L001

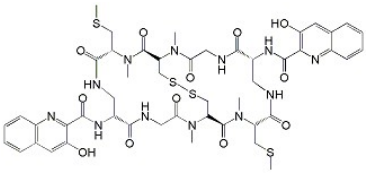
A SYMPHONY OF COUPLING REAGENTS FOR SOLID-PHASE SYNTHESIS OF AZATHIOCORALINE

F. Albericio[1], N. Bayó[1], A. Fernández[1], J. Tulla-Puche[1], E. Riego[1], C. Cuevas[2], E. Giralt[1], M. Álvarez[1]

[1]*Institute for Research in Biomedicals, Barcelona Science Park, University of Barcelona*
[2]*PharmaMar SAU, Colmenar Viejo, Spain*

Successful peptide synthesis is attained through proper management of the protecting groups and the concourse of the appropriate coupling reagents. Here we describe two optimized solid-phase (SP) strategies for the preparation of azathiocoraline, which contains a bicyclic symmetric structure; a moiety of 3-hydroxyquinaldic acid as DNA intercalator; a disulfide bridge; and the presence of several N-methylamino acids (NMAs). These aproaches overcome the problems caused by the presence of consecutive NMAs.

A key cornerstone of the syntheses is the concourse of orthogonal protecting groups and an arsenal of coupling facilitators. Thus, aminium and phosphonium salts were used in our SP strategies. While HATU was used for the stepwise elongation of the peptidic chain to assure complete acylation of NMAs, PyAOP was preferred for fragment condensation because it does not provoke capping of the amino function. For SP cyclization, HOAt gave better results that HOBt as an additive of DIPCDI. The cyclization and incorporation of 3-hydroxyquinaldic acid was performed in solution with EDC·HCl to allow easy work-up. While HOAt was the best additive for cyclization, the less reactive HOSu was preferred for the final acylation because it prevents over-incorporation of the carboxylic acid. These state-of-the-art strategies are valid for a broad range of thiocoraline analogues and will contribute to the discovery of new compounds with therapeutic applications.



L002

HOMOCHIRAL PEPTIDES FROM RACEMIC MONOMERS OF ACTIVATED AMINO-ACIDS. RELEVANCE TO BIO-CHIROGENESIS

G.J. Nery[1], R. Eliash[1], I. Rubinstein[1], G. Bolbach[2], I. Weissbuch[1], **M. Lahav**[1]

[1]*Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel*
[2]*Spectrométrie de Masse et Protéomique, Université Pierre Etmarie Curie, Paris, France*

One of the unsolved riddles in the field of origin of life is the emergence of the natural enantiopure bio-polymers of homochiral sequences from racemic precursors in pre-biotic chemistry. Polymerization of racemic monomers in an ideal environments yield generally peptides composed from repeating units of both handedness, randomly arranged. The aim of the present project is to design synthetic methods for the generation of homochiral peptides from racemic precursors.

Racemic monomers of activated amino acids where the L-enantiomer is labeled with deuterium atoms were polymerized in an aqueous environment to yield oligopeptides. The distribution of the oligopeptides and their sequences were analyzed by Maldi-Tof and Maldi-Tof-Tof mass spectrometry. Homochiral peptides were obtained from racemic precursors by the self assembly of the latter into crystalline arrays either at the air/water interface itself or within a membrane-like environment, followed by a lattice controlled polymerization taking place between homochiral molecules within the crystallites. These reactions were successfully extended for the generation of chiral peptides of Phe and Val via reactivity of 3D crystals suspended in water. More recently experimental conditions were invented for the generation of homochiral peptides via the polymerization of racemic monomers in aqueous solutions.

We demonstrate the feasibility to generate homochiral peptides from racemates, at conditions that might have been present at pre-biotic times.

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L003

HOW TO MAKE PEPTIDE MIMETICS

P.J. Cassidy[1,2], P.F. Alewood[1]

[1]Institute for Molecular Bioscience, University of Queensland [2]Mimetica Pty Ltd., Brisbane, QLD, Australia

The idea of making scaffold constrained peptide mimetics has been with us for more than 27 years. In that time a large amount of effort has been expended to make drug-like mimetics of peptide hormones. The results of this effort have not been impressive. Why?

In our work on peptide turn mimetics we sought to develop syntheses based on the following principles:
- the peptide side-chains at all positions are included
- the synthetic approach is general for different side-chains
- chirality is retained and controlled
- relative positioning of the side-chains is maintained.

Largely from synthetic expediency these principles have rarely been followed to-date. This factor may have contributed to the poor record achieved in bringing scaffold based peptide mimetics into the clinic, let alone to market.

We have developed methods that satisfy these principles. Our chemistry can produce tripeptide mimetics with a wide range of side-chains based on a 1,4-diazacycloheptane scaffold. These mimetics contain up to four chiral centres and our methods allow for the specific synthesis of all sixteen possible diastereomers. In addition we have demonstrated that the ability to control chirality is highly valuable in the process of optimising the biological activity and selectivity of mimetics. Application of the methods to hormone mimetics have produced compounds with activity at a variety of peptide receptors, some with low nanomolar affinity. Our chemistry will be described with a particular emphasis on the usefulness of chiral control in activity optimisation.

L004

ASSEMBLING OF THE EXTRACELLULAR CRF1 RECEPTOR DOMAINS BY MEANS OF CHEMICAL AND ENZYMATIC LIGATIONS

M. Beyermann[1], S. Pritz[1], J. Klose[1], S. Rothemund[2], A. Klose[1], K Fechner[1], E. Krause[1], M. Bienert[1]

[1]*Peptide Chemistry/Biochemistry, Leibniz-Institut Für Molekulare Pharmakologie, Berlin*
[2]*Interdisziplinaeres Zentrum Fuer Klinische Forschung, Universitaet Leipzig, Germany*

Ligand binding is the initial step of stimulation for G protein-coupled receptors (GPCRs), transmitting signals from the extracellular environment to the interior of the cell. Understanding how ligands bind to GPCRs at the molecular level is hampered by a lack of appropriate proteins for high resolution structural analysis, and the search for new ligands is limited by the need of cellular systems which exhibit appropriate receptors. Extracellular receptor domains (ECDs) play often a major role for ligand binding and can be, therefore, interesting tools to screening potential ligands and obtain first structural information on ligand binding mechanisms. Whilst single ECDs are easily available, chemically or by expression in E. coli, a construct of the four ECDs of a 7tm-GPCR has not been achieved yet. Here, we report on the first semi-synthesis of such a 23 kD-construct, consisting of ECD1-4 of the 7tm-GPCR Corticotropin-Releasing Factor receptor 1. The assembly was accomplished by the combination of protein expression in E. coli (ECD1), peptide synthesis (ECD 2, 3, 4), and chemical (loops to template) as well as enzymatic (ECD1 to template) ligations. This construct showed specific, nanomolar binding for natural receptor ligands and will be examined regarding its suitability for ligand search in future.

L005

"CLICK PEPTIDE": A NOVEL "O-ACYL ISOPEPTIDE METHOD" FOR PEPTIDE SYNTHESIS AND CHEMICAL BIOLOGY-ORIENTED SYNTHESIS OF ALZHEIMER'S DISEASE-RELATED Aß ANALOGUES

Y. Sohma[1], A. Taniguchi[1], Y. Chiyomori[1], F. Fukao[1], T. Yoshiya[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 [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] A clear understanding of pathological function of amyloid ß peptide (Aß) 1–42, a currently unexplained process, would be of great significance in the discovery of novel drug targets against Alzheimer's disease (AD).

[Methods] Based on our background regarding the "O-acyl isopeptide method" for peptide synthesis (Chem. Commun. 2004, 124; Tetrahedron Lett. 2004, 45, 5965; Biopolymers 2004, 76, 344; J. Peptide Sci. 2005, 11, 441; Bioorg. Med. Chem. 2005, 13, 6167; J. Am. Chem. Soc. 2006, 128, 696), we have developed a novel "Click Peptide" of Aß1–42, in which a native Gly25-Ser26 amide bond in Aß1–42 was isomerized to the ß-ester bond.

[Results] The "Click Peptide" did not exhibit the self-assembling nature under physiological conditions due to one single ester, and could migrate to the original Aß1–42 with a quick and one-way conversion reaction (so-called "click") via an O–N intramolecular acyl migration.

[Conclusions] Currently, the difficulties in handling Aß1–42, as a result of its highly aggregative nature, hamper the progress of Aß1–42-related AD research. The "Click Peptide" method would open doors for the investigation of the biological functions of Aß1–42 in AD.

