importantly, in the set up of diagnostic methods. It is well known that the method of peptide immobilization on the solid support is very important for a correct Ab recognition. This aspect is crucial when the antigens are covalently linked to the support, as in the SPOT assay, based on peptides covalently linked to cellulose [1].Therefore in our studies on the pathogenic relevance of auto-antibodies in Multiple Sclerosis (MS) and Atherosclerosis in patients infected with Helicobacter pylori (Hp) we synthesized appropriate oligopeptides immobilized on cellulose via N- or C-termini, using standard □-alanine linkers as well as a new linker, developed for this particular studies, based on isocyanuric acid derivatives [2].

In the case of the MS glycopeptide antigenic probe CSF114Glc (TPRVERN(Glc)GHSVFLAPYGWMVK) we found the strongest recognition when the peptide was linked to the cellulose support via the C-terminus. However, in the case of UreB F8 Hp urease smallest epitope (SIKEDVQF), and epitope UB-33 (321-339 Hp fragment: CHHLDKSIKEDVQFADSRI) the strongest reactions with sera of Atherosclerosis suffering patients were obtained for N-terminally anchored peptides.

[1]. Frank, R., J. Immun. Meth., 267, 13–26 (2002).

[2]. Kamiński, Z.J.; Główka, M.L.; Olczak, A.; Martynowski, D. Pol. J. Chem. 70, 1316-1323 (1996).

M481

PHARMACOKINETIC STUDY OF GAMMA-D-GLUTAMYL-L-TRYPTOHAN

A. Kolobov[1], A. Petrov[1], A. Trofimov[1], V. Trefilov[1], C. Tuthill[2]

[1]Peptide Chemistry Lab., State Research Institute of Highly Pure Biopreparations, St.-Petersburg, Russia [2]SciClone Pharmaceuticals, Inc., San Mateo, CA, USA

Background: The synthetic dipeptide gamma-D-glutamyl-L-tryptophan (SCV-07) has been shown to stimulate T-lymphocyte differentiation and specific immune responses, and enhance IL-2 and INF-gamma production. Due to this preferential activation of Th1 cytokine production, SCV-07 may show utility in treatment of infectious diseases. The purpose of this study was to evaluate the pharmacokinetics of SCV-07 in mice.

Methods: SCV-07 was injected via intravenous (i/v) or intraperitoneal (i/p) dosing, or given orally (p/o) to CBA mice at a dose of 2,500 µg/kg. The same animals were used for all three methods of administration with a dosing interval of 2 weeks. Blood samples were taken from the right retro-orbital sinus. For determination of the SCV-07 concentration in blood samples, an "EIA-SCV-07" competitive solid-phase immuno-enzyme assay was developed (LOQ 20 ng/ml). Mean concentration-time data were subjected to model-independent, compartmental and inflow profile analysis.

Results: SCV-07 after i/v administration was rapidly eliminated from the blood, with a mean residence time of 10 minutes. SCV-07 after i/p or p/o administration was observed at 1 and 5 minutes postdose, indicating very rapid absorption. Mean concentrations then declined and were measurable through 1.5 and 3 hours postdose (MRT 20 and 50 minutes, for i/p and p/o, respectively). The estimated bioavailability of SCV-07 after i/p and p/o administration was almost equivalent, representing 55-60%. The initial distribution of SCV-07 was 100-130 mL/kg and the apparent volume of distribution at steady state was 250-350 mL/kg.

Conclusions: SCV-07 has a significant potential to be used with oral administration.

Tu482

SEARCHING FOR THE PARATHYROID HORMONE (PTH) FRAGMENTS INDUCING HYPERTENSION

A. Lehmann[1,2], K. Boblewski[2], A. Jurska-Jaśko[2], A. Bonna[1], A. Rybczyńska[2], Z. Maćkiewicz[1]

[1]Department of Polypeptide Chemistry [2]Laboratory of Pathophysiology, Faculty of Pharmacy, Medical University of Gdansk, Poland

Background. Primary hyperparathyroidism (PHT) is characterized with increased parathyroid hormone (PTH) secretion and in 70% of PHT patients with hypertension. It was previously shown that pro-analogue of PTH with a reversed sequence (which include strong alkali sequence -Arg-Lys-Lys-) induced significant hypertensive response at dose 10-10M/kg b.w. One of the hypothesis attributed hypertension in PHT patients to the presence of fragments of degraded PTH possessing -Arg-Lys-Lys- sequence. Aim. To compare influence on mean arterial blood pressure (MAP) of analogue of 25-34 PTH fragment (amide) and 25-34 fragment of PTH, with -Arg-Lys-Lys- sequence and also responsible for binding to PTH receptor. Methods. Chosen peptides were synthesized manually by a solid phase peptide synthesis method. The purity of the products was tested by reversed-phase high performance liquid chromatography. The synthesized peptides showed the right molecular mass.

The influence on MAP of synthesized peptides was tested in Wistar rats. Sequential increasing boluses of each peptide: 10-10, 10-9 and 10-8M/kg.b.w. in the same animal were given i.v. Blood pressure was measured continuously in carotid artery. Results. Injection of synthesized analogue of 25-34 fragment of PTH does not show influence on MAP vs. control group.

Synthesized 25-34 fragment of PTH increased MAP in 92min. of experiment for 12mmHg ± 3mmHg vs. time of administration of first dose and for 17mmHg vs. control group. Conclusion. It seems to be possible, that in case of alternate degradation of PTH, accumulation of 25-34 fragment of PTH may partially play role in the mechanism inducing hypertension in PHT patients.

N-TERMINAL G17 ANALOGS ACTIVATE A PUTATIVE RECEPTOR ON HUMAN COLONIC CARCINOMA CELLS

J. Copps, S. Ahmed, R.F. Murphy, **S. Lovas**

Department of Biomedical Sciences, Creighton University, Omaha, NE, USA

Gastrin-17 (G17) is a peptide which promotes gastric acid secretion, cell proliferation, and occasionally gastrointestinal cancer in the gastric antrum. G17 also promotes the growth of cancerous colonic epithelial cells, but the CCK2/gastrin receptor, which mediates its activity, is largely not expressed on such cells. Instead, our previous studies have shown that some other receptor mediates stimulation of proliferation of DLD-1 and HT29 human colonic carcinoma cells by N-carboxymethyl gastrin (G17Gly) at namomolar concentrations and inhibition at micromolar concentrations, indication separate binding sites. We have shown previously that G17(1-12)-OH stimulates cell proliferation of HT-29 cells, but no binding studies have been conducted.

In this study, radioligand binding and cell proliferation assays are conducted on DLD-1 cells treated with G17(1-12)-OH and G17(1-6)-NH2, in order to determine their selectivity for and activation of the putative proliferation-stimulatory receptor. The results revealed that G17(1-12)-OH is not selective for a single receptor, but binds both sites as do G17 and G17Gly. G17(1-6)-NH2 promotes dose-dependent and non-biphasic proliferation of DLD-1 cells and binds a single receptor with low affinity.

This work was supported by NIH-BRIN grant (1 P20 RR16469) and the Carpenter Endowed Chair in Biochemistry, Creighton University.

M484

COMPARATIVE STUDY OF IGE-BINDING ACTIVITY OF SYNTHETIC PEPTIDES RNWD AND RNWDVYK

V.V. Yanchenko[2,3], D.K Novikov[2], P.D Novikov[2], **O.V. Melnik**[1],
V.P. Martinovich[1], V.P. Golubovich[1], L.K. Yanchenko[3]

[1]Department of Applied Biochemistry,Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk [2]Department of Clinical Immunology and Allergology, Vitebsk Government Midical University [3]Scientific Research Enterprise Resan, Vitebsk, Belarus

Biologically active domains of a high affinity receptor for Ig E (FcεRI) were determined, the fragments 111-114 and 111-117 of the receptor. The program of research of biological properties of synthesized tetrapeptide RNWD and heptapeptide RNWDVYK included the study of their binding with IgE, which was contained in standard solutions and in patients' blood serum.

The binding of peptides with IgE was explored by the IFA method using IgE antibodies labeled with horse-radish peroxidase (HRPO). Peptides in the concentration of 100 mkg/ml were used for sorption on immunological plotting boards.

Higher correlation between the IgE concentration and the optical density of the solution after introducing monoclonal antibodies labeled with HRPO and substrate chromogenic mixture (r=0.99) was found in the diagnostic system with sorption RNWDVYK-peptide than in the diagnostic system with sorption RNWD-peptide (r=0.94).

Similar investigations were conducted with the diagnostic systems with sorption RNWD and RNWDVYK peptides, but RWND peptides conjugated with HRPO were used as antibodies against immunoglobulin E, instead of HRPO-labeled monoclonal antibodies.

Almost equal correlation was found between the concentration of IgE in standard serum and serum of allergy patients with the known concentration of IgE and the optical density of the solutions after introducing the RNWD peptide, conjugated with HRPO.

After introducing allergy patients' blood serum in the holes on the plotting board, the heptapeptide bound 23.79% more IgE than the tetrapeptide.

Our experiments demonstrated a high IgE binding activity of synthetic RNWD- and RNWDVYK-peptides.

A STUDY OF LIGAND BINDING ACTIVITY OF THE RNWDVYK HEPTAPEPTIDE

V.V. Yanchenko[1,3], P.D. Novikov[2], D.K. Novikov[2], V.P. Golubovich[1],
V.P. Martinovich[1], **O.V. Melnik**[1], L.K. Yanchenko[3]

[1]Department of Applied Biochemistry, Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk [2]Department of Clinical Immunology and Allergology, Vitebsk Government Medical University [3]Scientific Research Enterprise Resan, Vitebsk, Belarus

The synthetic heptapeptide RNWDVYK is a fragment of a high affinity receptor (FcεRI) for immunoglobulin E (fragment 111-117). It is the active domain for binding with IgE.
The program of studies of biological properties of the heptapeptide included the investigation of its binding to IgE contained in standard solutions and in patients' blood serum.
The binding of RNWDVYK with IgE was investigated by the IFA method using the IgE antibodies labelled with horse-radish peroxidase (HRPO).
We determined the optimum sorption concentration of the peptide in this experimental immunoenzyme system to be 100 mkg/ml.
The ability of synthetic RNWDVYK peptides to bind with IgE was studied as a function of IgE concentration in standard serum (0.47 to 60 ng/ml IgE). A high correlation was found between the IgE concentration and the optical density of the solution after introducing monoclonal antibodies labeled with HRPO and the substrate chromogenic mixture (r=0.99).
Similar investigations were conducted using the allergy patients' blood serum. The serum with a known concentration of IgE was added to immunological plotting boards with sorbed synthetic RNWDVYK peptides. A high correlation was also found between the concentration of IgE in the patients' blood serum and the optical density of the solution after introducing monoclonal antibodies labelled with HRPO and substrate chromogenic mixture (r=0.94).
Our experiments showed the high IgE binding activity of synthetic RNWDVYK peptides. We demonstrated the possibility of construction of diagnostic systems for the quantitative determination of IgE and IgE-antibodies.

INVOLVEMENT OF L-NAME IN THE ANTINOCICEPTIVE EFFECTS OF NEWLY SYNTHESIZED ANALOGUES OF TYR-MIF-1 PEPTIDE IN RATS

A.I. Bocheva[1], E.B. Dzambazova[1], S.S. Pancheva[2], **T.I. Pajpanova**[2]

[1]Department of Pathophysiology, Medical University [2]Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Tyr-MIF-1 is a member of Tyr-MIF-1 family of peptides. Tyr-MIF-1 is able to interact with opioid receptors with a higher potency at m sites as well as to its specific non-opiate receptors in the brain. Nitric oxide and Tyr-MIF-1`s are potent modulators of opioid activities. Involvement of NO in nociceptive effects is well documented in various physiological and pathological processes in the CNS. L-NAME when administrated i.c.v. or systemically exhibit antinociceptive activity in rats as evaluated by the PP test. In the present study, we investigated the involvement of L-NAME in the antinociceptive action of newly synthesized analogues of Tyr-MIF-1 peptide: Nα-(Me)Tyr-MIF-1, D-Tyr-(Me)-MIF-1, Tyr(Cl2)-MIF-1 and Tyr(Br2)-MIF-1. The experiments were carried out on male Wistar rats (180-200 g). The changes in the mechanical nociceptive threshold were measured during acute pain by paw pressure test. In conclusion our results show that L-NAME is involved in antinociceptive activity of investigated newly synthesized analogues of Tyr-MIF-1 peptide.

M487

PACLITAXEL COVALENTLY BOUND TO PEPTIDES AND SEQUENTIAL OLIGOPEPTIDE CARRIERS: SYNTHESIS AND ANTITUMOR ACTIVITY

S. Papas[1], Ch. Kalogiros[1], L. Hadjiarapoglou[1], T. Akoumianaki[2], P. Theodoropoulos[2], V. Tsikaris[1]

[1]Department of Chemistry, University of Ioannina, Ioannina [2]Department of Basic Sciences, School of Medicine, University of Crete, Heraklion, Greece

Paclitaxel is one of the most important anticancer drugs used mainly in treatment of breast, lung, and ovarian cancer and is being investigated for use as a single agent for treatment of lung cancer, advanced head and neck cancers, and adenocarcinomas of the upper gastrointestinal tract. However, the development of resistance to paclitaxel, the side effects and low solubility of this drug remain major obstacles for its optimal use in the clinical practice. In this work, we present the synthesis of various analogues in which paclitaxel is covalently bound to peptides or as multiple copies to synthetic carriers. These peptide-paclitaxel derivatives possess greater solubility in water, could be suitable in producing anti-paclitaxel antibodies and inhibit the proliferation of human breast, prostate and cervical cancer cell lines. Although, no major differences were found concerning the extent of the antiproliferative effect between various paclitaxel derivatives and paclitaxel, the analogue with four molecules of paclitaxel covalently bound to synthetic carrier [Ac-(Lys-Aib-Cys)4-NH2] when used at low concentrations inhibited cell proliferation more potently than paclitaxel.

Acknowledgements: This work was supported by grants from EU and the Greek General Secretariat for Research and Technology (PENED 01ΕΔ376).

DEVELOPMENT OF DIAGNOSTICS AND VACCINE CANDIDATES FOR THE HIGHLY PATHOGENIC H5N1 INFLUENZA VIRUS

S. Zevgiti[1], D. Krikorian[1], E. Panou-Pomonis[1], V. Veljkovic[2],
M. Sakarellos-Daitsiotis[1], **C. Sakarellos**[1]

[1]*Department of Chemistry, Univrsity of Ioannina, Ioannina, Greece*
[2]*Institute of Nuclear Sciences VINCA, Belgrade, Serbia & Montenegro*

In this study, the Information Spectrum Method (ISM) of the HA1 subunit of the H5 hemagglutinin protein of the Influenza Virus, H5N1, of different reference isolates was performed in order to identify possible antigenic determinants resistant to virus mutations. Results of this analysis demonstrated that the primary structures of HA1 subunit of H5 hemagglutinins encode a common information corresponding to one characteristic frequency in their ISs, which is probably important for the biological function of these proteins, including their possible recognition by the immune proteins targeting this molecule. Besides, comparison of the ISs of HA1 proteins of H1 "Spanish flu" and H5N1 isolates demonstrated an informational similarity between them. Based on these results, a segment of the N-terminus of HA1 H5N1 was identified to play a crucial role in the inhibitory and immunological properties of all possible H5N1 variants. The identified core segment, being highly conserved in all H5 strains, was selected as an antigenic determinant and coupled to the Sequential Oligopeptide Carrier (SOCn), (Lys-Aib-Gly)n, to the Lys-NεH2 groups, in order to develop a diagnostic immunoassay and formulate a vaccine candidate for the highly pathogenic H5N1 influenza virus.

INVOLVEMENT OF THE HISTAMINERGIC SYSTEM IN THE NOCICEPTIN-INDUCED PAIN-RELATED BEHAVIORS IN THE MOUSE SPINAL CORD

S. Sakurada[1], H. Watanabe[1], H. Mizoguchi[1], A. Yonezawa[1], K. Yanai[2], T. Sakurada[3]

[1]Department of Physiology and Anatomy, Tohoku Pharmaceutical University [2]Department of Pharmacology, Tohoku University School of Medicine, Sendai [3]Department of Biochemistry, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan

Intrathecal (i.t.) injection of nociceptin elicited the behavioral responses consisting of scratching, biting and licking in mice. We recently reported that i.t. injection of histamine can elicit the behavioral responses which were characteristically similar to those of nociceptin. The purpose of the present study was to determine whether histamine-containing neurons in the spinal cord are involved in nociceptin-induced behaviors in mice. The i.t. injection procedure was adapted from the method of Hylden and Wilcox. Immediately following the i.t. injection, the time spent for nociceptive behaviors including scratching, biting and licking were measured. The i.t. administration of nociceptin resulted in nociceptive behavioral responses, which were eliminated by the i.t. co-administration of opioid receptor like-1 (ORL-1) receptor antagonists. The nociceptive behaviors were significantly attenuated by the i.t. co-administration of the H1 receptor antagonists, but not the H2 receptor antagonists. I.t. co-administration of the H3 receptor antagonist significantly increased the behavioral responses, whereas the behavioral responses were completely attenuated by the i.t. co-administration of the H3 receptor agonist. An antiserum against histamine injected i.t. reduced the nociceptin-induced behavioral responses. The same result was observed by i.p. pretreatment with histidine decarboxylase inhibitor. In conclusion, i.t.-administered nociceptin elicits the ORL-1 receptor-mediated nociceptive behavioral responses. The activation of the ORL-1 receptor by nociceptin may induce the release of histamine, which subsequently acts on the H1 receptor located on the substance P-containing neurons to produce the spinal cord-mediated nociceptive responses.

M490

SYNTHESIS AND ANTI-AGGREGATORY ACTIVITY OF (S,S) -CDC- AND GPIIB 313-320 DERIVED HYBRID ANALOGUES

K. Vitouladiti, K. Roufou, V. Moussis, D. Tsoukatos, A. Tselepis, **V. Tsikaris**

Department of Chemistry, University of Ioannina, Greece

The Arg-Gly-Asp (RGD) motif of adhesive proteins is recognized by the activated platelet integrin GPIIB/IIIA. Binding of fibrinogen (Fg) to activated αIIbβ3 causes platelet aggregation and thrombus formation. In previous studies, we demonstrated that highly constraint cyclic (S,S)-CXaaC- containing peptides inhibit platelet aggregation and Fg binding [1,2]. Cyclization reduces the allowed conformations, of both the backbone and the side chains, and possibly induces a favourable for the biological activity orientation of the charged side chains. Conformational studies revealed that orientation of the charged side chains toward the same side of the molecule increase the anti-aggregatory activity of the inhibitor. In this work we present the synthesis and the inhibitory activity of new cyclic compounds. For the design of the studied compounds we combined the available information from the -CDC- containing inhibitors and the GPIIB 313-320 (YMESRADR) sequence which has been shown to inhibit the ADP induced human platelet activation [3]. It was demonstrated that the hybrid molecules exhibit higher anti-aggregatory activity than the GPIIB 313-320 derived analogues. References:

1. Staurakoudis A. et al. (2001) Biopolymers 56, 20-26.
2. Kouki A. et al (2005) J. Thrombosis and Haemostasis 3, 2324-2330.
3. Mitsios J.V. et al. (2004) Eur. J. Biochem. 271, 855-862.

INVESTIGATIONS OF PROLINE- AND RGD-CONTAINING PROTHROMBIN-DERIVED PEPTIDES ON ISCHEMIA-REPERFUSION INDUCED HEART FAILURE ON RATS

M. Veveris[1], M. Pilmane[2], I. Kalvinsh[1], T. Keivish[1], A. Aubry[3], L. Polevaya[1]

[1]Latvian Institute of Organic Synthesis [2]Institute of Anatomy and Anthropology, Riga Stradins University, Riga, Latvia [3]Universite Henri Poincare, Nancy, France

Background and aims: Thrombin plays a key role in various disorders such as arterial thrombosis, atherosclerosis, restenosis, inflammation and myocardial infarction. Insights into the way in which thrombin interacts with its many substrates and cofactors have been clarified by crystal structure and site-directed mutagenesis analyses, but until recently there has been little consideration of how its non-proteolytic functions are performed. We investigated cardiovascular effects of seven modified proline- and RGD-containing peptides designed from three surface-exposed sites of prothrombin, corresponding to residues 218-223, 332-347, and 445-454. Methods: Cardioprotective effects of synthetic peptides were tested on the two rat models of heart failure produced by coronary artery occlusion (10- or 45-min) and reperfusion (30- or 240-min). Arterial blood pressures from left carotid artery, heart rate and ECG II standard lead were measured throughout experiment. At the end of second experiment hearts were morphologically investigated by light microscopy and electron microscopy methods. Results: On animal model with short-term ischemia investigated peptides did not protected from myocardium ischemia during occlusion, however, TP-L13, BK-mc and TP-H7 protected rat hearts from ventricular fibrillation, contributed more significant functional recovery during reperfusion and raising survival rate. On the model with prolong ischemia, acceptable cardioprotective effect revealed TP-H7 and BK-mc. These peptides significantly diminished necrotic zone of left ventricle, protected hearts from ischemia-reperfusion induced functional and morphological changes. Conclusions: Investigated proline-containing peptides revealed activity on cardiovascular system – decreasing of blood pressure, cardioprotective properties and improved recovery from ischemia.

CHARACTERIZATION OF INTRATHECALLY ADMINISTERED HEMOKININ-1-INDUCED NOCICEPTIVE BEHAVIOURS IN MICE

C. Watanabe[1], H. Mizoguchi[1], A. Yonezawa[1], T. Sakurada[2], S. Sakurada[1]

[1]Department of Physiology And Anatomy, Tohoku Pharmaceutical University, Sendai [2]Depatment of Biochemistory, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan

Hemokinin-1 (HK-1), a novel mammalian undecapeptide tachykinin, is cloned from mouse bone marrow. Recent report indicated that HK-1 is an agonist for the substance P specific receptor, NK1 receptor. However, its pharmacological effects and physiological functions are still unclear. The present study was designed to characterize the nociceptive behaviours induced by intrathecal (i.t.) administration of HK-1 in mice. The i.t. administration was made in conscious mice according to the method described by Hylden and Wilcox. Immediately after the i.t. administration, the accumulated time for nociceptive behaviours was measured for 10 min. The i.t. administration of HK-1 dose-dependently produced characteristic nociceptive behaviours consisting of scratching, biting and licking, which peaked at 0-5 min and almost disappeared by 15 min after injection. The subcutaneous pretreatment with morphine dose-dependently attenuated the HK-1-induced nociceptive behaviours. The nociceptive behaviours elicited by low-dose of HK-1 were significantly inhibited by i.t. co-administration with NK1 receptor antagonist, however, the nociceptive behaviours elicited by high-dose of HK-1 were not affected by i.t. co-administration with NK1 receptor antagonist. On the other hand, NMDA receptor antagonists significantly suppressed both high- and low-doses of HK-1-induced nociceptive behaviours in a dose-dependent manner. These results suggest that the nociceptive behaviours induced by low-dose of HK-1 may be mediated through both NK1 and NMDA receptors, whereas high-dose of HK-1 may induce the nociceptive behaviours through NMDA receptor. In conclusion, i.t. administration of HK-1 elicits the nociceptive behaviours through the various mechanisms, which are dependent on the doses of HK-1.

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STRUCTURAL DETAILS OF BACTERIAL LIPOPEPTIDES DETERMINING RECOGNITION BY TOLL-LIKE RECEPTOR DIMERS

U. Buwitt-Beckmann[1], H. Heine[1], G. Jung[2], S. Akira[3], R. Brock[4], A.J. Ulmer[1],
K.-H. Wiesmueller[5]

[1]Department of Immunology And Cell Biology, Research Center Borstel [2]Institute of Organic Chemistry, University of Tübingen, Germany [3]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan [4]Institute For Cell Biology, University of Tübingen [5]EMC Microcollections GmbH, Tübingen, Germany

Microbial compounds like lipopeptides/lipoproteins (LP) and lipoteichoic acid have been reported to signal through Toll-like receptor 2 (TLR2). The bacterial LP are strong modulators of the innate immune system. Until recently, it was generally assumed that triacylated LP, like the synthetic Pam3Cys-SK4, are recognized by TLR2/TLR1 heteromers, whereas diacylated LP, like FSL-1, induce signalling through TLR2/TLR6 heteromers. Contrary to this model, we could show that depending on the peptide moiety, diacylated LP also signal in a TLR6-independent and TLR1-dependent manner. The aim of this study was to analyse more closely the structural basis of this heteromer usage.

The synthesis of LP was carried out by fully automated solid phase peptide synthesis and Fmoc/tBu chemistry on TCP or Rink amide resin. Information on the structural factors determining the TLR2/TLR1 versus TLR2/TLR6 heteromer usage was obtained by testing of ligands with cells obtained from TLR2 , TLR6 , and TLR1-deficient mice.

When stimulating B-lymphocytes of wild-type mice we found that ester-bound long-chain fatty acids are necessary to induce considerable responses. For triacylated LP with long chain length ester-bound acyl residues (like Pam3C-SSNASK4) the response in TLR1-deficient cells was only slightly decreased, whereas for LP with short length ester-bound fatty acids (like PamOct2C-SSNASK4) the response was completely abolished.

In summary, a tri-acylation pattern is necessary but not sufficient to render an LP TLR1-dependent and a di-acylation pattern is necessary but not sufficient to render an LP TLR6-dependent. Contrary to the current model, ligand recognition by TLR2 may occur in a TLR1- and TLR6-independent manner.

CONFORMATIONALLY DRIVEN RATIONAL DESIGN OF GLYCOPEPTIDES AS SYNTHETIC PROBES FOR THE DETECTION OF AUTOANTIBODIES, BIOMARKERS OF MULTIPLE SCLEROSIS

A. Carotenuto[1], **M.C. Alcaro**[2], C. Bonaccini[3], F. Barbetti[2], F. Lolli[2], M. Chelli[2], A. Tramontano[3], E. Novellino[1], A.M. Papini[2], P. Rovero[2]

[1]Department of Pharmaceutical and Toxicological Chemistry, University of Naples "Federico II", Naples [2]Department of Organic Chemistry "Ugo Schiff", Department of Neurological Sciences, Department of Pharmaceutical Sciences, and Laboratory of Peptide and Protein Chemistry and Biology, University of Florence, Sesto Fiorentino [3]Department of Biochemical Sciences, University of Rome "La Sapienza", Rome, Italy

Sera from patients suffering from autoimmune disorders often contain multiple types of autoantibodies, some of which can be exclusive of a disease and thus used as biomarkers for diagnosis. Identification of these autoantibodies, as disease biomarkers, should be achieved using native antigens in simple biological assays. However, post-translational modifications, such as glycosylation, may play a fundamental role for specific autoantibody recognition.
In line with these observations, we previously described synthetic glycopeptides able to detect high autoantibody titers in sera of patients affected by multiple sclerosis, an inflammatory, demyelinating disease of the central nervous system. We also demonstrated that glycopeptides able to reveal high antibody titers in multiple sclerosis sera are characterized by a type I' beta-turn around the minimal epitope, bearing the relevant glycosylation [1].
We describe here the result of a conformationally driven rational design exercise, which led to the preparation of new, optimized glycopeptides endowed with enhanced antigenic properties. Most importantly, the same approach, based on structure alignment, was used to shed light on the native antigen(s), target of pathogenetic autoantibodies involved in demyelination processes, and recognized by the designed synthetic glycopeptide antigens.

[1] Lolli, F.; Mulinacci, B.; Carotenuto, A.; Bonetti, B.; Sabatino, G.; Mazzanti, B.; D'Ursi, A.M.; Novellino, E.; Pazzagli, M.; Lovato, L.; Alcaro, M.C.; Peroni, E.; Pozo-Carrero, M.C.; Nuti, F.; Battistini, L.; Borsellino, G.; Chelli, M.; Rovero, P.; Papini, A.M. Proc. Natl. Acad. Sci. USA 2005, 102, 10273-10278.

DETERMINATION OF HEPCIDIN CONTENT OF URINE USING ELISA AND MASS SPECTROMETRY

Sz. Bősze[1], G. Mező[1], G. Schlosser[1], G. Pócsfalvi[2], Á. Balogh[3], J. Prechl[4], F. Hudecz[1,5], B. Vásárhelyi[3,6]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Budapest, Hungary [2]Institute of Food Science and Technology, Avellino, Italy [3]First Department of Pediatrics, Semmelweis University [4]Department of Immunology [5]Department of Organic Chemistry, L Eötvös University [6]Research Laboratory of Pediatrics and Nephrology, Hungarian Academy of Sciences, Budapest, Hungary

Hepcidin is a liver-expressed cysteine-rich cationic 25-mer peptide (DTHFPICIFCCGCCHRSKCGMCCKT) which plays an important role in the regulation of iron metabolism. Human urine contains two predominant forms, comprised of 20 and 25 amino acids, that differ only by N-terminal truncation. We have investigated the hepcidin level in human urine samples. For these studies we have prepared linear peptides using Fmoc/tBu chemistry on Tenta Gel resin with DIC/HOBt coupling. Cyclisation was carried out in DMSO/water/0.2 M Tris.HCl (pH 8.6). Five hepcidin derived linear peptides corresponding to 1-7, 1-7-(Gly)5, 1-20, 1-25, 13-25 region were also prepared and modified with N-(+)-biotinyl-6-aminocaproic acid using PyBOP/HOBt/DIEA coupling methods for ELISA studies. The homogeneity and the primary structure of peptides and biotinylated peptides were checked by analytical RP-HPLC, amino acid analysis, ESI-MS. Mass spectrometric identification and quantification of hepcidin peptides from urine samples was performed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. Before MALDI analysis, urine samples were purified on an NP20 type Proteinchip having normal phase chromatographic support. Peptides were eluted from the surface of the chip and were directly spotted onto a MALDI target. Samples were analysed in positive, linear mode. We have detected protonated molecular ions of the 20-mer and 25-mer hepcidin in urine samples.

99M-TC LABELLING OF CCK8 PEPTIDES BY USING AMINODIPHOSPHINE COLIGANDS: IN VITRO AND IN VIVO EVALUATION FOR CHOLECYSTOKININ-B RECEPTOR IMAGING

E. Benedetti[1], A. Tornesello[1], D. Tesauro[1], S. Agostini[3], E. Didonè[3], M. Cavazza-Ceccato[3], L. Aloj[4], L. Tarallo[4], C. Bolzati[2], G. Morelli[1]

[1]Department of Biological Sciences, & CIRPeB University of Naples "Federico II", Naples [2]ICIS – CNR [3]Department of Pharmaceutical Sciences, University of Padua, Padova [4]Department of Nuclear Medicine, Istituto Nazionale per lo Studio E la Cura dei Tumori, Fondazione "G. Pascale", Napoli, Italy

The use of small radiolabelled compounds such as peptides is a very attractive tool for the diagnosis of several different pathologies, specially cancer, through the use of nuclear medicine techniques.1 Among the various membrane receptors, the two cholecystokinin receptors CCKA-R and CCKB-R are very promising biological targets for radiolabelled compounds due to their overexpression in many tumours2. In order to develop radiolabelled peptide derivatives able to target these receptors, the binding mode of the C-terminal cholecystokinin octapeptide (CCK8), toward the two cholecystokinin receptors CCKA-R and CCKB-R has been, recently, structurally characterized.3 The structural data suggest that modifications on the N-terminal end of CCK8 obtained by introducing chelating agents and their metal complexes should not affect the interaction of the derivatized CCK8 peptides with both CCKA-R and CCKB-R.

Here we report the labelling procedures and the in vitro and in vivo characterization of new 99mTc CCK8 derivatives. A stable 99mTc-nitrido complex is obtained by using the coordinative set formed by: 1) the N-terminal amino group and the SH cystein of the CCK8 derivative Cys-Gly-CCK8 peptide; and 2) a PNP aminodiphosphine used as coligand. Several phosphines are used in order to define the best labelling procedures and to optimize the in vivo biodistribution properties of the 99mTc labelled peptides.

References:

1 - Behr T M, Gotthardt M, Barth A, Behe M. Q J Nucl Med 2001; 45: 189.

2 - J.C. Reubi, J.C. Schaer, B. Waser, Cancer Res. 1997, 57, 1377.

3 - C. Giragossian, D.F. Mierke, Biochemistry 2001, 40, 3804.

Tu497

PEPTIDE BASED RADIOPHARMACEUTICALS FOR IMAGING OF TWO DIFFERENT RECEPTORS

R. Mansi, D. Tesauro, C. Pedone, **E. Benedetti**, G. Morelli

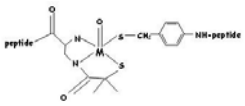
Department of Biological Sciences & CIRPeB, University of Napoli "Federico II", Naples, Italy

The widespread use of compounds containing the gamma-emitting radionuclide 99mTc in nuclear medicine for the scintigraphic imaging, as well as the recent introduction of the beta-emitting radionuclides 188Re and 186Re in radiotherapy, have led to a rapid development of their chemistries, in order to produce novel radiopharmaceuticals.

We have developed new peptide based radiopharmaceuticals based on a scaffold in which the radioactive metal ion is complexed by two different peptides that are able to bind two target receptors (see figure). The 3+1 mixed-ligand approach has been used for the preparation of neutral oxotechnetium(V) and oxorhenium(V) peptide complexes.

The complex preparation requires the simultaneous action of a dianionic tridentate ligand and a monoanionic monodentate thiolato on a suitable metal precursor. The dianionic tridentate ligand is based on the SNN donor set able to stabilize the metal complex. The chelating agent (HSC(CH3)2CONHCH2CH(CO-R)NH2) was coupled step by step to a bioactive peptide synthesized on solid phase. The second ligand, based on monodentate thiolato moiety, was coupled on N-terminus of the second peptide.

Labelling procedures and biological tests on tumour cells overexpressing receptors are described for 99mTc(O) complexed by CCK8 and octreotide peptide derivatives.