L006

SEMISYNTHESIS OF CAGED PHOSPHORYLATED AND FLUORESCENTLY LABELED STAT6 PROTEINS

S. Lahiri, R. Seidel, M. Engelhard, **C.F. Becker**

Max-Planck-Institut Für Molekulare Physiologie, Dortmund, Germany

Signal transducer and activators of transcription (STAT) proteins comprise a family of transcription factors that reside in the cytoplasm. Upon phosphorylation they form homo- and heterodimers that are transported into the nucleus. At this stage the STAT proteins bind to DNA promoter regions and induce expression of specific genes. This signal transduction pathway plays a key role in case of viral infections, inflammatory processes or cancer[1]. Our aim is to study DNA binding of STAT6 in vitro and its localization on the cellular level.

This is achieved by establishing semisynthetic access to STAT6 variants that carry a caged phosphor-tyrosine residue and/or a fluorescent label. A variety of phosphorylated and fluorescently labeled peptides comprising amino acids 635 to 661 of STAT6 with an additional His6-tag were synthesized by Fmoc-based SPPS. These peptides were linked to STAT6-thioester (aa1-634) by native chemical ligation[2].

Our approach combines the solution-phase synthesis of a caged amino acid with solid phase peptide synthesis and ligation of the resulting peptide to a multidomain protein expressed in E. coli. All peptides were obtained with reasonable yields and used in reactions with STAT6-thioester to give ligation yields of ~15-40%. Initial in vivo studies demonstrate that caged phosphorylated STAT6 resides in the cytoplasm but not in the nucleus. DNA-binding of caged phosphorylated and uncaged STAT6 is currently studied by single molecule fluorescent techniques.

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L007

EXPLORING SECONDARY STRUCTURE USING AZA- AND LACTAM-SCANNING

M. Bodas, R. Rao, D. Boeglin, F. Galaud, R. Kaul, S. Surprenant,
W.D. Lubell

Département de Chimie, Université de Montréal, Montréal, QC, Canada

Secondary structures responsible for peptide biology are traditionally identified by systematic scanning of each residue in the peptide sequence by replacement with another proteinogenic amino acid. For example, alanine, enantiomeric amino acid and proline scans have been respectively performed to study the importance of side chains, stereochemistry and conformation on the biological activity of the peptide. Our lab has been developing alternative scanning methods for characterizing the relationship between peptide structure and activity. For example, we have recently introduced effective solid-phase methods for introducing aza-amino acid residues systematically at different positions along the peptide chain [1,2]. Because aza-amino acid residues have been shown to adopt the i + 1 and i + 2 positions of beta-turns, aza-amino acid scanning offers potential for identifying regions of turn secondary structure which are important for biology. Similarly, we are pursuing solution- and solid-phase chemistry for introducing lactam constraints at different positions along the peptide [3] . Our presentation will focus on recent achievements in the development of new methodology for scanning various peptide sequences to identify secondary structures relevant for biological activity.

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L008

SYNTHESIS AND STUDIES OF A POLY-HISTIDINE-SPECIFIC CYSTEINE BASED FLUORESCENT LABEL

A. Szymańska[1], K. Beena[2], L.M. Gierasch[2,3]

[1]Department of Organic Chemistry, Faculty of Chemistry, University of GdańSk, Gdańsk, Poland [2]Department of Biochemistry and Molecular Biology [3]Department of Chemistry, University of Massachusetts, Amherst, MA, USA

Selective labeling of proteins enables observation of their structural changes, localization, and interactions, even in complex mixtures. The major limitations of many currently available labels are irreversibility and low selectivity. Therefore, new, more selective, reversible tags are needed. Affinity labeling utilizing transition metal complexes with ligands like nitrilotriacetic acid (NTA) offers a convenient starting point for design of such compounds. We have designed an NTA-based, fluorescent, reversible affinity tag, potentially useful in broad range of protein studies. We chose bimane as a fluorophore because of its small size and relatively high fluorescence quantum yield. Modification of the α-amino group of cysteine yielded the NTA "head", and reaction of the side chain thiol group with dibromobimane provided a new, small, neutral, water-soluble fluorescent reagent, BM-[Cys(NTA)]2. Steady state fluorescence and anisotropy measurements revealed micromolar affinity of the label for hexa- and deca-histidine tags. Reversal of labeling was achieved by EDTA addition. BM-[Cys(NTA)]2 also served as an energy donor for the fluorescein and bi-arsenical dye FlAsH in double labeling experiments. Thus, the promise of the new dye has been demonstrated in vitro, but we also anticipate that it will be useful in in vivo labeling because both NTA and bimane are membrane permeable.

Supported by a University of Gdańsk grant (BW to A. Szymańska) and NIH grants to L.M. Gierasch.

L009

A NEW GENERATION OF N-TRIAZINYLAMMONIUM COUPLING REAGENTS VERSATILE FOR PEPTIDE SYNTHESIS BY DIFFERENT STRATEGIES

B. Kolesinska[1], G. Sabatino[2,3], M. Chelli[2,3], P. Rovero[2,4], J. Kolesińska[1], K.G. Jastrzabek[1], Z.J. Kaminski[1], **A.M. Papini**[2,3]

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

Recently, we demonstrated the high efficiency and versatility of the "superactive esters" concept in rational design of the structure of coupling reagents. Two families of new generation of coupling reagents based on N-triazinylammonium tetrafluoroborates [1] and N-triazinylammonium sulfonates respectively were designed and obtained in high yield. Both families of coupling reagents were successfully used for the synthesis in solution of esters from appropriate acids, alcohols, and phenols, of Z-, Boc-, and Fmoc-protected dipeptides (derived from natural and unnatural sterically hindered amino acids) and for peptide fragment condensation [2]. We compared also the performance (in terms of yield, purity of the crude product, and racemization) of the series of N-(4,6-dimethoxy-1,3,5-triazinyl-2)ammonium, N-(4,6-dibenzyloxy-1,3,5-triazinyl-2)ammonium both tetrafluoroborates and sulfonates, using the classical in batch manual (Advanced ChemTech PLS) and automated multiple (Advanced ChemTech 396) solid-phase peptide synthesizers, and the innovative microwave-assisted monomode manual (Discovery, CEM) and automatic (Liberty, CEM) synthesizers, testing the synthesis of difficult peptide sequences, including Aib peptides, post-translationally modified peptides, and on-resin head-to-tail constrained cyclopeptides.

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L010

PROTEIN EPITOPE MIMETICS: AN INNOVATIVE APPROACH TO THE DISCOVERY OF SELECTIVE AND HIGHLY POTENT SERINE PROTEASE INHIBITORS

O. Sellier[1], F. Jung[1], S. DeMarco[1], J.W. Vrijbloed[1], J. Zumbrunn[1], C. Bisang[1], A. Decours[2], K. Moehle[2], J.A. Robinson[2], H. Henze[1], C. Ludin[1], D. Obrecht[1], **F.O. Gombert**[1]

[1]PEM-BU, Polyphor AG, Allschwil [2]Institute of Organic Chemistry, University of Zürich, Switzerland

The Protein Epitope Mimetics (PEM) technology, developed by Polyphor Ltd. in collaboration with Prof. John Robinson at the University of Zurich, provides access to β-hairpin and α-helix mimetics of proteins. PEM, cyclic peptide-like (1-2 kDa) β-hairpin molecules, are suitable to modulate protein-protein interactions. We successfully applied PEM technology for lead finding and optimization, such as CXCR4-antagonists and antimicrobials. Here we report the discovery of new serine protease inhibitors.

Starting from sunflower trypsin inhibitor (SFTI) we have established an integrated discovery platform for protease inhibitors. Potencies, ADME-properties and selectivity against a panel of 11 pharmacologically relevant proteases were optimized in iterative cycles. PEM-Technology thus enabled us to discover highly potent (Ki values between 1 and 24 nM) and selective (>1:1000) reversible cathepsin G, human neutrophil elastase (HNE) and tryptase inhibitors.

Interestingly, important contributions of inhibitor-target interactions distant to the active site could be confirmed by NMR and X-ray analyses. We synthesized more than 1`000 PEM molecules by an optimized solid-phase/liquid-phase process and LC-MS purification. Combining our SAR and ADME-properties data with structural information and homology modeling we were able to fine-tune potency, selectivity and pharmacological properties.

PEM molecules showing high stability (>90% after 4h) in rat and human plasma, liver microsomes and gastric juice (1h and 3h) were chosen for in vivo studies. Oral bioavailability in rats was demonstrated with a selected cathepsin G inhibitor. Pharmacokinetic results indicate that PEM protease inhibitors are sufficiently long-lived in vivo to further progress these molecules into a lead-development program.

L011

A CELL-BASED APPROACH FOR BIOSYNTHESIS/SCREENING CYCLIC PEPTIDE LIBRARIES AGAINST BACTERIAL TOXINS

J.A. Camarero, R.H. Kimura, A.T. Tran

Chemical Biology Division, Lawrence Livermore National Laboratory, Livermore, CA, USA

Available methods for developing and screening small drug-like molecules able to knock-out toxins or pathogenic microorganisms have serious limitations. In order to be useful, these new methods must provide high-throughput analysis and identify specific binders in a short period of time. To meet this need, we are developing an approach that uses living cells to generate libraries of small biomolecules, which are then screened inside the cell for activity. Our group is using this new, combined approach to find highly specific ligands capable of disabling Anthrax Lethal Factor (LF) as proof of principle.

Key to our approach is the development of a method for the biosynthesis of libraries of cyclic peptides, and an efficient screening process that can be carried out inside the cell. We have used two natural disulfide-containing cyclic peptides, the cyclotide Kalata B1 (KB-1) and the Bowman-Birk sunflower trypsin inhibitor (SFTI-1) as templates for library design. Cyclic peptide libraries based upon these molecular scaffolds have been biosynthesized using protein splicing in E.coli cells. At the same time, we have developed a FRET-based protein reporter that is able to detect LF activity in vivo. Both LF and its FRET-based substrate reporter have been co-expressed in E. coli. Strategies for the sequential co-expression of these three components of this new cell-based screening system will be presented.

L012

STRUCTURAL AND FUNCTIONAL DIVERSITY OF ENDOGENOUS REGULATORY OLIGOPEPTIDES

A.A. Zamyatnin[1,2]

[1]Computer Biochemistry Group, A.N.Bach Institute of Biochemistry, Russian Academy of Sciences and, Moscow, Russia [2]Department of Biotechnology, Universidad Tecnica Federico Santa Maria, Valparaiso, Chile

Natural oligopeptides may regulate nearly all vital processes. To date, the chemical structures of more than 6000 oligopeptides have been identified from more than 1000 organisms representing all the biological kingdoms.

We have compiled the known physical, chemical and biological properties of these oligopeptides — whether synthesized on ribosomes or by non-ribosomal enzymes — and have constructed an internet-accessible database, EROP-Moscow (Endogenous Regulatory OligoPeptides), which resides at http://erop.inbi.ras.ru. This database enables users to perform rapid searches via many key features of the oligopeptides, and to carry out statistical analysis of all the available information.

The database lists only those oligopeptides whose chemical structures have been completely determined (directly or by translation from nucleotide sequences).

It provides extensive links with the Swiss-Prot-TrEMBL peptide-protein database, as well as with the PubMed biomedical bibliographic database. EROP-Moscow also contains data on many oligopeptides that are absent from other convenient databases, and is designed for extended use in classifying new natural oligopeptides and for production of novel peptide pharmaceuticals.

L013

ENDOMORPHIN AS A TEMPLATE FOR DEVELOPMENT OF NEW OPIOIDS AGONIST-TACHIKININ ANTAGONIST CHIMERIC LIGANDS

A.W. Lipkowski[1,2], P. Kosson[1], A. Misicka[1,4], I. Bonney[1], G. Toth[3], V.J. Hruby[5], D.B. Carr[2]

[1]Medical Research Centre Polish Acad Sci, Warsaw, Poland [2]Tufts-New England Medical Centre, Boston, MA, USA [3]Institute of Biological Sciences, Hungarian Acad Sci, Szeged, Hungary [4]Department of Chemistry, Warsaw University, Warsaw, Poland [5]Department of Chemistry, University of Arizona, Tucson, AZ, USA

Endomorphins have been discovered and characterized as new endogenous opioid peptides that express unique selectivity to mu opioid receptors [1]. However, recently we have found that endomorphins express also low, but significant affinity to tachykinin receptors acting as antagonists [2]. Receptor ligands that express opioid agonist and tachykinin antagonist properties are very promising new analgesics. Therefore, we applied endomorphin template to obtain new opioid agonists with increased tachykinin antagonist properties. Small library of endomorphins analogue has been created. The biological studies showed that structural changes in primary endomorphin structures resulted in non-parallel opioid and tachykinin activities changes. Therefore, created library contains peptide analogues with various proportions between opioid and tachykinin activities.

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L014

STRUCTURE-INTESTINAL PERMEABILITY-RELATIONSHIP STUDIES OF BACKBONE CYCLIC PEPTIDES: FROM MODEL COMPOUNDS TO ORALLY ACTIVE DRUG LEAD

C. Gilon[1], Y. Linde[1], S. Hess[2], O. Ovadia[2], D.E. Shalev[3], H. Senderovich[4], T. Yehezkel[4], Y. Salitra[4], C. Haskel-Luevano[5], A. Hoffman[2]

[1]Organic Chemistry Department [2]Pharmaceutics Department [3]Wolfson Centre for Applied Structural Biology, TheHebrew University of Jerusalem [4]DeveloGen, Rehovot, Israel [5]Medicinal Chemistry, University of Florida, Gainesville, FL, USA

The effect of backbone cyclization on the intestinal permeability and intestinal metabolic stability of peptides was assessed, using three sub-libraries of backbone cyclic hexapeptides with the general sequence c(Phe(Cm)-Xaa-Xaa-Xaa-Xaa-(Nn)Phe-NH2), and two sub-libraries of linear analogs with the general sequence H-Phe-Xaa-Xaa-Xaa-Xaa-Phe-NH2. Peptides differed by: a) topology i.e. cyclic versus non-cyclic; b) the length of the bridge arms in the cyclic peptides; c) lipophilicity (N- or C-methylation). Peptides intestinal tissue permeability was investigated utilizing both side-by-side diffusion chambers and Caco-2 monolayer models. The transport mechanism was verified by colorimetric bilayer liposomes. Metabolic stability was determined with rat brush border membrane vesicles. Backbone cyclization dramatically improves peptide stability to proteolysis by the brush border enzymes. Moreover, it imposes substantial increase (five folds) on the intestinal permeability of the model cyclic peptides as compared to their linear analogs, regardless of the increase in lipophylicity. Both cyclic and linear model peptides lack the ability to penetrate a bilayer membrane; they therefore use the paracellular route for intestinal permeability. The same methodology was applied to the MC4R active parent sequence: Phe-D-Phe-Arg-Trp-Gly-NH2. Peptide in the library were studied for MC receptor functionality and selectivity as well as in-vitro intestinal permeability and intestinal metabolic stability. One of the backbone cyclic peptides (BBC-1) showed high mMC4R and mMC5R activity and selectivity (EC50 (nM) 3.97±0.63 and 7.27±0.40 respectively) while possessing very high intestinal metabolic stability and transcellular permeability. In-vivo studies in mice show reduced food consumption over a period of 24 hr of ~ 30% when administrated orally.

L015

NATURAL AND SYNTHETIC INHIBITORS OF THE 20S PROTEASOME: CRYSTALLOGRAPHIC KNOWLEDGE IN DRUG DESIGN STRATEGIES

M. Groll

Adolf Butenandt-Institute, Physiological Chemistry, Butenandtstr, Munich, Germany

The 20S proteasome is a large, cylinder-shaped protease that is found in all domains of life, playing a crucial role in cellular protein turnover. It has multiple catalytic centres located within a hollow cavity of the cylinder. This architecture prevents unwanted degradation of endogenous proteins and promotes processive degradation of substrates by restricting dissociation of partially digested polypeptides.

The regulation of proteasomal activity is particularly important for a vast amount of essential biological processes. Therefore, compounds that specifically inhibit the proteasome are potentially of great pharmacological interest. Taking the structural and functional approach obtained during the last years, Millennium Pharmaceuticals company could develop a proteasome inhibitor named bortezomib (Velcade®), which already passed clinical phase III study and now represents a newly approved prescription drug against multiple myeloma. Structural knowledge on proteasome inhibitors helps to elucidate new strategies for drug design development. We could show, that 1) the crystal structure of the eukaryotic 20S proteasome reveals a unique topography of the proteolytically active sites. Structure-derived active site separation distances were exploited for the design of homo- and heterobivalent inhibitors, based on peptide aldehyde-groups and polyoxyethylene as spacer element. Their inhibitory potencies, which are by two orders of magnitude enhanced, compared with pegylated monovalent inhibitors, result from their bivalent binding.