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ENDOSTATIN AS A NOVEL MARKER FOR HEPATOCELLULAR CARCINOMA

O. El Bendary[1], A. Anas[2], I. Mostafa[2]

[1]Clincal Chemistry Department [2]Gastroenterology and Hepatology Department, Theodor Bilharz Research Institute, Giza, Egypt

Background: Endostatin inhibits the proliferation of endothelial cells and induces their apoptosis. The measurement of serum endostatin can predict tumor vascularity. Tumor angiogenesis is a strong prognostic factor in patients with hepatocellular carcinoma(HCC). Significantly high levels of endostatin were noted in patients with trabecular-type tumors , and with hepatitis infection.

Methods : 20 patients with HCC, 16 patients with GIT malignancies, 8 patients with liver metastasis and 8 without metastasis, and 10 normal persons . All subjects were tested for alfa-feto protein (AFP) , CA19.9 , Carcinoemberyonic antigen (CEA), and Endostatin by ELISA

Results : Endostatin in normal control persons was 47.5 ± 14.22 ng/ml with a significant elevation ($p< 0.001$) between the HCC group and all the other tested groups .AFP was 1.9 ± 0.98 ng/ml in normal persons with a significant elevation between HCC and all the other tested groups ($p< 0.01$). CA19.9 was 8.14 ± 1.89 U/ml in normal persons with a significance elevation ($p< 0.01$) relative to HCC., and a significance of ($p< 0.001$) relative to GIT cancers with metastases. CEA was tested to be 1.12 ± 0.71 µg/l in normal persons , and had a significance of ($p< 0.001$) relative to GIT metastases. Endostatin was elevated in 2 of 8 patients with GIT cancers not proved to have metastasis.

Conclusion : Endostatin can be used to denote metaplasia and can also detect possibilities of metastasis or liver cell affection even before the frank development of metaplasia

SYNTHESIS OF TECHNETIUM-CHELATING AFFIBODY MOLECULES FOR DIAGNOSTIC IMAGING OF HER2-EXPRESSING TUMOURS

T. Engfeldt[1], T. Tran[2], A. Orlova[2,3], C. Widström[4], J. Feldwisch[3], L. Abrahamsén[3], A. Wennborg[3], A. Eriksson Karlström[1], V. Tolmachev[2,3]

[1]School of Biotechnology, Royal Institute of Technology, Stockholm [2]Unit of Biomedical Radiation Sciences, Rudbeck Laboratory, Uppsala University, Uppsala [3]Affibody AB, Bromma [4]Section of Hospital Physics, Department of Oncology, Uppsala University Hospital, Uppsala, Sweden

Affibody® molecules are a novel class of affinity proteins which are generated by combinatorial engineering of the 58 aa three-helix bundle scaffold, originating from the B domain of staphylococcal protein A. We have used Fmoc/tBu chemistry for total chemical synthesis of the Affibody ZHER2:342, binding with picomolar affinity to the cell surface receptor HER2. The synthetic protein was investigated for molecular imaging of HER2-overexpressing tumours. In vivo detection of HER2 in malignant tumours provides important diagnostic information which may influence patient management. To enable gamma camera imaging of the tumours, a panel of potential 99mTc-chelating sequences was designed and introduced into the Affibody. The well-studied Tc-chelating sequence mercaptoacetyltriglycyl (MAG3) was compared to serine-containing sequences with increased hydrophilicity, such as mercaptoacetyltriserinyl (MAS3).

The total synthetic yield was 14-16 % and the HER2-binding affinity of the Affibody conjugates were all in the range 200-400 pM. Binding specificity of Tc-labelled Affibody molecules was determined on HER2-expressing SKOV-3 ovarian carcinoma cells. All variants showed receptor-specific binding. The tumour-targeting properties were studied in SKOV-3 tumour-bearing nude mice. All conjugates demonstrated high tumour uptake, quick blood clearance and low uptake in most other organs. The biodistribution results further showed that the more hydrophilic, serine-containing chelators resulted in a reduced hepatobiliary excretion, which significantly decrease the background in the abdomen area and provide for more sensitive detection. Gamma camera images of mice with grafted tumours showed clear visualization of HER2-expressing tumours using the 99mTc-labelled MAS3-Affibody conjugate, suggesting a potential future application of this agent for diagnostic imaging.

ENGINEERING BIOINSPIRED LUMINESCENT PROBES: ANTIMICROBIAL PEPTIDES CHELATING LANTHANIDE IONS

E. Gatto[1], L. Stella[1], G. Bocchinfuso[1], A. Palleschi[1], M.E. Palleschi[1],
F. Formaggio[2], C. Toniolo[2], M. Venanzi[1]

[1]Department of Chemical Sciences and Technologies, University of Tor Vergata, Rome [2]Department of chemistry, University of Padova, Italy

Lanthanide chelates, due to their unique emission properties (large Stokes shifts, exceptionally long decay times), are actively explored for potential applications in medical diagnostics, drug discovery, imaging, and bioanalytical assays. On the other hand, oligopeptides offer attractive features as novel smart materials, principally because of their ability to fold into specific structures, to bind with high affinity to membranes or proteins, and to transport ions and small molecules. We recently studied the ion binding properties of trichogin GA IV, a natural peptide showing interesting antimicrobial activity. In particular, we synthesized the new fluorescent analogue

Fmoc -Aib-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Leu-OMe (F0)

(Aib, α-aminoisobutyric acid) to investigate its binding properties to Tb(III) ions. According to our published spectroscopic results F0 populate a set of ordered conformations involving 310/α-helical segments and compact structures generated by the formation of a turn around the flexible Gly5-Gly6 central motif. CD experiments showed that the binding of Tb(III) to F0 gives rise to a structural transition of the peptide chain from a helical to a folded conformation. Peptide binding is also responsible for the dramatic increase in the Tb(III) fluorescence intensity, suggesting that the Tb(III)/F0 complex may represent an interesting system for imaging applications or bioanalytical sensing. Fluorescence energy transfer experiments revealed that Tb(III) emission is also sensitized by the Fmoc N-terminal group. Molecular mechanics calculations allowed us to characterize the 3D-structure of the ion-peptide complex. In our model the Gly5-Gly6 torsional motions lead to a folded structure able to bind lanthanide ions.

SYNTHESIS AND IN VITRO EFFECT OF PEPTIDES AND PEPTIDE-CONJUGATES FROM 16 KDA PROTEIN OF MYCOBACTERIUM TUBERCULOSIS ON IFN-GAMMA PRODUCTION

K. Horváti[1], Sz. Bősze[1], G. Mező[1], N. Caccamo[2], F. Dieli[2], F. Hudecz[1,3]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Loránd University, Budapest, Hungary [2]Department of Biopathology, University of Palermo, Italy [3]Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary

The 16 kDa protein of Mycobacterium tuberculosis provokes specific immune response, therefore related epitope peptides and peptide-conjugates can be considered as potential diagnostics. In our previous study we have determined the functional human T-cell epitope within the 91-110 region. Based on this we synthesised two groups of peptides: a) N- and C-terminal alanine and beta-alanine elongated variants of the 91-104 epitope and b) 91-104 peptides with alanine substitution at different position according to the HLA DR and TCR binding sites. Peptides were prepared by solid phase synthesis using Boc/Bzl or Fmoc/tBu strategy. The homogeneity and the primary structure of peptides were checked by analytical RP-HPLC, amino acid analysis and ESI-MS. The T-cell stimulatory activity of the compounds was investigated using in vitro assays (proliferation and IFN-gamma production) on the 91-110 epitope specific human T-cell clones and PBMC (Peripherial Blood Mononuclear Cells) from patients and healthy (PPD+, PPD-) subjects. The effective peptides were conjugated to branched polypeptides with polylysine backbone (SAK, EAK), tetratuftsin derivative (H-[Thr-Lys-Pro-Lys-Gly]4-NH2) and lysine dendrimer (H-Lys-Lys(H-Lys)-Arg-Arg-beta-Ala-NH2) (MAP) carrier via thioether bond formation. The subtitution degree of the conjugates was determined by amino acid analysis. PBMC and human T-cell clones were stimulated with the free peptide alone or with peptide-conjugates containing an equimolar amount of peptide or with a mixture of free peptide and carrier. We found that conjugation of peptides enhanced human T-cell proliferation and IFN-gamma production.

Acknowledgement: the study was supported by Italian–Hungarian Intergovernmental Program (I-51/03) and Hungarian Research Fund (T043576).

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SYNTHESIS AND IN VITRO ANTIBACTERIAL ACTIVITY OF NATURAL ANTIMICROBIAL PEPTIDES AND CONVENTIONAL ANTIBIOTICS

W. Kamysz, P. Nadolski

Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of GdańSk, Poland

Antimicrobial peptides are molecules with a unique mechanism of action. They are widespread in nature and play the role of an effective weapon of innate immune system against bacteria, fungi and viruses.
The purpose of this study was to investigate the in vitro activity of natural antimicrobial peptides: citropin, piscidin, protegrin, temporin, uperin and the analogues of antimicrobial peptides: iseganan, pexiganan and omiganan. The peptides were synthesized using the solid-phase method and purified by high-performance liquid chromatography. The peptides were subjected to microbiological tests [MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration)] on reference strains of bacteria, according to the procedures outlined by the National Committee for Clinical Laboratory Standards (NCCLS). For comparison, conventional antibiotics (vancomycin, rifampycin, piperacillin, chloramphenicol) were included in this research.
Both the natural antimicrobial peptides and the analogues inhibited the growth of bacteria, but at higher concentrations than did conventional antibiotics. Nevertheless, both natural origin of antimicrobial peptides and their low toxicity constitute a considerable advantage and this is an argument for considering the antimicrobial peptides as good candidates for medicines.

THE LINEAR GRD PEPTIDE LABELED WITH A FLUORESCENT PROBE INTERACTS WITH β3 INTEGRIN

S. Bloch[2], B. Xu[2], Y. Ye[2], K. Liang[2], **G.V. Nikiforovich**[1], S. Achilefu[2]

[1]Department of Biochemistry and Molecular Biophysics [2]Department of Radiology, Washington University School of Medicine, St. Louis, MO, USA

The linear hexapeptide cypate-GRDSPK (compound 1; the cypate moiety is a near-infrared fluorescent label) whose RGD sequence was rearranged to GRD showed high uptake in the αvβ3 integrin-positive tumor tissues in vitro and in vivo. Despite low affinity of 1 to the integrin in the binding assays, the uptake was inhibited by equimolar amounts of the cyclic peptide c(RGDfV), which possesses high affinity to αvβ3 integrin. These observations led to hypothesis that cell internalization of compound 1 may be mediated mostly by only one of the integrin subunits, as the β3 one. Indeed, blocking of αv integrin by the specific antibody did not inhibit the internalization of 1 in tumor cells, which was in the contrast with successful blocking the cell internalization by the anti-β3 integrin antibody. Similar results were obtained in immunocytochemical assays employing the anti-αv and anti-β3 integrin antibodies. Also, studies utilizing the β3-knockout and wild-type mouse cell lines demonstrated that deletion of the β3 subunit markedly decreased internalization of compound 1 in the β3-knockout cells. The preferential interaction of compound 1 with the β3 subunit of integrins relative to the αv subunit was supported also by molecular modeling studies. Summarizing, the bulk of our experimental and modeling data emphasizes interaction with the β3 integrin as the primary mechanism of the uptake of cypate-GRDSPK by tumors. Since this compound showed the superior biodistribution profile in vivo, our results may provide a strategy to image and monitor the functional status of the β3 integrin in cells and live animals.

AN UNIVERSAL PEPTIDE SCAFFOLD TO DEVELOP ANTIGENIC PROBES SPECIFIC FOR AUTOIMMUNE DISEASES

I. Paolini, F. Nuti, E. Peroni, F. Lolli, M. Chelli, P. Rovero, A.M. Papini

Laboratory of Peptide & Protein Chemistry & Biology, Polo Scientifico, Università di Firenze, Sesto Fiorentino (FI), Italy

We demonstrated, for the first time, that an aberrant post-translational modification (PTM, N-glucosylation) is possibly triggering autoantibody response in Multiple Sclerosis. This was possible because of a "reverse approach", which led to CSF114(Glc), a structure based designed glycopeptide, as the first Multiple Sclerosis Antigenic Probe accurately measuring high affinity autoantibodies (biomarkers of disease activity) in sera of a statistically significant patients' population, compared to other autoimmune diseases [1].
Therefore, our "reverse approach" can be extended to other autoimmune conditions, such as Rheumatoid Arthritis and Systemic Lupus Erythematosus, proposing the beta-hairpin structure of CSF114 as an "Universal Peptide Scaffold" to be modified with a series of glycosyl amino acids (different in sugars and linkages), in the aim of developing personalized diagnostic/prognostic tests. The CSF114 beta-turn structure, exposing at the best the aberrant PTM specific for antibody-mediated forms of other autoimmune diseases, will lead to a family of Antigenic Probes to be used in diagnostic/prognostic immunoassays possibly useful for monitoring response to therapies.

[1] (a) Lolli, F. et al. P.N.A.S. U.S.A. 2005, 102(29), 10273-10278; (b) Lolli, F. et al. J. Neuroimmunology 2005, 167,131-137; (c) Papini, A.M. Nat. Med. 2005 11, 13; (d) Papini, A.M. et al. Granted U.S.A. Patent & PCT Application WO 03/000733 A2.

M505

TYROSINE-CONTAINED PEPTIDES AS BIOCHEMICAL MARKER OF OVARY TUMOR

N.F. Paulava

Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus, Minsk, Belarus

Background and aim: A growing tumor is accompanied by tumor intoxication development. Intoxication independs on tumor size and intensity of its break-up. Tumor intoxication is one of variant of endogenous intoxication. Concentration of tyrosine-contained peptides (TCP) in blood plasma have been proposed as biochemical marker of endogenous intoxication at different organs cancers. Our aim was to determine the TCP concentration in blood plasma patients with ovary tumor and its association with the severity of tumor.

Materials and methods: 178 patients with ovary tumor, mean age is 53 years, were studied. The control group consisted of 20 healthy women without tumor. Patients were divided into 2 groups: people with non-malignant and people with malignant ovary tumor. TCP content in blood plasma was estimated by our technique.

Results: TCP concentration in the control group were 0,32±0,13 mmol. The tested marker was present in increased concentration in blood plasma of the patients with ovary tumor. The mean concentration TCP in patients with non-malignant tumor was 0,53±0,16 mmol. The content of this marker in blood plasma of patients from second group was increased 1,32±0,20 mmol compared with healthy control group. After treatment a significant decrease in TCP content was observed.

Conclusions: The result indicate that content TCP in blood plasma depends of the type of tumor. It could be suggested that determination of TCP concentration in blood plasma could be useful for improve the diagnostic of ovary tumor and monitoring of its progression.

DEVELOPMENT OF A DIAGNOSTIC IMMUNOASSAY FOR THE DETECTION OF NATURAL ANTI-NTM/VIP ANTIBODIES OF HIV-1

D. Krikorian[1], S. Zevgiti[1], V. Veljkovic[2], M. Sakarellos-Daitsiotis[1], **C. Sakarellos**[1]

[1]Department of Chemistry, University of Ioannina, Greece [2]Institute of Nuclear Sciences VINCA, Belgrade, Serbia & Montenegro

According to the information spectrum (IS) concept the primary structure of a protein contains information determining its biological functions. This information is encoded by the distribution of the electron-ion potential (EIIP) of amino acids along the sequence and is represented by the frequency components in IS. Proteins with the same biological functions or interacting proteins (e.g. antibody/antigen) share the information corresponding to the common frequency components in their ISs. Investigation of the HIV-1 envelope glycoprotein gp120, as a model system for hypervariable proteins, revealed that this information is strongly conserved and is not significantly affected by natural mutations. The C-terminus of the second conserved region (C2) of gp120, encompassing NTM peptide is important for infectivity and neutralization of HIV-1, while human natural anti-vasoactive intestinal peptide (VIP) antibodies reactive with gp120 play an important role in control of HIV disease progression. NTM/VIP multiple copies were coupled to an artificial sequential oligopeptide carrier for developing an immunoassay (ELISA) as a reproducible, reliable and sensitive tool for the detection of anti-NTM/VIP derived antibodies.

CARDIAC TROPONINS DERIVED CONJUGATES AS TOOLS FOR PRODUCING SPECIFIC ANTIBODIES: SYNTHESIS AND IMMUNE RESPONSE

C. Strongylis[1], Ch. Papadopoulos[1], K. Naka[2], L. Michalis[2], K. Soteriadou[3],
V. Tsikaris[1]

[1]Department of Chemistry [2]School of Medicine, University of Ioannina [3]Hellenic Pasteur Institute, Athens, Greece

Troponin is a structural protein complex, which is responsible for the regulation of skeletal and cardiac muscle contraction. It consists of three components: troponin I (24kDa), troponin C (18kDa), and troponin T (37kDa), each of which carries out different functions in the striated muscles. Cardiac troponins are released into the bloodstream of patients after the onset of a cardiovascular damage. Even minimal elevations over the normal values, of serum troponin T and I are being used to diagnose acute myocardial infarction and also to rule out the patients' condition. The development and commercialization of highly specific biological assays for the detection of cardiac troponins is based on the production of specific antibodies against the whole complex or individual subunits. However, the specificity and sensitivity of these assays vary due to problems mainly originated from the fact that cardiac troponins have a high homology with the skeletal isoforms. The aim of this work is to select and synthesize appropriate regions of the cardiac isoforms of troponin I, C and T, suitable for the production of more sensitive and specific cardiac troponin detecting reagents. In order to construct the immunogenic complexes, the selected sequences were conjugated to the tetrameric Sequential Oligopeptide Carrier (SOC4), either by the classic solid phase step-by-step methodology or by chemoselective ligation reactions. Using the carrier conjugated troponin sequences, anti cardiac troponin complex specific antibodies in high titers were produced.