2) the class of TMC-95's, natural cyclic tripeptide metabolites, represent potent competitive proteasome inhibitors. Based on the crystal structure of the proteasome:TMC-95A complex, the synthetically challenging structure found in the natural product could be simplified, allowing easy synthetic accessibility. This new generation of TMC-95 analogues may represent promising lead structures for further optimization of affinity and selectivity of proteasome inhibitors.

3) the crystallographic analysis of the yeast 20S proteasome in complex with the inhibitor Homobelactosin C reveals a novel inhibitor binding mode and reveals information about composition of proteasomal primed substrate binding sites. The structural data provide an explanation for involvement of immuno-subunits in antigen generation and open perspectives for rational design of ligands, inhibiting exclusively constitutive or immuno-proteasomes.

L016

RATIONAL DESIGN OF CONSTITUTIVELY ACTIVE MUTANTS OF THE HUMAN C5A RECEPTOR

G.V. Nikiforovich[1], S. Sen[2], T.J. Baranski[2]

[1]Department of Biochemistry and Molecular Biophysics [2]Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA

Computational molecular modeling based on structural homology to rhodopsin yielded the 3D models of the transmembrane (TM) regions of the human C5a receptor and its mutants possessing constitutive activity in yeast, as F251A and I124N/L127Q (NQ). According to the models, molecular mechanism of constitutive activity in C5aR mutants may be associated with removing of bulky side chain groups, as that of F251, from the interface between TM helices 6 and 7. Specifically, in the NQ mutant, the side chain of F251 may move into the pocket between the TM3 and TM6 gated by the side chains of L127 in TM3 and F254 in TM6 of the wild type. Another bulky side chain in the TM6/TM7 interface is that of Y300 in TM7. Additional calculations showed that elimination of the side chains in positions 127, 254 and 300 (mutations L127A, F254A, Y300A and their combinations), as well as reducing of volume of the side chain in position 251 (as in F251N) may lead to constitutively active mutants. Corresponding mutants were expressed both in yeast and in mammalian cells. As predicted, several mutants, as Y300A, F254A, L127A/Y300A and F251N displayed constitutive activity in yeast, whereas mutants L127A, F251N, NQ-F251N and L127A/Y300A showed constitutive activity in mammalian cells, though constitutive activities of the designed mutants were generally lower than that of the NQ mutant. Summarizing, this study presents a convincing case of successful rational design of completely novel constitutively active mutants of a G-protein coupled receptor directly based on predictions from molecular modeling.

L017

SHAPE OF DE NOVO DESIGNED PROTEINS IN SOLUTION: SMALL ANGLE X-RAY SCATTERING

R.H. Nielsen, P.B. Tofteng, K. Sørensen, L. Arleth, **K.J. Jensen**

Department of Natural Sciences, KVL, Frederiksberg, Denmark

De novo design of proteins holds great promise for understanding protein folding and for new tailored protein structural motifs. It is also likely to become a powerful tool in nanobioscience. A key aspect is controlled self-assembly, either of sub-units to form the functional protein or oligomerization of proteins, in contrast to uncontrolled aggregation. We have previously described 4-helix bundle carboproteins, where carbohydrate templates were used to direct the organization of the attached peptide helices. However, characterization by NMR is made difficult by the structural economy often used in de novo design, with several copies of the same peptide sequence being used. Furthermore, if de novo designed proteins fail to crystallize, options for structural characterizations are limited.

Biophysical characterization of new proteins in solution is a crucial part of de novo design. Small angle X-ray scattering (SAXS) is uniquely suited for studying the aggregational state and shape of biomolecules in solution. Here we present comparative SAXS studies of de novo designed ‘hemi 4-helix bundles’, which dimerize to form 4-helix bundles, 4-helix bundles, and 2×4-helix bundles.

L018

SIGNAL SEQUENCE BINDING BY SIGNAL RECOGNITION PARTICLE

A. Szymanska[1], E. Clerico[2], **L.M. Gierasch**[3]

[1]Chemistry, University of Gdansk, Poland [2]Biochemistry & Molecular Biology [3]Biochemistry & Molecular Biology and Chemistry, University of Massachusetts, Amherst, MA, USA

Signal recognition particle (SRP)-mediated delivery of membrane and secretory proteins to the plasma or cytoplasmic membrane is one of the main protein targeting mechanisms in all cells. Interaction of SRP with the hydrophobic N-terminal signal sequence of a newly synthesized export protein starts a cascade of events. The later stages of the process of protein export are relatively well understood, but the mechanism of the very first encounter between signal peptide and SRP is still unclear. First, it is not known how SRP recognizes and effectively binds many different sequences. Which of the two SRP domains (NG or M) mediates signal peptide recognition also remains uncertain. To answer these questions, we have utilized synthetic signal peptides and both chemical cross-linking and biophysical approaches. Peptides based on the signal sequence of alkaline phosphatase were cross-linked to E. coli SRP, and products were analyzed by Western blot and LC/MS/MS following enzymatic digestion. Cross-linking of the signal peptides was observed primarily to the NG-domain when a short spacer arm was utilized, whereas cross-linking occurred predominantly to the M-domain with a longer spacer arm. We propose that the signal peptide binding pocket is localized in a cleft between the two domains, with the NG-domain serving as a primary signal peptide receptor and the M-domain serving as a shield for the hydrophobic part of the peptide. Further exploration of the binding mechanism is being pursued by fluorescence and nuclear magnetic resonance.

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L019

PALMITOYLATION OF BETA-SECRETASE IS INVOLVED IN ALZHEIMER'S DISEASE

B.M. Austen, R.B. Parsons, C. Sidera, G. Price

Department of Basic Medical Sciences, St George's University of London, London, UK

beta-Secretase is an acid proteinase that releases the neurotoxic peptide, beta-amyloid, which is involved in the neurodegeneration of Alzheimer's Disease. High cholesterol is known to be a risk factor for the disease. We have found by identifying a peak at 629.7m/z on Malditof mass spectrometry that in high cellular cholesterol, beta-secretase is palmitoylated on Cys residues within the sequence CLR, in a way that activates release of beta-amyloid. Mass spec showed that hydroxylamine cleaved the thioester bond, and removed the palmitoyl group. Inhibitors such as cerulenin, which prevent palmitoyl addition may have use as therapeutic agents.

L020

STRUCTURE ANALYSIS OF MEMBRANE ASSOCIATED ANTIMICROBIAL AND CELL PENETRATING PEPTIDES USING 19F-, 2H-, AND 31P-SOLID STATE NMR

P. Wadhwani[1], E. Strandberg[1], P. Tremouilhac[1], S. Afonin[1], A.S. Ulrich[1,2]

[1]*Institute of Biological Interfaces, Forschungszentrum Karlsruhe, Eggenstein-Leopoldshafen*
[2]*Institute of Organic Chemistry, University of Karlsruhe, Germany*

Solid state NMR is an important tool to investigate the structure and dynamic behaviour of antimicrobial peptides (AMPs) and cell penetrating peptides (CPPs) in their functionally relevant membrane environment. We have used highly sensitive 19F-NMR to compare various AMPs (Gramicidin S, PGLa, and K3) and CPPs (HIV-TAT, MAP) (Table 1). Peptide analogues were synthesized containing one specific 19F-labeled amino acid as a local structural reporter, such as 4-fluoro-phenylglycine (4F-Phg), 4-trifluoromethyl-phenylglycine (4CF3-Phg) or 3,3,3-trifluoro-alanine (F3-Ala). Their functional integrity was validated by testing their biological activities, and NMR samples were prepared by reconstituting the peptides into model membranes. Their three-dimensional structures were calculated from a set of local 19F-NMR parameters, such as chemical shift anisotropy and dipolar coupling, and the influence of the peptide on the lipid bilayer integrity was examined by 31P-NMR. Additional non-perturbing isotope labels such as 2H and 15N were used to verify the 19F-NMR-derived structures. In each system, we observed that the peptide would undergo a concentration-dependent self-assembly in the membrane or that the lipid components would re-arrange. As a general conclusion, the peptide concentration in the membrane plays a key role in determining their mode of action, and the respective threshold concentrations could be determined.

Table1: Investigated peptide sequences*

Peptides	Sequence
Gramicidin S (GS)	*cyclo*-[P<u>*V*</u>O<u>L</u>^{D}F]$_2$
PGLa	GM*A*SK<u>*AGA*</u>*I*<u>*AG*</u>K<u>*I*</u>*A*K<u>*VA*</u><u>*L*</u>KAL-amide
K3	KIAGKI*A*K<u>*I*</u>*A*<u>G</u>K<u>*I*</u>*A*KI*A*GKIA-amide
MAP	KLALK<u>L</u>*A*<u>L</u>K*A*<u>L</u>K*AA*<u>L</u>KLA-amide
HIV-Tat	GRKKRRQRRRPPQ

*underlined positions labeled with 4F-Phg, 4CF$_3$-Phg or F$_3$-Ala and isotope-labelled positions are shown in italics.

L021

A RHEGNYLOGIC STRATEGY FOR THE SYNTHESIS OF SIGNAL TRANSDUCTION MODULATORY, CELL PENETRATING PEPTIDES

S. Jones[1], P. Ostlund[2], U. Langel[2], J. Howl[1]

[1]Research Institute in Healthcare Science, School of Applied Sciences, University of Wolverhampton, Wolverhampton, UK [2]Department of Neurochemistry and Neurotoxicology, Stockholm University, Stockholm, Sweden

Many cell-penetrating peptides (CPP) have been utilised as biologically inert vectors. A majority of these studies have employed sychnologically organised constructs in which a bioactive cargo (message) is chemically conjugated to the CPP (address) prior to intracellular delivery. However, a QSAR-based algorithm can now be used to predict cell penetrant peptides within the primary sequences of proteins. Thus, it should be feasible to identify rhegnylogic sequences that contain discontinuously organized pharmacophores that enable the effective cellular penetration of a biologically active peptide.

To further investigate the rhegnylogic concept, we identified a 20AA fragment (RKLTTIFPLNWKYRKALSLG) within the first intracellular loop of the human type(a) calcitonin receptor (hCT(a)) that was predicted to be an excellent cell penetrating sequence. This sequence, hCT(a)174-193, includes a splice variant 16AA insert located within the first intracellular loop of the hCT(a). This sequence modulates the pharmacology of human calcitonin receptors to inhibit receptor-stimulated inositol phosphate metabolism. Exogenously applied hCT(a)174-193 modulated cAMP formation in ECV304, a cell line that endogenously expresses hCT(a). Moreover, hCT(a)174-193 enhanced both receptor- and non-receptor-mediated formation of cAMP in these cells. Our future aims are to further establish the biological and therapeutic significance of rhegnylogically organised cell penetrating modulators of signal transduction.

L022

UPTAKE OF THE CELL-PENETRATING PEPTIDE PEP-1 IS DRIVEN BY ELECTROSTATIC INTERACTIONS. A BIOPHYSICAL STUDY CARRIED IN VITRO AND IN VIVO

S.T. Henriques, M. Castanho

Centro de Química E Bioquímica, FCUL, Lisbon, Portugal

The cell-penetrating peptide pep-1 (Ac-KETWWETWWTEWSQPKKKRKV-cysteamine) is capable of introducing large proteins into different cell lines, maintaining their biological activity. Although, it has been used as a carrier, the uptake mechanism used by pep-1 is unknown. The principal aim of our work is to understand pep-1 interaction with biological membranes and to elucidate the peptide cellular uptake.

The study of peptide interaction with membranes was carried out in vitro with model membranes: large unilamellar vesicles (LUVs); black lipid membranes and giant unilamellar vesicles. HeLa cells have been used to follow the translocation of peptide associated with a protein. Fluorescence spectroscopy methodologies, electrophysiological measurements and fluorescence microscopy have been used to perform the experiments.

Pep-1 has a high affinity for membranes, which is enhanced in the presence of negatively-charged phospholipids. Pep-1 is able to induce aggregation and fusion of LUVs but this occurs without membrane leakage, however, in the presence of high peptide/lipid ratios membrane is disrupted. Pep-1 translocation in vitro and in vivo only occurs in the presence of transmembrane potential without evidence for a mechanism endosomal-dependent, which suggest that the underlying translocation mechanism is physically-mediated.

In conclusion, peptide uptake is ATP-independent and is driven by electrostatic interactions between peptide and membrane, where the charge net between inner and outer layer and local disturbs induced by pep-1, promotes translocation. Peptide uptake occurred without evidence for pore formation.

L023

TRANSLOCATION AND CELLULAR TRAFFICKING OF CELL PENETRATING PEPTIDES (CPP). DESIGN OF A MASS STABLE REPORTER TAG

D. Delaroche[1,3], B. Aussedat[1,3], S. Aubry[1,3], G. Chassaing[1,3], F. Burlina[1,3], G. Bolbach[1,2,3], **S. Lavielle**[1,3], S. Sagan[1,3]

[1]UMR7613 [2]Plateforme De Spectrométrie De Masse Et De Protéomique, Université Pierre Et Marie Curie-Paris 6 [3]CNRS, UMR 7613, FR 2769, Synthèse, Structure et Fonction de Molécules Bioactives, Paris, France

Since the discovery of penetratin corresponding to the third helix of the Antennapedia homeodomain, many CPP have been described. Some of them (Trojan peptides) are able to shuttle to the different cellular compartments, cytoplasm and/or nucleus of living cells various kinds of compounds (proteins, oligonucleotides, antibodies nanoparticles drug carriers….), which are called cargoes. All the processes that allow transduction of one molecule from the extracellular medium to the intracellular one have been stated: inverted micelles, charged lipid vesicles, membrane potential, clathrin- or caveolin-dependent endocytosis, macropinocytosis. It becomes more and more obvious that the mechanism of cell-entry depends strongly on the nature and size of the CPP.

For pharmacological applications, it is crucial to know the intracellular fate of both the CPP and the linked cargo that depend on their translocation pathway, cellular trafficking and final locations (lysosomal vs. proteasome degradation). We have designed and synthetized a mass stable reporter (msr) tag, trifluoroacetyl-(diethyl)Gly-Lys(biotin)-(D)Lys-Cys, that might be incorporated either in CPPs or cargoes, such as the protein kinase pseudo-substrate (PKCi= RFARKGALRQKNV) a protein kinase C phosphorylation peptide inhibitor. Using MALDI-TOF mass spectrometry, this mass stable reporter tag permitted an accurate tracking of either the full-length CPP and PKCi or their degradation products inside and outside cells. Furthermore, the use of the deuterated forms of the reporter ((diethyl)Gly: 10D vs. 10H) allowed an absolute quantification of both the CPP and PKCi peptides. The two peptides are likely located within the cytoplasm of the cells but they are degraded at different rates.

L024

NATURAL PEPTIDE-MEDIATED SELECTIVE GENE DELIVERY INTO ACTIVELY PROLIFERATING CELLS

M.A. Hayashi[1], F.D. Nascimento[2], A. Kerkis[3], V. Oliveira[4], E.B. Oliveira[5], G. Rádis-Baptista[6], H.B. Nader[1], T. Yamane[7], I.L. Tersariol[8], I. Kerkis[9]

[1]Center for Applied Toxinology, Instituto Butantan [2]Departamento de Bioquímica, Universidade Federal de São Paulo (UNIFESP) [3]Clínica E Centro de Reprodução Humana "Roger Abdelmassih" [4]Laboratório de Neurosciências, Universidade da Cidade de São Paulo (UNICID), São Paulo [5]Departamento de Bioquímica E Imunologia, Universidade de São Paulo (USP), Ribeirão Preto [6]Centro de Ciências Biológicas, Universidade Federal de Pernambuco (UFPE), Recife [7]Centro de Biotecnologia Molecular, Instituto de Pesquisas Energéticas E Nucleares (IPEN) [8]Centro Interdisciplinar de Investigação Bioquímica (CIIB), Universidade de Mogi Das Cruzes [9]Laboratório de Genética, Instituto Butantan, São Paulo, Brazil

Crotamine is a natural peptide found in the venom of a South American rattlesnake. Recently, we have shown that crotamine belongs to the family of cell penetrating peptides (CPPs), which currently are extensively used as carriers of different molecules into the cells [FASEB J (2004) 18: 1407-1409]. However, the use of CPPs is mainly limited by their unspecific nature and unclear mechanisms of uptake. Herein we show that crotamine is capable of binding electrostatically to plasmid DNA forming DNA-peptide condensates, overcoming the usually employed chemical conjugation or fusion protein approach required by the majority of known CPPs. DNA binding to crotamine was demonstrated by means of both gel retardation assay and circular dichroism analysis. Moreover, differently from other known CPPs, crotamine demonstrates cell specificity and a selective delivery of plasmid DNA into actively proliferating (AP) cells. We also show, for the first time, the delivery of a plasmid DNA in vivo into various tissues using CPPs technology. After 24hrs of intraperitoneal injection of DNA-peptide condensates, a strong green fluorescence signal was evidenced in a variety of actively regenerating tissues of mice. The mechanism of crotamine penetration into the cells was also investigated, demonstrating the involvement of heparan sulfate proteoglycans in uptake, followed by endocytosis and peptide accumulation within the lysosomal vesicles. Finally, our data also suggest the same uptake mechanism for both crotamine alone and crotamine-plasmid DNA condensates.