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DEVELOPMENT OF CHEMOSENSORS FOR ENDOCRINE DISRUPTING CHEMICALS : SYNTHESIS AND EVALUATION OF SOLID PHASE BOUND MODELRECEPTORS

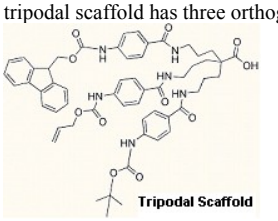
S.E. Van der Plas, A. Madder

Organic and Biomimetic Chemistry, Ghent University, Ghent, Belgium

The increasing problems with the reproductive systems of man and animals are recently linked to the presence of polluting chemicals with endocrine activity, the so called Endocrine Disrupting Chemicals or EDC's .
The family of EDC's is a heterogeneous one and consists of natural and synthetic hormones (like estradiol, ethynylestradiol and diethylstilbesterol), phyto-estrogens (like genistein and coumestrol) and industrial chemicals (like bisfenol-A, ftalates and various pesticides). Because of the complexity of the environmental matrices and the low physiologically active concentrations of the EDC's there is still a need for an efficient routine analysis protocol.

We want to develop a solid-phase bound receptor that possesses a high selectivity for EDC's and thus can be used in a simple solid-phase extraction protocol. This receptor must have the right functional groups that bind the EDC's with a strong affinity and must be able to create a cavity in which the EDC's can fit. By looking at nature's own estrogenic receptor for humans we have found the different amino acids responsible for the specific interactions .

In order to create the cavity which mimics the behaviour of the hormone-binding domain of the human estrogenic receptor we have made a tripodal scaffold. This tripodal scaffold has three orthogonal protected amino groups that will allow the generation of three independent peptide chains.



SENSITIVE IMMUNOPEPTIDOMETRIC ASSAY FOR ACTIVE PSA

L. Zhu[1], H. Koistinen[1], A. Närvänen[2], P. Wu[1], U.-H. Stenman[1]

[1]Department of Clinical Chemistry, Biomedicum, University of Helsinki [2]Department of Chemistry, University of Kuopio, Finland

Background and aims: We have previously developed peptides binding specifically to active PSA by phage display. These peptides have been utilized in an immunopeptidometric assay for specific measurement of active, noncomplexed PSA. However, this assay has not been sensitive enough for the measurement of active PSA in clinical samples. Therefore, we aimed to develop an improved assay utilizing the same principle as previously, but using a more sensitive detection method based on proximity ligation assay.

Methods: In the assay, PSA is first captured on a solid phase by a PSA antibody. After washing, a PSA binding peptide and a PSA antibody are added. Both the peptide and the antibody are biotinylated and complexed with streptavidin coupled with DNA strands which, when in close proximity to each other, can be ligated and subsequently amplified by quantitative real time PCR, facilitating sensitive detection of active PSA.

Results: Using this method we were able to measure 0.07 µg/l active PSA in a volume of 50 µl, a concentration that is about ten times lower than the sensitivity of previous assay. The assay does not cross-react with inactive proPSA or the highly similar kallikrein hK2.

Conclusions: Our results show that highly sensitive immunopeptidometric assay can be developed using proximity ligation. This assay principle should facilitate the establishment of specific assays for active forms of other proteases.

SYNTHESIS OF PEPTIDES DERIIVED FROM GNRH AND THEIR ANALYSIS AND CHARACTERIZATION BY CAPILLARY ZONE ELECTROPHORESIS

V. Solinova[1], **T. Barth**[2], V. Kasicka[1], J. Barthova[3], L. Lepsa[4]

[1]Department of Structural Analysis [2]Department of Tissue Plant Cultures, IOCB [3]Department of Biochemistry, Faculty of Sciences, Charles University, Prague [4]VIDIA Ltd., Vestec, Czech Republic

Rapidly increasing knowledge of new gonadotropin-releasing hormones (GnRH)of different species of the animal kingdom induces the need to prepare new synthetic derivatives and fragments of these peptides with higher potency and metabolic stability and suitable for the formulation of new immunogens. The species related differences in the sequence of the native mammalian GnRH

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH2 concern predominantly the positions 5, 7 and 8, particularly Tyr in position 5 is replaced for His or Leu, Leu in position 7 by Val or Trp, and Arg in position 8 is substituted by Lys, Ser, Asn or Gln. Several GnRH derivatives with with the above substitution and GnRH fragments were prepared by solid phase peptide synthesis and purified by RP-HPLC. Purity of the synthetic peptides was checked by capillary zone electrophoresis (CZE); peptides were analysed as cations in acidic backround electrolytes (pH 2.25 -2.5) and/or as anionic anions in weakly alkaline backround electrolyte (pH 8.1). Analyses were carried out in a home-made apparatus equipped with fused silica capillary (L.D. 50 um effective/total lenght19/30 cm) and UV-photometric detector at 206 nm. Picomole to femtomole amounts of peptides in nanoliter sample volumes were sufficient for thei qualitative and quantitative analyses for determination of their effective electrophoretic mobilities and the estimation of their effective charges.Supported by grant of Ministry of Agriculture of CR-NAZV QF 3028 by rants of GA CR nos. 203/05/2539, 203/06/1044 and Research project Z40550506 of AS CR.

M511

PEPTAIBIOMICS: A SURVEY OF THE FUNGAL GENUS HPOCREA

C. Krause, J. Kirschbaum, **H. Brückner**

Interdisciplinary Research Center, Department of Food Sciences, University of Giessen, Germany

We propose the names "peptaibiome" and "peptaibiomics" for the complete expression of peptaibiotics and methods for their analysis. Peptaibiotics are defined as fungal peptides that contain Aib (α-aminoisobutyric acid). We use peptaibiomics for the structural determination of peptaibiotics from fungi grown on single agar plates thus avoiding time-consuming isolation and purification procedures. The method comprises fast and effective solid-phase extraction followed by on-line RP-HPLC coupled to tandem ESI-MS. Here we present a survey of the peptaibiome of Hypocrea species. In extracts of Hypocrea semiorbis, H. vinosa, H. dichromospora, H. gelatinosa, H. nigricans, H. muroiana and H. lactea a multitude of short and long-chain peptides containing Aib could be characterized. The formation of new and known peptaibiotics could be established by comparison with sequences stored in data bases. Notably, H. vinosa and H. lactea produce peptides which are new analogs of peptaibiotics trichogins and trikoningins from species of Trichoderma. The data establish Hypocrea as a rich source of peptaibiotics.

SUPERIOR HPLC PACKING MATERIALS: HIGH RESOLUTION AND EXCELLENT DURABILITY FOR PEPTIDE AND PROTEIN SEPARATION

K. Tsujimura[2], O. Nakajima[2], **G. Krautz**[1]

[1]*Duesseldorf Representative Office, Daiso Co., Ltd., Duesseldorf, Germany*
[2]*Research Laboratories, Daiso Co., Ltd., Amagasaki, Japan*

Process scale RP HPLC purification of peptides and proteins is increasingly important in bio-pharmaceutical production. Besides selectivity, other crucial factors are loadability, recovery, mechanical stability and chemical robustness in the process and its cost.
DAISOGEL SP-200-C8-BIO, the latest generation octylsilyl(C8)-phase with 20 nm pore diameter is fine tuned for the separation of bigger peptides or smaller proteins. Loadability is believed to depend on the surface area of the packing material. Consequently, smaller pores providing larger surface area should lead to increased loadability. This principle is misleading in the case of large molecules, because they cannot penetrate smaller pores. Therefore the chromatographically accessible surface area has to be taken into account.
Recovery problems like irreversible adsorption or aggregation are frequently caused by hydrophobic surface properties of ODS phases. The less hydrophobic C8 is a substituent to avoid considerably these problems. However C8 is less durable than ODS under extreme acidic conditions. Our new proprietary C8 modification technology combined with a perfect end-capping minimizes the presence of residual silanol groups and protects the silica surface. Under pH 12 leaching is reduced by 90% compared to commercially available C8 phases.
SP-200-C8-BIO demonstrates high mechanical stability by no obvious alteration of back pressure and particle size after 10 repeated packing cycles in DAC columns.
By overcoming the common weaknesses of the conventional C8 RP silica phases, DAISOGEL SP-200-C8-BIO opens new avenues for process scale separation of peptides and proteins.

ANTAGONISTIC OPIOID PEPTIDES DERIVED FROM MILK PRODUCTS

S. Krawczuk, B. Jarmołowska, M. Iwan, K. Bielikowicz

Department of Biochemistry, Institute of Biology, University of Warmia and Mazury, Olsztyn, Poland

Milk proteins are a source of opioid peptides. These peptides are liberated from milk proteins during enzymatic hydrolysis. Some of these peptides are characterized with agonistic
(β-Casomorphins) and some with antagonistic (Casoxins) properties.
The aim of the investigations was to determine the presence of opioid peptides with antagonistic properties in milk products.
The experimental material included cheeses, yogurts and kefirs. Peptides were extracted with a methanol-chloroform mixture (2:1 v/v). The peptide extracts were purified by SPE method on C18 or StrataX columns and characterized by SDS-PAGE electrophoresis. The agonist opioid peptides (Casoxins) were identified by HPLC using standard agonist peptides. The opioid activity was measured by examining the effects of peptide extracts on the motor activity of isolated rabbit intestine.
The results of SDS-PAGE electrophoresis indicated the presence of 5 to 9 fractions in peptide extract derived from cheeses and yogurts and 17 to 20 ones from kefirs. The presence of casoxin A (0.22- 0.68 μg/mg of extract) was proved in all examined the milk products. Lactoferroxin A (0.31-1.88 μg/mg of extract) was identified only in kefirs and yogurts. Those products were also found to contain trace amounts of casoxin C. All peptide extracts showed the antagonistic activity in the relation to motor activity of isolated rabbit intestine. The highest antagonistic activity was reported of peptide extract from kefirs (3.62-17.20%) and Gouda cheese (15.68-16.36%), as compared to morphine.
The physiological and nutritional function of these antagonist peptides requires elucidation.

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DETERMINATION OF PEPTIDE: PROTEIN MOLECULAR RATIO IN CONJUGATES BY SELDI-MS METHOD

T.E. Kuraeva, S.A. Moshkovskiy, E.Y. Aleshina, T.I. Kuzmina, E.F. Kolesanova

V.N. Orekhovich Institute of Biomedical Chemistry RAMS, Moscow, Russia

Synthetic peptides are widely used as antigens in various research and practical areas of biology and medicine. Peptides with molecular masses < 5000 kDa should be conjugated with carrier proteins in order to ensure their immunogenicity and protect from proteolysis. In these cases the comparison of peptide immunogenicities and immunotest system development should be performed having in mind exact peptide-to-protein ratios.

23 conjugates of peptide fragments of hepatitis C virus envelope protein E2 with ovalbumin, bovine serum albumin, and myoglobin were prepared using glutaraldehyde (GA), m-maleimidobenzoyl N-hydroxysuccinimide ester, dimethyl suberimidate (DMS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide as conjugating reagents. The rough evidence of the peptide-protein conjugate formation was obtained by PAGE. The exact peptide:protein molar ratio was estimated in all 23 conjugates by SELDI-MS. Almost all conjugates had oligomeric structures due to the formation of intermolecular linkages between proteins. The peptide : protein molar ratio in conjugates varied from 1:1 to 13,6:1. Conjugates obtained with the GA were more diversified in the number of peptide molecules linked to carrier proteins (peptide:protein ratios ranged from 3:1 to 13:1) than other conjugation reagents. The least peptide:protein ratios (1:1 – 3:1) were obtained using DMS as a conjugation reagent. The latter conjugates were scarcely useful for the generation of antipeptide antibodies: we failed to obtain antibodies to a peptide conjugated via DMS although the same peptide conjugated with the same protein via GA displayed a reasonable immunogenicity. The research was supported by the Federal research program "Live systems. Lot 1 GS-KP.6/003".

IMPROVED METHOD DEVEROPMENT FOR THE EFFICIENT HPLC SEPARATION OF PEPTIDES

N. Kuriyama, N. Shoji, M. Omote, K. Morishita, M. Moriyama

Research & Development Division, YMC Co., Ltd., Komatsu City, Japan

Various combinations of pore size and chemistry of silica-based materials were investigated for high performance liquid chromatography (HPLC) of peptide separation. Incorrect pore size and ligands have been suggested to cause peak broadening, poor resolution and poor recovery. Our study suggests that an appropriate combination of pore sizes and ligands is necessary to obtain the most efficient usage of reversed-phase HPLC columns according to the molecular weights of peptides and proteins. We will also show the possibilities of an improved method development for the separation of complex peptide mixture by pH or additives.

NODULARINS, CYCLIC PENTAPEPTIDE HEPATOTOXINS PRODUCED BY NODULARIA SPUMIGENA FROM THE BALTIC SEA

H. Mazur-Marzec[1], J. Meriluoto[2], M. Plinski[1], J. Szafranek[3]

[1]Department of Marine Biology and Ecology, University of GdańSk, Gdynia, Poland [2]Department of Biochemistry and Pharmacy, Åbo Akademi University, Turku, Finland [3]Faculty of Chemistry, University of GdańSk, Poland

Nodularin (NOD) is a cyclo[-D-erythro-β-methylAsp(iso-linkage)-L-Arg-Adda-D-Glu(iso-linkage)-2-(methylamino)-2(Z)-dehydrobutyric acid], where Adda is the C20 β-amino acid, (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8,-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid. This pentapeptide hepatotoxin is a protein phosphatase inhibitor and liver tumour iniciator (LD50 = 50 μg/kg). The planktonic cyanobacterium Nodularia spumigena is the only producer of the toxin; its blooms are of public health significance as they are formed in drinking and recreational waters.

Water extracts of the Baltic strain of N. spumigena were analysed with Hybride Quadrupole-TOF LC-MS/MS instrument. Ionspray (ISP) and collision induced dissociation (CID) were used to characterize nodularin and its analogues. Amino acid structures and sequence were derived from fragmentation pattern of the [M+H]+ ions. Apart from unmodified nodularin-R (NOD-R), three demethylated variants have been found. The sites of demethylation were located on aspartic acid [Asp1]NOD, Adda residue [DMAdda3]NOD, and dehydrobutyric acid [dhb5]NOD. Two other NOD variants with the additional methyl group located in Adda [MeAdda]NOD and Glu [Glu4(OMe)]NOD residues were detected. The presence of linear NOD and the geometrical isomer of NOD-R, reported earlier in N. spumigena from New Zealand have also been revealed. In the Baltic Sea, so far, only the unmodified NOD and [Asp1]NOD have been detected. Out of the eight nodularin variants characterised in the present study, the following two: [dhb5]NOD and [MeAdda]NOD have not been described earlier. The ISP/MS/CID analyses showed that the variety of NOD analogues produced by one N. spumigena strain is larger than it was previously thought.

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CAPILLARY ELECTROPHORESIS ANALYSIS OF POSTTRANSLATIONAL MODIFICATION CONTAINING PEPTIDES

P. Mucha, A. Kozlowska, P. Rekowski

Department of Chemistry, University of Gdansk, Poland

Posttranslational modifications (PTMs) like phosphorylation, acetylation, or methylation have been shown to play a significant role in directing the function of various proteins [1]. In eukaryotes, most of proteins have been shown to be posttranslationally regulated by a variety of different modifications. Many effects of PTMs include a change of enzymatic activity, ability to bind ligands, subcellular localization, targeted degradation or a lifetime of proteins. Due to a wide spectrum of effects, identifying and associating PTMs with a protein activity is a challenging task [2].

Capillary electrophoresis (CE) has been used to study electrophoretic behavior of PTM-peptides. We have analyzed following fragments of proteins: Ac-Tat(49-57)-NH2, [Lys(Ac)51]Tat(49-57)-NH2, Tat(49-57)- NH2, Tat(52-57)-NH2, Ac-Tat(52-57)-NH2, [Ac-Arg(Me)252]Tat(52-57)-NH2, [Arg(Me)252]Tat(52-57)-NH2, PPAC(301-309)-OH, [Tyr(P)307]PPAC(301-309)-OH, [Ac-Phe148,Lys(Ac)150]gp120(148-150)-NH2, [Ac-Phe148]gp120(148-150)-NH2, [Lys(Ac)150]gp120(148-150)-NH2, [Tyr(PO3)9]GAL-NH2, GAL(1-15)-NH2 by capillary electrophoresis. Using a phosphate buffer most of PTM-peptides were poorly separated at acidic or neutral pH. The best results were obtained using trifluoroethanol containing separation buffers. Optimization of CE separation of maps of peptides containing PTMs should allow to detect PTM-proteins and characterize their role in the living cell. Comparison of modification events occurring in diseased and healthy cell may help to identify diagnostic or prognostic markers for a particular disease state.