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L025

THE PEPTIDE 3-10-HELIX AS A SPACER AND A TEMPLATE

C. Toniolo

Department of Chemistry, University of Padova, Italy

Beside the classical α-helix and pleated β-sheet conformations, the only other principal long-range structure that occurs significantly in peptides and proteins is the 3-10-helix. A complete characterization of this helix in solution has been recently achieved by our group using a number of physico-chemical techniques, including CD and NMR, thus allowing its unambiguous differentiation from the conformationally closely related α-helix. Unique examples of 3-10-/α-helix conversions and equilibria have been found.

Recent applications of this rigid, helical peptide structure have focused on its role as a spacer for studies of N- and C-terminal probe…probe interactions. In particular: (i) CD exciton coupling as a function of separation and angle between two porphyrin chromophores; (ii) fluorescence measurements of protoporphyrin…naphthyl interactions; (iii) photophysics of the electron transfer from a ruthenium complex to a fullerene (C60) derivative; (iv) dissociative electron transfer from a phthalimido group and a labile dialkylperoxide σ-bond (electrochemistry); (v) induction and propagation of axial chirality in biphenyl-based peptides (CD). The 3-10-helix has also been exploited as a template in numerous investigations involving two side chains, e.g. (i) interactions between two (or among three) nitroxyl radicals (ESR); (ii) interaction between a binaphthyl fluorophore and a nitroxyl (fluorescence quenching); (iii) interaction between an azulenyl fluorophore and a nitroxyl (CIDEP); (iv) mini-receptor in the non-covalent binding of fullerene (C60) (HPLC and photophysics); (v) transphosphorylation catalysis and oligonucleotide cleavage; (vi) ring-closing metathesis.

L026

THE BETA-PEPTIDE DOMAIN: FROM CONTROLLED SECONDARY STRUCTURES TO NANOSTRUCTURED ASSEMBLIES

T.A. Martinek[1], I.M. Mándity[1], A. Hetényi[1], L. Fülöp[2], G.K. Tóth[2], F. Fülöp[1]

[1]Institute of Pharmaceutical Chemistry [2]Department of Medical Chemistry, University of Szeged, Hungary

Control over folding and self-assembly is a major current goal of biopolymer and biomimicking polymer research: it can result in biopolymer-like activities and new structural classes of nanostructured materials. The self-organizing beta-peptides exhibit stable secondary structures that can be exploited to construct bioactive substances.[1,2] We set out to establish a general approach to the stereochemical control over secondary structures and the secondary structure-dependent self-assembly of the beta-peptides into nanostructured tertiary structure motifs.
The models were synthetized via solid-phase techniques, and they were characterized by using NMR, IR, ECD and VCD spectroscopy. The supramolecular structures were studied by using transmission electron microscopy, dynamic light scattering and diffusion-ordered NMR.
It was found that systematic control of the secondary structure can be attained by tuning the configuration of the backbone atoms within the cyclic beta-amino acid residues. The tailor-made beta-peptide helices and strands are prone to self-assemble into tertiary structure motifs leading to nanosized fibrils and vesicles (Figure).[3]

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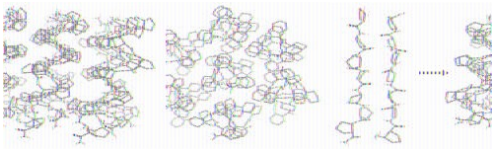

L027

SYNTHETIC BETA-PROTEINS: FROM DESIGN TO STRUCTURE

W. Haehnel[1], K. Fritzemeier[1], P. Pollet[1], G. Gemmecker[2], H. Kessler[2]

[1]Institute of Biology II/Biochemistry, University of Freiburg [2]Institute of Organic Chemistry, TU Munich, Germany

The design of β-proteins requires an extended understanding of amino acid packing if compared to our previous synthesis of α-helical proteins on a cyclic template (TASP) with functional groups [1]. The TASP concept is extended to the assembly of four β-hairpins folding either into a β-sandwich or a β-barrel. While the β-sandwich with two four-stranded sheets started from the backbone of a known protein that of eight-stranded antiparallel β-barrels started from first principle of an elliptical cylinder. A common strategy is to pack the amino acid side chains in a way that they form a tight hydrophobic core and to allow optimal H-bonds between neighboring β-strands. Some parameters of the force field CHARMM have been modified to adapt it to the requirements of protein design. The selection of amino acid side chain rotamers by efficient algorithms leads to structures near the global energy minimum. The criteria to select proteins are discussed. A few proteins were assembled from synthetic peptides and found to fold into a well defined structure. Improvement of surface residues reduced the aggregation of the β-sandwich protein to a stable dimer as determined by equilibrium ultracentrifugation and 1D 1H NMR. Spectroscopic analyses show a fold with a free energy near -25 kJ/mol and confirm the β-structure by circular dichroism, FTIR and 2D-NMR. The 2D-1H,1H-DQF-COSY spectrum at 750 MHz shows a typical β-sheet distribution. We hope to contribute to a detailed understanding of β-structures and to the association of β-strands which is the basis of several wide spread diseases.

L028

HIGH RESOLUTION SCANNING TUNNELLING MICROSCOPY OF THE ß-AMYLOID PROTEIN (Aß1-40) OF ALZHEIMER'S DISEASE SUGGESTS A NOVEL MECHANISM OF OLIGOMER ASSEMBLY

D. Losic[2], L.L. Martin[3], A. Mechler[3], D.H. Small[1], **M. Aguilar**[1]

[1]Department of Biochemistry & Mol Biol, Monash University, Clayton, Vic. [2]School of Chemistry, Physics and Earth Sciences, Flinders University, Adelaide, SA [3]School of Chemistry, Monash University, Clayton, Vic., Australia

The aggregation of the ß-amyloid protein (Aß) is an important step in the pathogenesis of Alzheimer's disease. There is increasing evidence that lower molecular weight oligomeric forms of Aß may be the most toxic species in vivo. However, little is known about the structure of Aß oligomers. In this study, scanning tunnelling microscopy (STM) was used to examine the structure of Aß monomers, dimers and oligomers. Aß1-40 was visualised by STM on a surface of atomically flat gold. At low concentrations (0.5 uM) small globular structures were observed. High resolution STM of these structures showed them to be monomers of Aß. The monomers measured approximately 3-4 nm in diameter. Internal structure was seen in many of the monomers consistent with a conformation in which the polypeptide chain is folded into 3 or 4 domains. Oligomers were seen after ageing the Aß solution for 24 hr. The oligomers were also 3-4 nm in width and appeared to be formed by the end-to-end association of monomers with the polypeptide chain oriented at 90o to the axis of the oligomer. The results suggest that the oligomer formation can proceed through a mechanism involving the linear association of monomers.

L029

ADDRESSING THE PROBLEM OF CANCER DETECTION AND TREATMENT WITH MULTIVALENT LIGANDS THAT ADDRESS THE CELL SURFACE: CANCER VS. NORMAL CELLS/TISSUES

V.J. Hruby[1], R.J. Gillies[2], E.A. Mash[1], J. Vagner[1], H. Handl[2], Y. Monguchi[2], R. Sankaranarayanan[1], J. Josan[1]

[1]Chemistry Department [2]Cancer Center, University of Arizona, Tucson, AZ, USA

The early detection and treatment of many cancers such as melanoma cancer and pancreatic cancer is very difficult due to the complexity of the disease which limits the effectiveness of any treatment or detection method. In an effort to overcome these problems, we have developed a new approach to the development of ligands that can address these issues. Comparison of the expressed genome of cancer vs. normal cells demonstrate that numerous differences exist. We have directed our efforts against the expressed differences at the cell surface. We have designed and synthesized a number of linkers of varying lengths and chemical properties and combined them to produce templates with various physical-chemical properties and various lengths. We then attach one, two, or more ligands to the various termini at the linear or brached linker assembly. These ligands are specific pharmacophores or epitopes for specific cancer cell surface receptors/acceptors that distinguish cancer cells from normal cells. The linker templates are designed to also accommodate imaging agents and/or drugs. Synergistic activities are observed for some of these assemblies with unique biological activity profiles. The use of systematic structural modification provides insights into the requirements for these synergistic effects.