This work was partly supported by a grant no. BW-8000-5-0fly-6 from the University of Gdańsk.

[1] R. B. Parekh, Ch. Rohlff, Current Opinion in Biotechnology, 8, 718-723, 1997.

[2] G.T. Cantin, J.R. Yates III, Journal of Chromatography A, 1053, 7-14, 2004.

Tu518

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ENANTIOSEPARATION OF β-AMINO ACIDS

A. Péter[1], R. Berkecz[1], F. Fülöp[2]

[1]Department of Inorganic and Analytical Chemistry [2]Institute of Pharmaceutical Chemistry, University of Szeged, Hungary

The past decade has seen a growing interest in β-amino acids, which are important intermediates for the synthesis of compounds of pharmaceutical interest and can be used as building blocks for peptidomimetics. Oligomers of β-amino acids (β-peptides) fold into compact helices in solution. Recently, a novel class of β-peptide analogues adopting predictable and reproducible folding patterns (foldamers) was evaluated as a potential source of new drugs and catalysts.

Studies on synthetic β-amino acids can be facilitated by versatile and robust methods for determining the enantiomeric purity of starting materials and products. High-performance liquid chromatography (HPLC) is one of the most useful techniques for the recognition and/or separation of stereoisomers including enantiomers.

The aim of the present work was to evaluate HPLC methods for the separation of enantiomers of eighteen 3-amino-3-aryl-substituted propanoic acids (β-amino acids). Direct separations were carried out on different macrocyclic glycopeptide based stationary phases, such as ristocetin A containing Chirobiotic R, teicoplanin containing Chirobiotic T, teicoplanin aglycon containing Chirobiotic Tag, vancomycin containing Chirobiotic V columns and on a chiral crown ether based column.

The effects of different parameters on selectivity, such as the nature of the organic modifier, the mobile phase composition, the flow rate and the structure of the analytes are examined and discussed. The separation of the stereoisomers was optimized by variation of the chromatographic parameters. The efficiency of the different methods and the role of molecular structure in the enantioseparation were noted. The elution sequence of the enantiomers was determined in most cases.

Th519

MULTIPLEX RT-PCR DETECTION OF PREVALENT TRANSLOCATIONS IN IRANIAN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

A. Shabani[1], **Y. Riahi**[1], Z. Afraz[2]

[1]Azzahra University,Faculty of Science,Department of Biochemistry [2]University of Tehran,Faculty of Medicine, Tehran, Iran

The purpose of this study was to use the application of multiplex reverse transcription polymerase chain reaction(RT-PCR) assay for detecting the two most common leukemia translocations t(1;19) and t(9;22) in childhood acute lymphoblastic leukemia in Iranian children. 32 cases of leukemia patients were screened with the RT-PCR assay. This assay will identify the ALL type BCR-ABL transcripts encoded by the t(9;22) and all described variants of the E2A-PBX1 transcript encoded by the t(1;19). RNA was isolated from leukocyte cells of patient's samples. Through the construction and optimization of specific primers for each translocation,we have been able to set up multiplex RT-PCR reactions.Then PCR products was electrophoresed on agaros gel and were compared with size markers and expected fragtments.The results were correlated with the cytogenetic findings and showed that there was one positive case for t(1;19) and no positive case for t(9;22) translocations.
This study illustrates the utility of multiplex RT-PCR assay for the molecular diagnosis and monitoring of childhood acute lymphoblastic leukemia and the clinical relevance of our findings will have to be evaluated with larger patient numbers.

Key words: Acute Lymphoblastic Leukemia – Multiplex RT-PCR

M520

A RATIONAL APPROACH TO EVALUATING PEPTIDE PURITY

A. Swietlow[1], R. Lax[2]

[1]Amgen, Inc., Pharmaceutics, Thousand Oaks, CA [2]PolyPeptide Laboratories, Inc., Torrance, CA, USA

Recent years have seen an enormous increase in interest in peptide therapeutics. New peptide leads are often chosen by screening procedures using microgram to milligram quantities of peptides, frequently provided by specialized manufacturers utilizing automatic synthesizers to maximize output. The purity of the resulting compounds is often not very high. The use of SPPS synthetic procedures predetermines that most impurities are closely related and difficult to resolve by reverse-phase purification. These factors, combined with the use of generic analytical methods not specifically optimized for the peptide in question (e.g. the ubiquitous 0.1% TFA/water/acetonitrile system), lead to erroneous results that frequently severely overestimate the purity of the peptide. The use of poorly characterized materials in pharmaceutical development leads to significant risks of obtaining false negative or false positive results that may cause potential leads to be overlooked or misinterpretation of their pharmacological profiles.

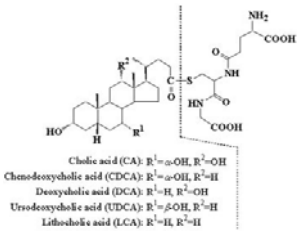
We describe a rapid, systematic and reliable HPLC procedure for evaluation of peptide purity. Utilizing the increased separation efficiency by increasing the column temperature and adjusting the gradient in two steps in reverse-phase buffers containing TFA, $NaClO_4$, or ion-exchange buffers containing KCl, we demonstrate that methods - suitable for preclinical research – can be developed rapidly. The proposed approach will be illustrated with examples of peptides ranging between 9 and 28 amino acids and a model peptide VYPNGA. It will be demonstrated that peptides showing an HPLC purity close to 100% are often 10 – 25% less pure.

STUDY ON THE SYNTHESES AND LC/ESI-MS ANALYSES OF THE GLUTATHIONE CONJUGATES OF BILE ACIDS

T. Wakamiya[1], M. Sogabe[1], Y. Yamaguchi[1], K. Mitamura[2], S. Ikegawa[2]

[1]Faculty of Science and Technology [2]Faculty of Pharmaceutical Sciences, Kinki University, Higashi-osaka, Osaka, Japan

Clofibric acid (p-chlorophenoxyisobutyric acid), a carboxylic acid-containing drug, is metabolized to a glutathione (GSH) conjugate in vivo, and the conjugate is excreted in human urine [1]. Although bile acids, compounds with carboxylic acid in molecules, are also expected to form GSH conjugates in liver, no evidence is so far obtained to confirm such metabolism, since there are no suitable standard samples for the research. In the present paper we report the syntheses of the GSH conjugates of main bile acids in human, i.e., cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), ursodeoxycholic acid (UDCA) and lithocholic acid (LCA) as shown below, and the detailed analyses of these synthetic conjugates by means of linear ion trap LC/ESI-MS. Furthermore, the evidence for conversion of cholyl adenylate [2] and CA-CoA thioester into CA-GSH conjugate will be presented. [1] L. J. Shore et al., Drug Metab. Dispos., 23, 119 (1995). [2] S. Ikegawa et al., Anal. Biochem., 266, 125 (1999).



Th522

SEARCH FOR IMMUNOSUPPRESSANTS WITH ENHANCED BIOAVAILABILITY BASED ON THE SEQUENCES OF CYCLOLINOPEPTIDE A, CLX AND UBIQUITIN

M. Cebrat[1], H. Bartosz-Bechowski[1], A. Kluczyk[1], M. Lisowski[1], P. Stefanowicz[1], A. Staszewska[1], B. Picur[1], P. Ruchala[2], E. Micewicz[3], I. Woznica[4], M. Zimecki[5], Z. Szewczuk[1]

[1]Faculty of Chemistry, University of WroclAw, Poland [2]Department of Cancer Immunology & AIDS, Dana Farber Cancer Institute [3]Division of Infectious Diseases, Children's Hospital Boston, Harvard Medical School [4]Department of Physiology, Tufts University School of Medicine, Boston, MA, USA [5]Institute of Immunology and Experimental Therapy, PAS, Wroclaw, Poland

Immunosuppressive drugs like cyclosporin A (CsA), FK506, and rapamycin are used in clinical practice to prevent transplant rejection and in treatment of autoimmune disorders. Application of these compounds has serious limitations due to their side effects and poor bioavailability.

We have previously shown that cyclolinopeptide A and many of its analogues, as well as a decapeptide fragment of ubiquitin (LEDGRTLSDY), possess immunosuppressive activities similar to that of CsA. These peptides do no exhibit such strong side effects as CsA, but their practical application is hindered because of their poor solubility in water.

The 49-57 fragment of Tat protein and its analogs, including oligoarginine sequences, are known for their unusual ability to cross cell membranes, skin, and blood-brain barriers. Moreover, these peptides are able to transport other substances into cells. This strategy was successfully applied in cases of CsA, taxol, and other drugs to improve their bioavailability.

Now we have synthesized a series of analogues of cyclolinopeptide A, CLX, and the immunosuppressive fragment of ubiquitin, covalently bound to the cell-penetrating fragment of Tat and its analogues. The ability to cross the biological membranes and the immunosuppressive activities of these conjugates were tested. The conformation of the peptides was determined by circular dichroism.

Acknowledgements: This work was supported by the Ministry of Scientific Research and Information Technology grant 2P05A04928.

M523

THE EFFECT OF THE IMPORT MECHANISM OF CATIONIC CELL-PENETRATING PEPTIDES ON THE BIOACTIVITY OF CARGO PEPTIDES

M. Fotin-Mleczek, R. Fischer, F. Duchardt, H. Hufnagel, R. Brock

Group of Cellular Signal Transduction, Institute for Cell Biology, University of Tuebingen, Germany

Background and aims: Cell-penetrating peptides (CPPs) possess the highly attractive ability to accomplish a non-invasive cellular delivery of membrane impermeable cargo molecules. The CPP-based approach provides the possibility of a selective interference with cellular signal transduction by import of peptides interfering with protein-protein interaction. However, the endocytosis-dependent mechanism of cell uptake represents a major challenge for the efficient delivery of peptides into the cytoplasm. In this context we investigated the implications of the endocytic uptake mechanism for the bioactivity of cargo peptides.
Methods: We used fluorescein-labelled cationic cell-penetrating peptides and analyzed the uptake efficiency (flow cytometry) as well as the intracellular distribution (confocal laser scanning microscopy). The bioactivity of a proapoptotic cargo-peptide, delivered into the cells either via electroporation or via CPPs was quantified using a caspase-3 activity assay and cellular assays. To address the integrity of CPPs during their trafficking, a fluorescent double-labelled Antp peptide was designed and used as an intracellular FRET-sensor.
Results: Endocytosis-mediated uptake of the CPP-cargo conjugate led to a significant reduction of cargo bioactivity compared to its direct transfer via transient membrane permeation. This finding was related to the sequestration of peptides within endocytic vesicles but also, in the case of the TNF response, to the induction of receptor internalization during cell entry. Moreover, during endolysosomal passage peptides undergo significant proteolytical degradation.
Conclusions: The endocytosis-dependent uptake mechanism of cationic CPPs interferes with the bioactivity of the delivered cargo at several stages. Moreover, CPPs themselves act as bioactive molecules interfering with cellular signalling.

HIV-TAT PEPTIDE-MODIFIED CHOLESTEROL PULLULAN: SYNTHESIS AND ACTIVITY AS A GENE VECTOR

T. Fukuda[1], Y. Takashima[2], A. Kimura[2], Y. Kamo[2], N. Murata[2], T. Kanazawa[2], H. Okada[2]

[1]Nagahama Institute of Bio-Science AND Technology, Nagahama [2]Pharmaceutics and Drug Delivery, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

Cell penetrating peptides have been known as a useful tool for nucleic acid, protein and drug delivery into cells. Cholesterol pullulan (CP), in which maltose moieties are partially modified by cholesterol, is unique in forming self-assembled nanoparticles (20-30 nm) in water. Combination of these characteristics is considered to be promising for development of effective non-viral vectors without toxicity. A conjugate of HIV-Tat and CP was synthesized and its gene expression efficiency was evaluated. Fully protected HIV-Tat-(48-57)-Cys(Snm)- Gly-NH-R was obtained by conversion of the corresponding Cys(Acm) peptide which was synthesized by the solid-phase method [Snm: (N-methyl-N-phenylcarbamoyl)-sulfenyl] [1]. The sulfhydryl function was introduced to the hydroxyl groups of CP by acylation with Trt-3-mercaptopropionic acid followed by acid treatment. Resulting 3-mercaptopropionyl-CP was coupled with Cys(Snm) peptide to form disulfide bridge and the protecting groups of the peptide were removed to give the CP-Tat conjugate. CP-Tat and pCMV-Luc complex was transfected into COS7 cells and luciferase activity was analyzed after 24 h. CP-Tat elicited remarkable cytoplasmic luciferase activity and low toxicity. It showed particle diameter of approximately 80 nm and positive charge (+20mV). These results suggest that CP-Tat is a safe and useful gene vector, and could be also effective as a siRNA carrier. [1]Chen, L et al., J. Med. Chem., 40, 864-876 (1997).

A PEPTIDE ARRAY WITH A FUNCTIONAL LINKER TOWARD A CELL ASSAY CHIP FORMAT

T. Kakiyama, K. Usui, K. Tomizaki, H. Mihara

Department of Bioengineering and The COE21 Program, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Midori, Yokohama, Japan

To approach a high-throughput cell assay format using peptides, we attempt to design and construct a peptide microarray for examination of cell activities of peptides including apoptotic cell death. Peptides were immobilized onto solid surfaces via a novel multi-functional linker. The linker enable us to examine various types of peptide cell assays in an array format. We also designed and synthesized peptidyl capture agents on the basis of the cell-active sequences suitable for the peptide microarray.

M526

CELL PENETRATING NANOPARTICLES FOR FLUORESCENT IMAGING AND THERAPEUTIC APPLICATIONS

J.K. Mall, J. Darling, B. Patel, J. Howl

RIHS, University of Wolverhampton, UK

The utility of targeted nanoparticles as fluorescent probes for tissue imaging has recently been subject to widespread interest. One exciting prospect is the further development of nanoparticles conjugated to both targeting peptides and cytotoxic cargoes. These nanodevices could preferentially bind to specific cells and/or tissues to provide effective tools for drug delivery. Hence, such multifunctional nanoparticles could provide both diagnostic and therapeutic functions by acting as fluorescent probes that offer targeted delivery of therapeutic agents.

We have coated the surface of quantum dots (Qdots) with cell-penetrating peptides (CPP) to target and label U251M cells for fluorescence imaging. Qdots were initially coupled to polyethylene glycol linkers via carboxyl functionalities on their surface. A heterogeneous mixture of poly-arginine peptides of varying lengths (Arg(6)-Arg(10)) were covalently coupled via amide bonds to the polyethylene glycol linkers, conferring a cell penetrating capacity to the modified Qdots. Fluorescence imaging of U251M cells, after incubation with the conjugated Qdots at concentrations of 20nM, gave clear signals indicating cell binding and internalisation of the modified Qdots across the plasma membrane.

We aim to further expand this work by employing racemic mixtures of CPP and cytotoxic agents to engineer conjugates that will facilitate both imaging and the therapeutic delivery of cytotoxic moieties.

Tu527

ENDOCYTIC PATHWAYS OF TRANSPORTAN-MEDIATED PROTEIN CELLULAR DELIVERY

K. Padari[1], K. Koppel[1], M. Hansen[2], Ü. Langel[2], **M. Pooga**[1]

[1]Institute of Zooloogy and Hydrobiology, University of Tartu, Estonia [2]Department of Neurochemistry and Neurotoxicology, Stockholm University, Stockholm, Sweden

The ability of cell penetrating peptides (CPP) to deliver biologically active cargoes into different cell types has been successfully applied in several experimental systems. Despite the progress and growing number of described CPPs, reports about the internalization mechanisms and the intracellular routes of CPPs still remain controversial. We have characterized the membrane interaction and cellular localization of proteins delivered into HeLa cells by cell penetrating peptide transportan (TP) and its shorter analogue TP10 on ultrastructural level. Our previous results obtained by transmission electron microscopy showed that complexes of transportans with gold-labeled streptavidin translocated into cells inducing large invagination of plasmamembrane, suggesting the uptake by macropinocytosis. The complexes of transportan with gold-labeled neutravidin, in contrary, were taken into cells mostly via caveosomes and clathrin-coated vesicles. The cell-transduced transportan-protein complexes localized mainly in the vesicular structures of different size and morphology. Most of the complexes-containing vesicles in the perinuclear area contained also Lamp2 protein, marker of late endosomes and lysosomes. Still, the transportan-protein complexes were not confined in the membrane-surrounded vesicles, but spread in the cytosol suggesting the escape of transportan-protein complexes from endosomes.

Our findings show the involvement of different endocytic pathways in the transportan-mediated uptake process of proteins. The concentration of a CPP and the properties of cargo protein seem to determine the pathway for the cellular uptake of a particular construct.

ACTIN-DEPENDENT CPP-MEDIATED PROTEIN CELLULAR DELIVERY TAKES PLACE VIA INDUCED ENDOCYTOSIS

P. Säälik[1], M. Hansen[3], Ü. Langel[3], M. Pooga[2]

[1]Institute of Molecular and Cell Biology [2]Institute of Zoology and Hydrobiology, University of Tartu, Estonia [3]Department of Neurochemistry and Neurotoxicology, Stockholm University, Stockholm, Sweden

Studies in the field of cell penetrating peptides has shifted from the cellular uptake to finding methods for increasing the escape of endocytically entered peptides/cargos into cytoplasm. Identification of the utilized endocytic pathway might suggest methods for induction the endosomal escape. Therefore we mapped the type(s) of endocytosis exploited by transportan (TP) and TP10 in the protein delivery eukaryotic cells, by using a set of endocytosis inhibitors. Wortmannin, bafilomycin A1 or brefeldin A did not interfere with the internalization of TP/TP10-avidin complexes as estimated by FACS analysis. However, the vesicles with CPP-avidin complexes were more evenly distributed and not concentrated in perinuclear area in wortmannin-treated cells, suggesting that the translocation of early endosomes and continuity of endosomal pathway was interfered by wortmannin. Amiloride, the inhibitor of macropinocytosis decreased the peptide-mediated protein cellular delivery at very high concentrations only and could interfere with other uptake processes also. Depolymerization of the actin cytoskeleton with cytochalasin inhibited both TP- and TP10-mediated protein transport decreasing the uptake about 75-80% for both peptides. In parallel, a strong induction of peptide-mediated avidin delivery by Ca2+ and chloroquine was observed. To assess whether this induction leads to higher concentration of complexes located in cytosol or are the complexes still trapped in endosomal vesicles, a fractionation of cellular material was carried out.

M529

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW GNRH-III DERIVATIVES

I. Szabó[1], Sz. Bősze[1], J. Reményi[1], G. Schlosser[1], F. Hudecz[1,2], G. Mező[1]

[1]*Research Group of Peptide Chemistry, Hungarian Academy of Sciences*
[2]*Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary*

GnRH-III (Pyr-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH2) is a decapeptide hormone analogue which was isolated from sea lamprey. GnRH-III possesses antiproliferative activity on hormone-dependent tumors. The effect is developed by the signal transduction pathway after binding of the compound to the GnRH receptor. In order to monitor the binding and uptake by MCF-7 human breast carcinoma and C26-H mouse colon carcinoma cells by flow cytometer, fluorescent labelled GnRH-III derivative was prepared. 5(6)-carboxyfluorescein through an enzyme labile spacer (Flu-GFLGC-NH2) was attached to the chloroacetylated GnRH-III ((Pyr-His-Trp-Ser-His-Asp-Trp-Lys(ClAc)-Pro-Gly-NH2) by thioether bond formation. The results suggested that the hormone analogue was internalised by C26-H and MCF-7 cells depending on the incubation time. This finding provides a possibility to use GnRH-III as a targeting moiety for intracellular drug delivery. Therefore we have prepared methotrexate and doxorubicine conjugates of GnRH-III. The drug molecules were attached to the Lys side chain in position 8 of GnRH-III by thioether bond formation through the GFLG spacer elongated with Cys either at the C- or N-terminus. Since we found earlier that the dimer derivative of GnRH-III was more effective, new dimer derivatives with a combination of antitumour agents were also prepared. Branched GnRH-III derivative (PyrHWSHDWK(ClAc-GLFGC(Acm))PG-NH2]) was synthesised by SPPS. The drug molecules were attached to this compound by thioether bond and finally disulfide bridge was formed between two peptide chains. The cytotoxicity of new derivatives was characterised by MTT test.

Acknowledgement: This work was supported by grants from the Hungarian National Science Fund (OTKA T 032533, T 043576 and T 049814) and "Medichem 2" 1/A/005/2004.

SYNTHESIS OF NEW CYCLIC RGD PEPTIDES FOR SPECIFIC TUMOR CELL TARGETING

J. Schmidt[1], L. Rocheblave[2], J. Martinez[2], A Pèlegrin[1], F. Cavelier[2], **E. Vivès**[1]

[1]Immunociblage des Tumeurs et Ingénierie des Anticorps, INSERM-EMI0227, Centre Régional de Lutte Contre le Cancer Val D'Aurelle Paul Lamarque [2]Laboratoire des Aminoacides, Peptides et Protéines, UMR 5810 CNRS-UM1-UM2, Université Montpellier 2, Montpellier, France

RGD peptides (R = arginine; G = glycine; D = aspartic acid) have been found to promote cell adhesion upon interaction with alphaV-beta3 receptors, which are strongly overexpressed during neoangiogenesis by solid tumor associated cells compared to healthy cells. In this study we designed new targeting motifs aimed to deliver various antitumoral drugs specifically to cells involved in tumor vascularization. We inserted this short RGD sequence in tetracyclopeptides closed with various means. We expect these new cyclotetrapeptides to be more specific for the targeted receptor. Moreover, these new type of cyclic peptides were multimerized on different scaffold to further improve the receptor avidity. Our purpose is first to scrutinize and to quantify the efficient cellular uptake of these molecules and second, to address the specific cell targeting of a fluorescent cargo by differents tools such as Fluorescence Activated Cells Sorting (FACS) analysis or fluorescence microscopy. These new targeting units were evaluated on two different cell lines: Human Umbilical Vein Endothelial Cells (HUVEC) with an over expression of the alphaV-beta3 integrin receptor and A549 cells expressing a much lower level of this receptor. Preliminary results about the selectivity and the efficacy of these new targeting units will be presented.

Th531

OLIGOPEPTIDE ANTIFUNGALS ARE EXCEPTIONALLY ACTIVE AGAINST MULTIDRUG-RESISTANT YEAST

R. WakieĆ[1], J.W. Payne[2], J. Becker[3], S. Milewski[1]

[1]Department of Pharmaceutical Technology & Biochemistry, Gdańsk University of Technology, GdaŃsk, Poland [2]School of Biological Sciences, University of Wales, Bangor, UK [3]Department of Microbiology, University of Tennessee, Knoxville, TN, USA

Peptide permeases of human pathogenic fungi are able to bind and transport a range of oligopeptides incorporating non-proteinogenic amino acids. These broad substrate specificities of oligopeptide transporters make them particularly attractive carriers for the transport of antifungal agents into pathogen cells, according to the "warhead delivery" concept.

In our current studies we have shown that a number of oligopeptidic compounds, such as: peptide-nucleoside antibiotic nikkomycin, synthetic FMDP-peptides and oxalysyl-peptides, demonstrate a broad spectrum of antifungal activity against human pathogenic fungi, including the multidrug-resistant (MDR) strains. We have found that the presence of Cdr1p, Cdr2p or Mdr1p drug-effluxing proteins does not diminish the susceptibility of model recombinant S. cerevisiae and clinical isolates of C. albicans cells to the action of several peptidic antifungals. Moreover, most of the tested compounds demonstrate enhanced antifungal in vitro activity against MDR cells containing ATP-dependent (ABC) drug-exporting proteins. The higher growth-inhibitory activity of oligopeptide antifungals against MDR yeast is correlated with the increased rate of their intracellular accumulation mediated by peptide permeases. It has been found that the presence and activity of ABC-type drug exporters results in the increased proton gradient across the yeast cell membrane, thus providing an additional proton motive force for H+-dependent peptide permeases. Finally, the peptide antifungals are not extruded from yeast cells by Cdr1p, the major drug-effluxing membrane protein of C. albicans.

Our present findings indicate that the observed supersusceptibility of MDR yeast to oligopeptide antifungals is due to the enhanced uptake of these agents by the oligopeptide/H+ symporters.

PEPTIDE-MEDIATED DELIVERY OF SIRNA VIA COVALENT CONJUGATES

R.T. Witkowska, M. Ahmadian, L.J. Purvis, S. Roth, S. Ludmann, H. Wang,
J.W. Dattilo, K.W. Farber, S.J. Mayer, L. Chen, Y. Chen, K. Cui, M.E. Houston, P.H. Johnson, S.C. Quay

Nastech Pharmaceutical Company Inc., Bothell, WA, USA

Previous studies have demonstrated that longer siRNAs that are processed by Dicer can result in more potent knockdown than the corresponding standard 21-mer siRNAs. Dicer-substrate 25-27-mer siRNAs were conjugated with different structural classes of peptides and their cell uptake properties evaluated. Peptides were conjugated to the 5' end of the siRNA sense or antisense strand via a thioether bond under denaturing conditions to prevent aggregation and precipitation. The ability of conjugates to translocate fluorescently-labeled siRNA across the plasma membrane was evaluated by flow cytometry. The results indicate that some peptides can mediate higher efficiency uptake of siRNA into cells compared with Lipofectamine or cholesterol-conjugated siRNA. The peptide-siRNA formulation with 27-mer siRNA conjugates showed higher knockdown of TNF-alpha mRNA and protein levels in activated human monocytes in vitro compared to the conjugated 21-mer siRNA species. The products resulting from in vitro digestion of peptide-conjugated RNA duplexes with recombinant human Dicer were identified using ESI-MS and consisted predominantly of the desired 21-mer siRNA. We postulate that enhanced activity of the 27-mer Dicer conjugate may be a result of Dicer processing of the delivery peptide prior to RISC loading. For the 21-mer conjugate, lower activity may be the consequence of steric hindrance between the peptide and RISC.

NOVEL TUMOR-TARGETED PEPTIDE-CAMPTOTHECIN CONJUGATES: SYNTHESIS AND BIOLOGICAL EVALUATION

A. Dal Pozzo[1], M.H. Ni[1], E. Esposito[1], C. Pisano[2], L. Vesci[2], M. Castorina[2],
F. Bucci[2], C. Aulicino[2], M. Guglielmi[2], G. Giannini[2], D. Alloatti[2], S. Dallavalle[3], G.L. Beretta[4]

[1]Istituto di Ricerche Chimiche E Biochimiche G. Ronzoni [2]Research and Development, Sigma-Tau [3]DISMA, Università di Milano [4]Istituto dei Tumori di Milano, Milan, Italy

Several peptides containing the sequence Arg-Gly-Asp (RGD) were studied and developed for their nanomolar affinity to the membrane receptor alfa v beta3 and alfa v beta 5 integrins, which are over-expressed by endothelial cells during proliferation and by tumor cells. To improve the pharmacological profile of some Camptothecin derivatives (CPTs), five conjugates were designed, where the cytotoxic drugs were covalently attached to the RGD peptide analogues for preferential uptake into tumor cells. The peptides to be used have been selected among a series of new pentacyclic peptides bearing at 5-position a trifunctional pseudoamino acid with a carboxy-terminal side-chain. Peptide analogues showing the highest affinity to alfa v beta 3 and alfa v beta 5 integrins were coupled with CPTs at different positions. The conjugates have been optimized for binding to the receptors, proteolytic stability and an overall improvement in tumor selectivity. The nature of the linkage between RGDs and CPTs has a major impact on stability and biological activity of the conjugates. The conjugates with amide bond, but not those with ester bond, are sufficiently stable and show in vitro antitumor activity against A498 and A2780 cell lines. Experimental results in vitro suggest that the conjugates are taken up by the tumor cells studied through an active transport mechanism. Attachment of the cytotoxic drugs to the RGD peptide analogues is expected to increase the therapeutic index by limiting the side effects on normal tissues. In vivo experiments are in progress.

SYNTHESIS OF MODIFIED AMYLIN- AND ABETA-PEPTIDES TO DESIGN SELF-ASSEMBLED PEPTIDE-BASED BIONANOMATERIALS

R.C. Elgersma, D.T.S. Rijkers, R.M.J. Liskamp

Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Utrecht, The Netherlands

Amyloid fibrils are characterized by the (anti)parallel organization of beta-pleated sheets, which leads to a reduced solubility of the protein and to the formation of deposits of amyloid plaques. Amyloid formation in living organisms is a highly undesired process and is a (co)causative factor in several diseases e.g. Alzheimer's disease, Parkinson's disease and late onset diabetes. On the contrary, the driving force of amyloid formation can also be exploited in the design of self-assembled bionanomaterials, which find application in tissue engineering, as drug delivery systems and as hydro- or organogels. We have synthesized several backbone-modified amylin(20-29; SNNFGAILSS) derivatives in which N-alkyl amino acids, peptoids, alpha-hydroxy acids and (N-alkylated) beta-aminoethane sulfonyl moieties have been incorporated as amide bond isosteres. These amylin derivatives did not fold into amyloid fibrils but formed large (up to 10 microm in length) supramolecular assemblies such as helical ribbons and tapes, twisted lamellar sheets, and ribbons progressing to closed tubes, depending on the amide bond isostere. Furthermore, the highly amyloidogenic peptide Abeta(16-22) (KLVFFAE) was utilized to direct the self-assembly of a newly designed beta-hairpin mimic into shape specific nanoarchitectures. Depending on the pH, either broad linear non-twisted lamellar sheets or entangled fibrous assemblies were observed by electron microscopy. Striking differences in CD- and FTIR-spectra of the supramolecular assemblies imply that self-assembly of these Abeta-derivatives was based on different driving forces. Insight into these driving forces opens up new avenues for the design of tailor-made peptide-based bionanomaterials.

M535

PHOTO-INDUCED GEL-TO-SOL TRANSITION OF DNA NETWORK CROSS-LINKED WITH FULLERENE-CONJUGATED PEPTIDE ASSEMBLY

N. Higashi, Y. Mizuguchi, T. Koga

Department of Molecular Science & Technology, Doshisha University, Kyoanabe, Kyoto, Japan

Hydrogels are a class of materials integral to biotechnologies such as tissue engineering and drug delivery, some of which are environmentally responsive to temperature, pH, and light (photo). We report herein a new photo-responsive hydrogel consisting of DNA and peptide that is prepared via self-assembling process.

As a peptide, fullerene-tagged 16-mer sequential peptide, C60-(KL)8, has been designed. The fullerene moiety is known to have DNA cleaving ability in the presence of visible light and the lysine residues of (KL)8 segment can interact with phosphate groups of DNA electrostatically. C60-(KL)8 was prepared by using solid phase synthesis based on Fmoc chemistry.

Detailed analyses of the conformation and nanostructure of C60-(KL)8 were first of all performed by using CD, UV, DLS, AFM, and TEM measurements. Experimental results showed that C60-(KL)8 self-assembled spontaneously in water and formed versatile nanostructures with different size and/or morphology, depending on the conformation of (KL)8 segment. For instance, at an appropriate pH, C60-(KL)8 self-assembled into multi-layered, vesicular structure. Incorporation of this vesicle into aqueous DNA solutions resulted in hydrogel formation, in which the vesicle would serve as a cross-linker of DNA strand. The visible-light irradiation induced gel-to-sol transition, probably because of cleavage of DNA strand by singlet oxygen generated through interaction of photo-excited C60 aggregates with molecular oxygen. The cleaved fragments were detected by electrophoresis. These DNA/peptide networks linked with C60 is expected to have an application potential not only in fullerene technology but also biological and clinical fields.

THE PROTEIN CONCENTRATES FROM AMARANTH

J. Jelinek[1], K. Kolomaznik[2], **M. Jelinek**[3]

[1]*Technical University of Liberec, Faculty of Mechatronics and Interdisciplinary Studies, Measurement Department, The University of Hradec Kralove, Hradec Kralove*
[2]*Tomas Bata University in Zlin, Faculty of Applied Informatics, Zlin*
[3]*AMR AMARANTH A.S., Hradec Kralove, Czech Republic*

1. Amaranth grain contains high amount of protein (grain till 18%, young leaves till 28%) in comparison with common cereals (8, 5 - 12%). Amaranth protein is very rich in essential amino acids - lysine - (3 times higher then cereals and legumes) and gluten free. Combination of amaranth protein with other plant proteins (cereals) enables to formulate the composite protein (near to milk or beef protein, but exclusively on vegetal basis). It is shown on graphs. The aim of the projects' proposals is a development and realization of the technology for fractionation of amaranths defatted flour (or young biomass) to nutritionally important products, i.e. oil, protein concentrate, starch and fibres concentrate.
2. Projects observe basic aspect of separation. There are shown procedural diagrams and details about the pilot unit. Product is a top protein obtained by removing starches and next polysaccharides decomposed on soluble monosaccharide by specific enzymes. There are shown the chromatograph measuring. We can see complete disintegration of starch and the unchanged proteins. The separate solution monosaccharide is usable for others fermentative processes or as a nutrient solution for yeasts. We used the Measuring System for the Fermentative Process Study for developing of technologies.
3. There are description methods for isolation amaranth protein - extraction processes, enzymatic removal starch. The product is a isolate protein rich in essential amino acids. The waste monosaccharide solution was used to production yeasts biomass rich in proteins vitamins.
4. Amaranth plants are a perspective source of high quality proteins. Amaranth protein isolate have high nutritional value and can be used as food ingredients, for functional, probiotic formulation.