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L030

LAPP6, A NEW PEPTIDE POLYMER ABLE TO STIMULATE NATURAL PLANTS DEFENSES

F. Cavelier[1], N. Margheria[1], E. Vernière[1], C. Cabanes[2], C. Maquin[3], J. Martinez[1]

[1]Lapp-Umr 5810, Montpellier [2]Agronutrition, Carbonne [3]De Sangosse, Pont-du-Casse, France

To protect cultures against pathogens, actual treatments use pesticides, insecticides or fungicides that are toxic and leave pollutants after degradation. Investigations on new safe molecules in agriculture lacking environment problems are of great importance. Elicitors offer a promising new strategy in crops protection by stimulating the natural plants defenses. Peptaibols present elicitation properties, generally attributed to their pore forming ability. We have designed helicoidal peptide mimicking the peptaibol structure easy to synthesize at low cost. We have developed a very simple preparation allowing solvent recycling and easy scale-up. We have tested a series of polymers of various amino acids and identify a leader (LAPP6).

Our results demonstrate that this new synthetic peptide polymer was able to stimulate the natural defenses of melon, cucumber, zucchini and grapevine (Chardonnay), as indicated by stimulation of enzymatic activities such as peroxydase and chinase. Compound LAPP6 have been also tested against mildew (Plasmopara vitocola) in vineyards. All these results will be presented and discussed.

L031

CHALLENGING ALZHEIMER'S DISEASE: IN VIVO ACTIVE BACE INHIBITORS AND AMYLOID ß "CLICK" PEPTIDES

Y. Kiso[1], T. Kimura[1], Y. Sohma[1], A. Taniguchi[1], Y. Hayashi[1], S. Ishiura[2]

[1]Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, Kyoto Pharmaceutical University, Kyoto [2]Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Japan

[Background and Aims] Based on the substrate transition state, we designed and synthesized novel classes of inhibitors of aspartic proteases such as HIV protease, malarial plasmepsin II, containing the hydroxymethylcarbonyl (HMC) isostere. Among them, tripeptide KNI-272 was a highly selective and superpotent HIV protease inhibitor. Physicochemical studies suggested that the HMC isostere is an ideal transition-state mimic[1,2]. We applied the substrate transition state concept to develop inhibitors against ß-secretase (BACE1) targeting Alzheimer's disease (AD).

[Methods] We designed and synthesized small-sized BACE1 inhibitors KMI-420 and KMI-429 that contained phenylnorstatine as a substrate transition-state mimic[3]. The native Aß1-42 tends to aggregate due to uncontrolled polymerization complicating AD research. On the basis of our study with the "O-acyl isopeptide method"[4], we developed novel photo- and pH-triggered "click" peptides that readily convert to the native Aß1-42 upon activation.

[Results] We successfully designed a novel BACE1 inhibitor, KMI-492 that reduced amyloid ß peptide production in transgenic and wild-type mice[5]. Click peptide Aß1-42 analogs migrated to generate Aß1-42 with a 'click' reaction via an O-N intramolecular acyl migration[6].

[Conclusions] The BACE1 inhibitors and amyloid ß 'click' peptide that we developed will pave the way to defy Alzheimer's disease.

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L032

HIGH N-METHYLATION OF PEPTIDES FOR IMPROVEMENT OF ACTIVITY AND ADME

H. Kessler[1], J. Chatterjee[1], E. Biron[1], V. J. Hruby[2], C. Gilon[3], D. Hoyer[4], D. Mierke[5]

[1]Department Chemie, TU München, Garching, Germany [2]University of Arizona, Tucson, AZ, USA [3]Hebrew University, Jerusalem, Israel [4]Novartis, Basle, Switzerland [5]Brown University, Providence, USA

Peptides are usually considered to be no good drug candidates due to enzymatic cleavage and lack of oral availability. However, cyclic penta- or hexapeptides are not cleaved in vivo. The remaining problem is oral availability.

Usually Lipinski`s “rule of five” is used as exclusion criterion in drug research to have at least a chance for oral uptake. But there are examples such as Cyclosporin (CsA) that violate all of the rules and are orally available. We speculated that the high N-methylation is the reason for CsA´s good ADMET properties. N-Methylation not only reduces enzymatic cleavage but also changes the lipophilicity profile of the peptide and may induce conformational restriction. The latter has recognised as an important factor to govern oral availability. To investigate this approach we used the following:

1. Exploring the influence of N-methylation to cyclic peptide conformation. 30 differently N-methylated peptides of the basic structure cyclo(-D-Ala-Ala4-) were synthesized and analysed by NMR and MD. The thus identified conformationally homogeneous peptides can serve for structural design and for spatial screening.
2. Synthesis in solution and in solid phase for N-methylated amino acids and peptides was improved.
3. A library of 30 N-methylated peptides derived from the well known Veber-Hirschmann peptide cyclo(-DTrp-Lys-Thr-Phe-Pro-Phe-) was synthesized. Among them eight exhibited high activity for the somatostatin receptor subtype II (SSR-II) and one of the active compounds is taken-up into the blood when orally administered.

A similar approach was applied to the MT-II peptide of Hruby et al.(melanocortin receptor ligand).

L033

HLDF-6 HEXAPEPTIDE NEUROPROTECTIVE EFFECT ON RAT HIPPOCAMPAL NEURONS AT THE ALZHEIMER'S DISEASE MODEL IN VIVO AND IN VITRO

V.M. Lipkin[1], S.S. Zhokhov[1], Z.I. Storozheva[2], A.T. Proshin[2], E.A. Surina[1], I.I. Babichenko[3], I.A. Kostanyan[1]

[1]Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry [2]Anokghin Institute of Normal Physiology, Russian Academy of Medical Sciences [3]Russian People's Friendship University, Moscow, Russia

Previously we have isolated the HLDF (human leukemia differentiation factor), a small protein with molecular mass of 8.2 kDa from the culture medium of human promyelocyte leukemia HL-60 cells treated with retinoic acid. This protein induces differentiation of original cells via the granulocytic pathway. It was shown that HLDF had no specific receptors on the surface of HL-60 cells but could affect the fluidity of cell membrane and thus affect the binding of cytokines, involved in process of proliferation and differentiation.

A hexapeptide TGENHR (HLDF-6) was identified in the C-terminal part of the protein which retained the ability of the full-size factor to induce differentiation of HL-60 cells. This peptide was shown to possess antitumor and neuroprotective properties.

HLDF-6 hexapeptide neuroprotective effect has been studied on Alzheimer's disease model both in vivo and in vitro. The syndromes of this disease in male rats were induced by administration of beta-amyloid peptide (25-35) and ibotenic acid into the hippocampus. HLDF-6 peptide precludes long-term memory loss and exploratory activity decrease in such animals; furthermore, it reduces significantly the amount of pyknotic neurons in the CA1 area of hippocampus. The neuroprotective effect of this peptide upon beta-amyloid toxicity was also demonstrated in vitro on the initial cultures of rat hippocampal and cerebellar neurons. A possible mechanism of the peptide neuroprotective effect was suggested, which includes its influence on sexual steroid hormones biosynthesis and metabolism. Therefore HLDF-6 could be perspective remedy in Alzheimer's disease treatment.

L034

PACAP PEPTIDES AS NEW α-SECRETASE ACTIVATORS

E. Kojro[1], R. Postina[1], K. Gehrig-Burger[1], E. Wieczerzak[2], F. Fahrenholz[1]

[1]*Institute of Biochemistry, Johannes Gutenberg-University, Mainz, Germany*
[2]*Faculty of Chemistry, University of Gdansk, Poland*

Proteolytic cleavage of the amyloid precursor protein (APP) by α-secretase within the Aβ sequence precludes formation of amyloidogenic peptides and leads to a release of soluble APPsα, which has neuroprotective, anti-apoptotic and growth promoting properties. Therefore, pharmacological up-regulation of the α-secretase could be an approach for treatment of Alzheimer disease (AD). The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) has neurotrophic, neuroprotective as well as anti-apoptotic properties and is involved in learning and memory processes. Its specific G protein-coupled receptor PAC1 is expressed in several CNS regions including the hippocampal formation. Here we examined the effect of PAC1 receptor activation on α-secretase cleavage of amyloid precursor protein and the production of secreted APP (APPsα). Stimulation of endogenously expressed PAC1 receptors with PACAP in human neuroblastoma cells increased APPsα secretion which was completely inhibited by the PAC1 receptor specific antagonist PACAP (6-38). In HEK cells stably overexpressing functional PAC1 receptors PACAP-27 and PACAP-38 strongly stimulated α-secretase cleavage of APP. The PACAP-induced APPsα production was dose-dependent and saturable. This increase of α-secretase activity was completely abolished by hydroxamate-based metalloproteinase inhibitors including a preferential ADAM 10 inhibitor. By using several specific protein kinase inhibitors we show that the MAP-kinase pathway (including ERK1 and ERK2), and PI3-kinase mediate the PACAP-induced α-secretase activation. As PACAP neuropeptides are able to cross the blood brain barrier, they might be of therapeutic value for the treatment of AD. Therefore, we are currently testing PACAP peptides in an AD mouse model.