CELLULOSE PEPTIDE MICROARRAYS IN SERODIAGNOSIS OF PARVOVIRUS B19

J. Hepojoki[1], K. Hedman[2], N. Zander[3], A. Dickmans[4], L. Kaikkonen[2], R. Frank[3,4], **H. Lankinen**[1,2]

[1]Peptide and Protein Laboratory [2]Department of Virology, Haartman Institute, University of Helsinki, Finland [3]AIMS Scientific Products GmbH German [4]Research Center for Biotechnology, Braunschweig, Germany

Peptide microarrays are expected to enhance the value of peptide-based serodiagnostics. We here show that epitope mappings performed with cellulose peptide microarrays correspond to the previously identified parvovirus B19 Spot epitope mapping recognition patterns (1). To begin with, we made epitope mapping with the highly sensitive Spot array method (2) in order to study antigenic regions of parvovirus B19 VP1 and VP2 capsid proteins. Epitope mapping identified highly reactive, immunodominant early epitope on parvovirus surface that centered to KYVTGIN residues of VP1. In the subsequent phases we developed the KYVTGIN epitope type specific (ETS) IgG serodiagnostics. A correlation between enhancing IgG avidity to B19 capsid and a transient reactivity with the point-of-care KYVTGIN peptide was clear. Together the two assays enhanced the value of early diagnosis of B19 infections (3). Enzyme immunoassay (EIA) is the prevailing assay format in the majority of serodiagnostic applications and is commonly used to detect antibody responses to one protein or peptide antigen at a time. Peptide microarrays will allow parallel detection of antibody responses that may represent post-infection time points of early, late or past immunity. Thus, parvovirus serology is a good example of the type of new opportunities that peptide microarrays present for ETS serodiagnosis and development of dedicated microarray platforms.

1. Kaikkonen, L., Lankinen, H., Harjunpää, I., Hokynar, K., Soderlund, M., Oker-Blom, C., Hedman, L. & Hedman, K. Acute phase-specific heptapeptide epitope for parvovirus B19 diagnosis. J. Clin. Microbiol. 37: 3952-6, 1999

2. Frank, R. The SPOT-synthesis technique. Synthetic peptide arrays on membrane supports-principles and applications. Journal of Immunological Methods. 267: 13-26, 2002

3. Enders, M., Schalasta, G., Baisch, C., Weidner, A., Pukkila, L., Kaikkonen, L., Lankinen, H., Hedman, L., Söderlund-Venermo, M., Hedman, K. Human parvovirus B19 infection during pregnancy--value of modern molecular and serological diagnostics. J Clin Virol. 35: 400-6, 2006

M538

COVALENT CONJUGATION OF PEPTIDE EPITOPS OF VP1 PROTEIN OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) WITH MEMBRANE ACTIVE ANIONIC POLYELECTROLYTES

M. Mustafaev, Z. Mustafaeva, B. Mansour

Department of Bioengineering, Yildiz Technical University, Istanbul, Turkey

The use of peptide epitops of viruses particles in the composition of polymeric conjugates as vaccine has several potential advantages over whole viral or bacterial preparation.

Recently, we report a novel approach to a totally synthetic vaccine which consists of FMDV VP1 peptides, prepared by covalent conjugation of peptide biomolecules with membrane active carbochain PEs.

In the present study, peptide epitops of VP1 protein both 135-161(P1) amino acid residues (Ser-Lys-Tyr-Ser-Thr-Thr-Gly-Glu-Arg-Thr-Arg-Thr-Arg-Gly-Asp-Leu-Gly-Ala-Leu-Ala-ala-arg-Val-Ala-Thr-Gln-Leu-Pro-Ala) and triptophan(Trp) containing 135-161 amino acid residues (Trp-135-161)(P2) were synthesized by using the solid-phase methods. We investigated the mechanism for the formation of polymer-peptide and polymer-WSC- peptide complexes and covalent condensation reaction at different ratio of components (nPeptide/n Polymer), and pH value of solution by using different physicochemical analyses methods (HPLC;VİSCOTEK-with Ultra-viole Visible, Refractive Index, Light Scattering, and Viscosity Quadruple Detector Systems; Fluorescence Spectroscopy).

It was found that in general case the system PE-P1(P2) is characterized by a bimodal distribution of polymer and peptide components of mixtures on chromatograms. Peptide oligomers bind to polymer macromolecules by cooperative mechanism: all added peptide molecules is strongly bound by the polyanione and the existence of the free polymer chains in the system under these conditions unambiguously indicates a non-random distribution of the peptide molecules between the anionic polyelectrolytes. The dynamics of peptide-specific antibody formation induced by these conjugates was investigated.

Tu539

WATER-SOLUBLE POLYMERIC BIOCONJUGATES OF HEPATITIS B SURFACE POLYPEPTIDE ANTIGENS AND THEIR IMMUNOGENICITY

Z. Mustafaeva

Department of Bioengineering, Yildiz Technical University, Istanbul, Turkey

We have recently developed new approaches for obtaining highly immunogenic peptide conjugates: synthetic polyelectrolytes (PE) were used for the conjugation with peptide molecules in which PE carry out the carrier and adjuvant roles simultaneously.
In this study, 4 epitopes of antigenic parts of surface antigen of Hepatitis B virus (2-16, 22-35, 95-109 and 115-129 of the s gene.) had been synthesized.The synthesis of peptides was performed by Explorer PLS ® Automated Microwave Synthesis Workstation (CEM). Peptide conjugates of synthetic anionic polyelectrolytes (copolymers of acrylic asid and N-vinylpyrrolidone) were synthesized by carbodiimide condensation following the modification procedures described early. Composition and structure of bioconjugates were characterized by HPLC (Shimadzu), NanoSPR-3, Zetasizer Nano ZS, Steady State Fluorescence Spectrometer QM-4 and Viscotek TDA 302 size exclusion chromatography. It was obtained that a single immunization of mice with PE-peptide conjugates without classical adjuvant increased the primary and secondary peptide-specific immune response to HBsAg. Moreover, these conjugates possess own selectivity for recognizing the antibody in blood sera of hepatitis virus injected people.

Th540

CELL ADHESIVE PEPTIDE-CONJUGATED CHITOSAN MEMBRANE: AS A BIOMEDICAL BASEMENT MEMBRANE FOR TISSUE ENGINEERING

M. Nomizu[1], M. Mochizuki[1], Y. Kadoya[2]

[1]Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo [2]Kitasato University School of Medicine, Sagamihara, Japan

Tissue engineering requires delivery of transplanted cells to organ sites needing repair/regeneration. We have demonstrated that several active laminin peptide-conjugated chitosan membranes enhanced the biological activity and promoted cell adhesion in a cell-type specific manner. The most active laminin peptide (AG73: RKRLQVQLSIRT)-conjugated chitosan membrane could deliver keratinocytes to a wound bed. When human keratinocytes were seeded onto the AG73-chitosan membranes under serum-free condition, more than 70% of the cells attached within 2 hrs. The membranes carrying keratinocytes were stable enough for handling with forceps and were inverted onto the muscle fascia exposed on the trunk of nude mice. Keratinocyte sheets were observed after 3 days and colonies appeared after 7 days on the fascia of host mice. These cells were multilayered on day-3 and expressed various keratinocyte markers, including cytokeratin-1, involculin, and laminin ?2-chain. These results suggest that the AG73-conjugated chitosan membrane is useful as a therapeutic formulation and is applicable as a cell delivery system, such as delivering keratinocytes to the wound bed. The peptide-chitosan approach may be a powerful cell transplantation tool for various tissues and organs.

M541

IMPROVEMENT OF AUTOANTIBODY DETECTION IN AUTOIMMUNE DISEASES BY INNOVATIVE SOLID-PHASE GLYCOPEPTIDE BASED TECHNOLOGIES

F. Nuti[1,2], E. Peroni[1,2], E.S. Bulukin[3], B. Mulinacci[1,2], M. Chelli[1,2], P. Rovero[1,4], A.M. Papini[1,2]

[1]Laboratory of Peptide & Protein Chemistry & Biology [2]Department of Organic Chemistry "Ugo Schiff" and CNR-ICCOM [3]Department of Chemistry [4]Department of Pharmaceutical Sciences, Università Di Firenze, Sesto Fiorentino (FI), Italy

An ELISA diagnostic kit (MSPepKit), based on the glycopeptide CSF114(Glc) able to recognize specific autoantibodies in Multiple Sclerosis (MS) patients' sera has been developed by the Laboratory of Peptide & Protein Chemistry and Biology [1]. Detection of autoantibodies by synthetic peptide antigens in solid phase assays requires immobilisation of the post-translationally modified peptides as synthetic probes.
The aim of this study was to compare ELISA with different techniques to increase specificity and efficiency in autoantibody detection for early diagnosis and or prognosis of autoimmune diseases.
As alternative techniques we selected a biosensor technology based on surface plasmon resonance and Bio-Plex suspension array system, BioRad.
The biosensor technology and Bio-Plex suspension array system will offer advantages such as rapid analysis, and high sensitivity for a high throughput screening.
Immobilisation will be based on different strategies that are anchoring the synthetic antigen on different solid supports such as polystyrene well plates (ELISA), dextran coated gold chip (BIAcore), and carboxy-coated polystyrene beads (Bio-Plex).
Optimisation of the different techniques was performed with anti-CSF114(Glc) autoantibodies isolated using affinity chromatography from MS patients' sera. The analytical parameters such as specificity, sensitivity, and matrix effect were evaluated. The different technologies have been used for a high throughput screening of MS sera.

[1] (a) Lolli, F. et al. P.N.A.S. U.S.A. 2005, 102(29), 10273-10278; (b) Lolli, F. et al. J. Neuroimmunology 2005, 167,131-137; (c) Papini, A.M. Nat. Med. 2005 11, 13; (d) Papini, A.M. et al. Granted U.S.A. Patent & PCT Application WO 03/000733 A2.

BIODEGRADABLE BLOCK COPOLYMERS CARRYING CELL ADHESION PEPTIDE SEQUENCES

V. Proks, L. Machova, F. Rypacek

Institute of Macromolecular Chemistry, Academy of Sciences of The Czech Republic, Prague, Czech Republic

Biodegradable aliphatic polyesters obtained by polymerisation of lactones, such as polylactide (PLA), polyglycolide (PGA), ε-polycaprolactone (PCL) are often used for three-dimensional porous scaffolds in tissue engineering. Bioactive surfaces of these biomaterials can be prepared by surface deposition of amphiphilic block copolymers1. Important biomimetic features can be introduced by using copolymers containing fibronectin-and laminin-derived peptide sequences (e.g.,GRGDSG, PHSRN, REDVDY,SIKVAVS, YIGSR, etc.) which control specific cell adhesion.2 Here we discuss the route for preparation of amphiphilic block copolymers composed of hydrophobic polylactide and hydrophilic polyethylene oxide (PEO) blocks, carrying various cell-adhesion oligopeptide sequences at the end of PEO block.

Fully protected peptide fragments were prepared by solid-phase peptide synthesis by using Fmoc strategy and chlortrityl resin. The side-chain protected peptides were cleaved from resin by 25% HFIP solution in DCM. The copolymers peptide-polytehyleneoxide were prepared by coupling of the activated peptide fragments with α-amino-ω-hydroxy-PEO in DMF using PyPOP as an activation reagent. Subsequently, the polylactide block was grafted to the ω-hydroxy end group of the peptide-PEO copolymer via a controlled ROP polymerisation of lactide. The deprotection of the peptide side-chains provided a functional peptide-block-PEO-block-PLA copolymer.

NEW SYNTHETIC APPROACHES OF POLYPROLINE-BASED DENDRIMERS

A.G. Torres[1], E. Giralt[2,3], F. Albericio[2,3], M. Royo[1]

[1]Combinatorial Chemistry Unit [2]Biomedicine Rearch Institute, Barcelona Science Park - University of Barcelona [3]Department of Organic Chemistry, University of Barcelona, Spain

The development of new biopolymer materials as drug delivery systems is of enormous interest on biomedicine. Dendrimers are polymers with particular properties; they are highly branched polymers with well-defined chemical composition, and show compact globular shape, monodisperse size and controllable surface functionalities. Peptide dendrimers incorporates amino acids in their structures and have additional features such as biocompatibility and biodegradability.

In previous studies we described the solid-phase synthesis of a new class of polyproline-based dendrimers. These biopolymers have the capacity to cross the mammalian cell membrane and moderate toxicity. These promising results open up the possibility to explore these dendrimers as delivery systems.

To design more versatile polyproline dendrimers, we have developed a methodology that involves the combination of solid-phase and solution strategies. Diverse multivalent PEG- and proline-based cores were synthesized to attain dendrimers with distinct topologies. Dendrimers were synthesized by iterative building block addition [(GlyPro5)2ImdOH], around an inner core, using a peptide solution convergent approach. A variety of coupling methodologies and protecting groups for the N-terminal function were explored.

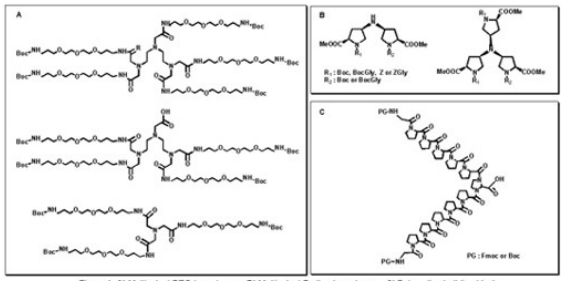


Figure 1. **A)** Multivalent PEG-based cores, **B)** Multivalent Proline-based cores, **C)** Polyproline building block.

M544

SYNTHESIS AND RADIOLABELLING OF NEW ENDOMORPHIN ANALOGUES CONTAINING UNNATURAL ALFA AND BETA AMINO ACIDS

G. Tóth[1], A. Keresztes[1], E. Szemenyei[1], E. Kató[2], A.Z. Rónai[2], F. Fülöp[3]

[1]Institute of Biochemistry, Biological Research Center of Hungarian Academy of Sciences, Szeged [2]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Faculty of Medicine, Budapest [3]Institute of Pharmaceutical Chemistry, University of Szeged, Hungary

New endomorphin analogues containing 2',6'dimethyltyrosine (Dmt), (1S,2R)-2-aminocyclopentanecarboxylic acid (Acpc) and beta-methylphenylalanine (beta-MePhe) were designed and synthesized to obtain more potent and selective mu-opioid receptor ligands with higher stability against proteolytic enzymes. We have prepared the peptides by SPPS methods using racemic amino acids. The diastereomeric peptides were separated by HPLC. The configuration of the unnatural amino acids in the peptides was determined by chiral TLC using enantiomeric standards. Radioligand binding assays and in vitro GPI and MVD assays indicated that several analogues showed high, subnanomol affinity and high selectivity for mu-opioid receptors having agonist or antagonist properties. The incorporation of alicyclic amino acids into the endomorphins resulted in enzyme resistant peptides. The most promishing analogues (Dmt-Pro-Phe-Phe-NH2 and Tyr-(1S,2R)Acpc-Phe-Phe-NH2) were labeled with tritium using precursor peptides containing dehydroproline or dehydro-(1S,2R)Acpc amino acids and tritium gas and Pd/BaSO4 catalyst. The novel peptides and their radiolabelled analogues with high specific radioactivity (1.4-2.8 TBq/mmmol) have become useful pharmacological and biochemical tools for the opioid research.

This work was supported by OTKA T 46514, OTKA TS 049817 and RET 08/2004 grants.

FORMULATION OF AN INJECTABLE IMPLANT FOR PEPTIDE DELIVERY

R. Astaneh[1], H.M. Moghimi[1], M. Erfan[1], H. Mobedi[2]

[1]School of Pharmacy, Shaheed Beheshti University of Medical Sciences [2]Iran Polymer and Petrochemical Institute, Tehran, Iran

Background and aims: Injectable drug delivery based on polymer solution platforms has gained in resent years,
Particulary for protein-based therapies. The influence of polymer molecular weight (RG 502H, RG504H) on the morphology, erosion of matrices and also on their in-vitro drug release behavior over a period of 28 days was assessed for leuprolide acetate in this study.
Methods: Each formulation was composed of 33% (w/w) polymer and 3% (w/w) leuprolide acetate dissolved in NMP. Release studies were performed in a home-made diffusion cell at 37°C. The polymer erosion was studied using two different methods as follows. (a): L-lactic acid detection (b): pH change study. The morphology of the matrices was then analyzed by scanning electron microscope (Cambridge S360) after gold coating of sample.

Results and discussion: Figure1 shows scanning electron micrographs (SEM) of surface of two solidified formulations. As is shown, the lower molecular weight polymer formulation shows higher porosity and pore diameter due to a rapid phase inversion.

As is seen in Figure 2, the release profile after the burst phase (phase I) can be divided into three more phases with different release rates. Results showed that burst effect
for RG 502H, 32%, was significantly ($P<0.05$) higher than RG 504H (13%). The pH drop behavior, which is related to both lactic and glycolic acid monomer, and lactic acid release profile are in good agreement with drug release profile as well.

Figure 1. Surface morphologies (a) RG502H, (b) RG504H

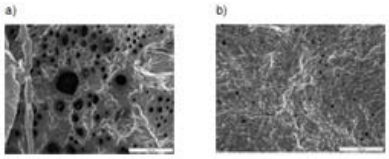
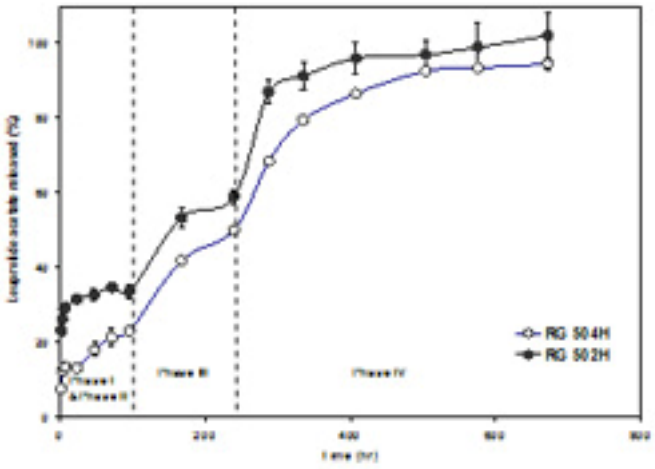


Figure 2. Release of leuprolide acetate from RG 502H RG 504H



PEPTIDIC FLUORESCENT NANOCRYSTALS AS PROBES FOR LIPID OR BIOLOGICAL MEMBRANES

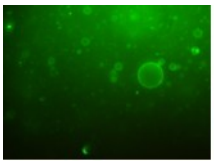
M. Baudy-Floc'h[1], A. Dif[1], P. Bauchat[1], C. Luccardini[2], M. Dahan[2], V. Marchi-Artzner[1]

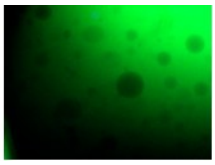
[1]UMR CNRS 6226, Université de Rennes 1, Rennes [2]Departement Physique, Ecole Normale Supèrieure, Paris, France

The fluorescent semi-conductive (CdSe/ZnS) nanocristals possess very attractive optical properties that could be used for tracking individually biological receptors in vivo. Our aim is to design functionalized water-soluble semi-conductive nanocristals (or quantum dots) that interact selectively with lipidic or biological membranes. To valid our approach, the interaction between the decorated QD and giant vesicles were observed by optical fluorescent and dark field microscopies.

In view to solubilize and selectively bind fluorescent nanocristals to a lipid membrane, heterobifunctionalized peptidic ligands (LiIPe) that presented an adhesion domain for the nanocristal surface, an hydrophilic spacer and a terminal recognition function, were synthetized. The colloïdal stability of the water-soluble nanocristals (NC-LiPe) was checked by dynamic light scattering, optical and electron microscopies

The interaction of grafted nanocristals (NC-LiPe) with positive or neutral giant vesicles was observed by optical fluorescence and dark field microscopies. As shown in figure, negatively charged nanocristals (NC-LiPe) selectively adsorbed onto the surface of positively charged giant vesicles without altering the morphology of the vesicle. The nanocristals appeared as fluorescent patches growing on the surface of the vesicle until completely recovering. Therefore these ligands (LiPe) permitted to chemically functionalize the nanocristals by keeping their colloïdal stability and their fluorescence in water. Furthermore it was possible to selectively label vesicle membrane.





M547

PHOTOCURRENT GENERATION BY SELF-ASSEMBLED PEPTIDE MONOLAYERS ON INTERDIGITATED GOLD MICROELECTRODES

E. Gatto[1], M. Venanzi[1], L. Stella[1], L. Lorenzelli[2], **F. Formaggio**[3], C. Toniolo[3], G. Marletta[4]

[1]Department of Chemical Sciences and Technologies, University of Tor Vergata, Rome [2]ITC-Irst Microsystem Division, Trento [3]Department of Chemistry, University of Padova [4]Department of Chemistry, University of Catania, Italy

Fabrication of photocurrent-generating systems based on bioinspired organic-inorganic hybrid materials is currently of great interest. More specifically, the photoelectronic properties of nanometric films formed by peptide self-assembled monolayers have been actively investigated. In this work interdigitated gold microelectrodes were modified by covalently linking a hexapeptide ester functionalized by a lipoic acid (Lipo) at the N-terminus. The peptide chain [Lipo-(Aib)4-Trp-Aib-OtBu] comprises five α-aminoisobutyric acid (Aib) residues and one Trp, a fluorescent amino acid with strong absorptions in the UV region. Due to the very high percentage of conformationally constrained Aib residues in the chain, the peptide adopts a rigid 3-10-helical structure. Cyclic voltammetry measurements indicate that the peptide forms a homogeneously and densely packed monolayer on the gold surface, while current/voltage curves exhibit interesting rectifying properties of the peptide SAM. Photocurrent generation experiments, performed on the peptide-layered microelectrode, show peculiar modifications of the spectrum. At 240 nm a notably higher photocurrent/voltage response was observed for the peptide-modified electrode, suggesting that a photoinduced electron transfer process from Trp to gold does take place with high efficiency. This conclusion was confirmed by a comparison of the electric and photoelectric conduction properties of a undecathiol SAM on the same microelectrodes. The role of the peptide chain in mediating the electron transfer process was also investigated by determining the influence of the secondary structure on the efficiency of the photocurrent generation.

IMMOBILISATION OF ALDO/KETO REDUCTASES BY PROTEIN LIGATION STRATEGIES

K. Holland-Nell, A.G. Beck-Sickinger

Institute of Biochemstry, University of Leipzig, Germany

New strategies for the specific enzyme immobilisation play a key role in the development of biosensors and in the emerging research field of bioelectronics today. The major drawback of common immobilisation methods is the lack of any specific site directed position of the enzyme on a surface. This may lead to randomly orientated enzymes and subsequently limited activity. The aim of this work is to selectively activate enzymes at their C-terminal position in order to allow specific immobilisation. We chose AKR1A1, an enzyme of the aldo/keto reductase superfamily, for the synthesis of an artificially monolabeled redoxprotein. AKR1A1 is a monomeric enzyme and catalyzes the NADPH dependent reduction of aliphatic/aromatic ketones and aldehydes. To produce monofunctionalized enzymes we applied the strategy of Expressed Protein Ligation (EPL). Accordingly, we used the IMPACT®-system and cloned the aldo/keto-reductase as fusion protein with an additional intein/chitin binding domain. Through intein mediated splicing we could produce C-terminal thioester of the AKR1A1. In the next step, the thioester was coupled to a biotin containing peptide by native chemical ligation. This specifically modified enzyme was immobilised on avidin coated surfaces. The attachment on the surface was tested by tryptic digestion, followed by MALDI-TOF-MS analysis. The enzyme activity was proven to be intact as shown by the determination of the Michaelis-Menten constant within a photometric assay.

SOLID PHASE PEPTIDE SYNTHESIS USING NANOPARTICULATE AMINO ACIDS IN WATER

K. Hojo, H. Ichikawa, M. Maeda, Y. Fukumori, **K. Kawasaki**

Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Nishi-Ku, Kobe, Japan

Solid phase peptide synthesis has many advantages compared with solution peptide synthesis. However this procedure requires a large amount of organic solvents. Since safe organic solvent waste disposal is an important environmental problem, we aimed to perform peptide synthesis in water. We have reported solid phase peptide synthesis in water using water-soluble N-protected amino acids, such as 2-[phenyl(methyl)sulfonio]ethoxycarbonyl and 2-(4-sulfophenylsulfonyl)-ethoxycarbonyl amino acids. Following to study on water-soluble N-protected amino acids, we developed a new technology based on nanochemistry for solid phase peptide synthesis in water. The new technology is based on coupling reaction of suspended nanoparticle reactants in water. Fmoc-amino acids are used widely in peptide synthesis, but most of them show poor water-solubility. We prepared well-dispersible Fmoc-amino acid nanoparticles in water by pulverization using a planetary ball mill in the presence of poly(ethylene glycol) (PEG). The size of Fmoc-amino acid particles was 300-500 nm. To evaluate the utility of this technique, Leu-enkephalin was prepared using the nanoparticulate Fmoc-amino acids on a PEG-grafted Rink amide resin in water. Coupling reactions were performed with a water-soluble carbodiimide in the presence of N-hydroxy-5-norbornene-2,3-dicarboximide. The synthesis was successful and this methodology will be useful for "environment-friendly synthesis" in the future.

M550

NANOSTRUCTURES FORMED FROM LIPIDATED OLIGOPEPTIDES IMMOBILIZED ON CELLULOSE AS COMBINATORIAL ARTIFICIAL RECEPTORS ARRAYS

J. Kolesinska[1], B. Kolesinska[1], S. Wysocki[2], Z.J. Kaminski[1]

[1]Institute of Organic Chemistry [2]Institute of General Food Chemistry, ŁÓdź University of Technology, ŁÓdź, Poland

Supramolecular structures formed from N-lipidated oligopeptides immobilized in the regular pattern on the cellulose surface are able to bind ligand molecule, thus acting like artificial receptors. Due to the conformational flexibility of lipidated oligopeptide chains, the supramolecular structure is highly flexible, forming the cavities with the shape and prosperities adjusted most effectively to requirements of the guest molecule.

Structural requirements for a peptide providing the most efficient fit the guest molecules are not known, therefore an array of the artificial receptors have been synthesized and used in the studies. Thus, even in the case, when the single receptor in an array does not necessarily have selectivity for a particular analyte, the combined fingerprint response can be extracted as a diagnostic pattern visually, or using chemometric tools.

In the previous studies we found, that interactions of any colorless guest or the multi-component analyte with an array could be visualized by the processes involving competitive adsorption of appropriate dye [1].

In order to improve the sensitivity of the competitive binding and to study the mechanism of molecular recognition, experiments involving fluoresceine and fluoresceine marked ACP fragment were performed.

We found that λmax and intensivity of fluorescence depends on the structure of the peptide motif and lipidic fragment of receptor. This confirmed, that a binding process depends on the length of fatty acid chain, the size of the receptor pocket, and the orientation of fluoresceine inside the receptor.

[1] Kamiński, Z.J.; Kolesińska, J.; Kolesińska. B., Acta Pol. Pharm., 61, 48-51 (2004).

THE EFFECTS OF APX MIMICS IN CARDIAC MUSCLE CELLS

Y. Shan, Y. Yan, L. Wang, J. Hu, X. Fang, **W. Li**

College of Life Science, Jilin University, Changchun, Jilin Province, China

We have previously synthesized and shown an ascorbate peroxidase (APX) mimics, deuterohemin Histidine peptide-6 (DhHP-6) [1] was able to clear reactive oxygen species (ROS) in mitochondria (MT), had anti-cataract effect. In this study, a biotinylated signal peptide, mitochondria targeting sequence (MTS)[2], was designed and synthesized by solid phase peptide synthesis (SPPS). This MTS was linked with DhHP-6 by disulfide bond, and the new molecular was named MTS~DhHP-6. The peroxidase activity of MTS~DhHP-6 (2.1x103 U•μmol-1) was tested and similar to that of MP-11 (4.2x103 U•μmol-1). MTS~DhHP-6 coated with quantum dots (QDs) [3] were observed to accumulate into Neonatal rat cardiomyocytes (NRCMs) of Wistar rats and co-localized with MitoTracker Red in MT. These results suggest that MTS~DhHP-6 is an excellent APX mimics and may have potential clinical use in the protection and therapy of the diseases caused by free radical damage.

References

1. Liping Wang, Yali Liu, Hui Yang, Wei Li, Chemical Journal of Chinese Universities, 2004, 11(25): 2171-2173.
2. Lisa M. DiPilato, Xiaodong Cheng, and Jin Zhang, Proc Natl Acad Sci U S A., 2004; 101(47): 16513–16518.
3. Yaming Shan, Wei Cui, Liping Wang and Wei Li, Proceedings of the Twenty-Eighth European Peptide Symposium, Kenes International, Israel, 2005, 551.

FURTHER DEVELOPMENT OF PRACTICAL PRODUCTION SYSTEM FOR LABELED PEPTIDE ARRAYS FOCUSING ON HIGH THROUGHPUT PROTEIN DETECTION

K. Nokihara[1], T. Ohyama[1], H. Kawakami[1], N. Kawahira[1], Y. Kodama[1], N. Miyazato[1], N. Ono[1], Y. Oka[2]

[1]HiPep Laboratories, Kyoto [2]Nippon Light Metal Company, Ltd., Shizuoka, Japan

The novel high throughput protein detection system using designed peptide arrays has been successfully indicated on their capabilities as the "protein-chip" [1-4]. Our concept has many advantages especially for high-quality industrial production and practical applications compared to arrays with antibodies or recombinant proteins. The deposited peptide solution can be dried without covalent immobilization, although, when the resulted arrays are exposed in protein-solution they showed planned conformation [5]. Based on these basic results, several hundreds of α-helical, β-loop and β-sheet peptides, which involved a cysteinyl residue for covalent immobilization and TAMRA as a fluorescent label, were successfully synthesized, characterized and used as capture molecules. A novel material for chips made from amorphous carbon suitable for our concept has been developed, which has significant advantages over conventional glass or polymer plates, such as no self-fluorescence, mechanically more stable, easy manufacturing in the aspects of precised and high throughput processing. Thus, chip-plate with nano-L wells could also be easily manufactured. Peptides were deposited on these chips surface covalently as well as non-covalently in 350 picoL/spot (diameter: ca 200 μm). The resulted chips were used for protein detection. A part of this work was funded by the Okinawa-Bio-Project and NEDO-Grant.

References: 1. Takahashi M., et al., Chemistry and Biology, 10, 53 (2003); 2. Usui K., et al., Biopolymers, 76, 129 (2004) ; 3. Usui K., et al., Molecular Diversity, 8, 209 (2004) ; 4. Nokihara, K., et al., Kobunsi Ronbunshu, 61, 523 (2004) ; 5. Usui, K., et al., Molecular BioSystems, 2, 113 (2006).

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NANOPARTICLES FROM ELASTIN-BASED POLYMERS AS DRUG RELEASE DEVICES

K. Okamoto[1,2], A. Doi[2], S. Uehara[2], I. Maeda[1,2], M. Furuta[3]

[1]Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, Iizuka [2]Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu [3]Research Institute of Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka, Japan

Elastin is the core protein of the elastic fibers which furnish the resilience for the elastic tissues such as arterial walls, ligaments, lungs, skin, etc. Elastin-based polypeptide, poly(Val-Pro-Gly-Val-Gly), undergoes self-assembly called coacervation, in which
microcoacervate droplets with approximately 1000 nm diameters are formed [1]. Nanoparticles cross-linked by cobalt-60 γ-irradiation of these microcoacervate droplets are useful as drug release devices. To investigate the size optimization of nanoparticles, the stability of nanoparticles in the treatment of enzyme, and the drug release profiles from nanoparticles, the three copolymers; poly[10(Val-Pro-Gly-Val-Gly), (Val-Ala-Pro-Gly-Val-Gly)], poly[4(Val-Pro-Gly-Val-Gly), (Val-Pro-Gly-Phe-Gly), and poly[8(Val-Pro-Gly-Val-Gly), 2(Val-Pro-Gly-Phe-Gly), (Val-Ala-Pro-Gly-Val-Gly)] were synthesized and cross-linked with cobalt-60 γ-irradiation in coacervate state. As results, the sizes of nanoparticles obtained were small at low temperature and large at high temperature, showing that the swelling and shrinking of nanoparticles are temperature-dependent. The microcoacervate droplets of these copolymers before γ-irradiation were susceptible to elastase digestion, while the nanoparticles of these copolymers after γ-irradiation were resistant to elastase digestion. The release profiles of anti-tumor drug, adriamycin, from nanoparticles appeared to be diffusible.

Ref.

1. Kaibara, K., Okamoto,K. and Miyakawa,K. in Handbook of Polyelectrolytes and Their Applications, Volume 3: Application of Polyelectrolytes and Theoretical Models (S.K. Tripathy, J. Kumar and H. S. Nalwa, Eds). American Scientific Publishers, pp143-194 (2002).

ELECTRON TRANSFER TO FLAT PEPTIDES

C. Toniolo[1], M. Crisma[1], C. Peggion[1], F. Polo[1], S. Antonello[1], F. Maran[1],
R. Improta[2], V. Barone[2], Y. Tsuji[3], Y. Yamaguchi[3], T. Wakamiya[3]

[1]Department of Chemistry, University of Padova, Padova [2]Department of Chemistry, University of Naples, Italy [3]Faculty of Science and Technology, Kinki University, Osaka, Japan

We have already reported the synthesis and chemical characterization of a set of Nα-para-cyanobenzoylated (pCNBz) α,β-didehydroalanine (ΔAla) homo-peptide methyl esters from monomer to pentamer. In the present study we first examined their 3D-structural propensities by use of FT-IR absorption, NMR, and X-ray diffraction techniques. All oligomers are found in a completely flat, highly intramolecularly H-bonded, fully extended (multiple C-5) conformation.
A major outcome of our subsequent cyclic voltammetry study is that the initial one-electron uptake by all ΔAla homo-oligomers occurs at the same potential. This value is significantly less negative than that measured for the (Aib)n peptide spacers. We believe this is due to a different delocalization of the LUMO (and SOMO). While for the (Aib)n spacers the LUMO is localized on the redox moiety, for the (ΔAla)n peptides the LUMO expands onto the diffuse π-system offered by the N-terminal residue. Interestingly, the reduction potential of the pCNBz-NH- moiety is unaffected by peptide main-chain lengthening. We conclude that the peptide bridge does not include a stabilization of the LUMO of the donor through a cooperative increase of the strength of the intramolecular H-bonds, as previously observed for the (Aib)n spacers. In agreement with the electrochemistry data, the results of our theoretical study show that the SOMO delocalization only involves the C=C bond of the N-terminal ΔAla unit and that the orbital shape and energy are independent of peptide length.