L035

POST-TRANSLATIONALLY MODIFIED PEPTIDES – NEW TARGETS IN AUTOIMMUNE DISEASE?

A.W. Purcell[1], K. Lau[1], B. Drummond[1], N.A. Williamson[1], L.J. Roberts[2], S.I. Mannering[3], L.C. Harrision[3]

[1]The Department of Biochemistry and Molecular Biology, The Bio21 Molecular Science And Biotechnology Institute [2]Department of Medicine, The University of Melbourne [3]The Walter and Eliza Hall Institute for Medical Research, Parkville, Vic., Australia

Background and Aims: With the elucidation of the human genome much attention has turned to post-transcriptional and post-translational mechanisms to generate the functional diversity thought to be required to explain the complexity of human life. Both these mechanisms result in a substantially more complicated array of expressed human proteins (proteome) than simply inferred from the genome. It has been estimated that up to 1 million different protein species may be found in a cell or tissue-specific proteome. Given the enormous complexity of the proteome, surprisingly little is known about how this diversity impacts on the immune system, and if recognition of such modified proteins by T lymphocytes occurs in health and disease. The formation and accumulation of post-translationally modified autoAgs in an inflammatory context has recently emerged as a new paradigm in human autoimmune disease.

Methods: We have used an immunoproteomic approach whereby T lymphocyte reactivity towards antigen preparations derived from recombinant and endogenous sources are used to highlight immunologically active material. Mass spectrometry is then used to characterise the peptide epitopes of interest.

Results: We observe T cell responses that are disease specific towards oxidatively modified autoantigens present in human pancreatic islet lysates in patients with type 1 diabetes and responses towards citrullinated peptides in patients with rheumatoid arthritis.

Conclusion: These studies highlight the important role post-translationally modified antigen plays in human autoimmune diseases. In addition to identifying novel peptides targeted by autoreactive T cells, the definition of the modification provides new insights into the pathophysiology of these diseases.

L036

MULTIPLEXED PROFILING OF PROTEASE AND KINASE SUBSTRATES WITH READOUT ON OLIGONUCLEOTIDE MICROARRAYS

M. Lebl, D.L. Barker, I.A. Kozlov, P.C. Melnyk, J.P. Hachmann, A. Srinivasan, M. Shults, N. Nelson, C. Zhao

Illumina, Inc., San Diego, CA, USA

We have developed a miniaturized and multiplexed assay for the measurement of protease and kinase activity in complex samples. This technology will accelerate research in functional proteomics and enable multiplexed protease inhibitor screens on a large scale.

The assay readout is based on Illumina's universal Sentrix® BeadArrays. The peptide sequences are conjugated to oligonucleotide sequences complementary to the oligo tags on randomly assembled and decoded bead arrays. The unique oligonucleotide part of each oligonucleotide-peptide conjugate is attached to the amino terminus of the peptide sequence. In the case of the kinase assay, peptides phosphorylated by kinases in solution are selectively chemically modified and detected after hybridization to an array of randomly assembled beads. For the protease assay, the peptide portion is C-terminally labeled with a biotin residue and contains a specific peptide sequence on the amino terminus. Upon protease cleavage, the biotin residue is detached from the oligonucleotide-peptide conjugate. Following the reaction, all biotin containing species are captured and removed by incubation with streptavidin beads. The cleaved conjugates that remain in solution are captured by hybridization of their oligo sequence to BeadArrays and detected by labeled antibody against a specific peptide sequence tag.

We have generated multiple sets of oligonucleotide tagged peptide substrates of different complexity (100 to 1500) and have shown that the response of individual substrates is independent of the mixture complexity. Our results demonstrated the possibility to perform the protease and kinase assay in a multiplexed environment with high sensitivity and minimal consumption of biological sample.

L037

CRYPTOMICS: IDENTIFICATION OF NOVEL BIOACTIVE PEPTIDES FROM INTACT PROTEINS

A.I. Smith[1], D.J. Autelitano[2], A. Rajic[2], M.C. Berndt[1], L.L. Ilag[2], M. Vadas[3]

[1]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Vic 3008, Australia Cryptome Pharmaceuticals [2]Cryptome Pharmaceuticals, Melbourne, VIC. [3]Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide, SA, Australia

Background: There is increasing evidence that proteolytic cleavage gives rise to 'hidden" peptides with bioactivities that are often unpredicted and totally distinct to the parent protein. The liberation of these cryptic peptides or crypteins has so far been shown to be prevalent in proteins associated with endocrine signalling, extracellular matrix, the complement cascade and milk. A broad spectrum of proteases has been implicated in the generation of natural crypteins that appear to play a role in modulating diverse biological processes such as angiogenesis, immune function and cell growth.

Method: Cryptomics is a new systematic and integrated approach of finding crypteins in vitro. It involves a reiterative proteomic based process of systematic proteolyitic fragmentation, chromatographic fractionation, screening for bioactivity and then proteomic identification of functional crypteins focussing on (but not limited to) the human cryptome.

Results: Novel crypteins with potent anti-coagulation and anti-proliferative properties derived from circulating human plasma proteins have been identified using this approach.

Conclusion: It is very likely that the human cryptome contains a plethora of undiscovered crypteins involved in regulating disease processes yet to be discovered and representing potential human therapeutics. It is also possible that additional crypteins in search of function or have not yet been subjected to the forces of natural selection reside in the cryptome.

L038

IANUS PEPTIDE ARRAYS: A NEW METHOD FOR DETECTION OF PROTEIN-LIGAND INTERACTION SITES

M. Malesevic[1], C. Yu[2], G. Jahreis[1], M. Schutkowski[3], C. Schiene-Fischer[1], C. Lücke[1], G. Fischer[1]

[1]Max Planck Research Unit for Enzymology of Protein Folding, Halle/Salle, Germany [2]Nuffield Department of Clinical Medicine, John Radcliff Hospital, Headington, Oxford, UK [3]Jerini AG, Berlin, Germany

Protein-protein and protein-ligand interactions are between few of the most important processes in living organisms. The understanding of those interactions gives us insights into fundamental events of the life cycle. Among others, peptide libraries prepared by spot synthesis on different solid carriers are powerful biotechnological tools for the study of these interactions. It is well known that the tertiary structure of proteins is essential for their biological functions, a property which is lacked by smaller peptides. However, it has already been shown that small peptide fragments can (i) adopt a well-defined secondary structure and (ii) retain some of the activity of the parent protein. In this work we are showing that protein-ligand interactions can be represented by and investigated as peptide-peptide interaction using peptide pairs that are immobilized on a solid support (Ianus peptide arrays).1 As a model system is the Streptavidin/Strep-tag II complex applied. Using a standard spot-technique, the streptavidin scans are synthesized on to one side of the orthogonal protected linker and labeled with a fluorescent dye. The labeled Strep-tag II peptide is synthesized on another side of the linker. Subsequently, peptide-peptide interactions in-between each peptide pair are analyzed by measuring the fluorescence intensity of dye labels. The Ianus-peptide array provides a novel high-throughput screening method for a preliminary mapping of unknown protein-ligand interaction sites.

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L039

NMR VIEWS OF PEPTIDES AND PROTEINS IN SOLUTION

K. Wüthrich

The Scripps Research Institute, La Jolla, CA, USA

Nuclear magnetic resonance (NMR) spectroscopy was first reported in 1946. For many years it was one of the focal areas of physics and chemistry, and then evolved as a pivotal method in biology and medicine. In my field of specialization, the use of solution NMR techniques for studies of peptides and proteins has been pursued at an ever increasing pace during the past 35 years. Over the decades, the "NMR view" of these biological macromolecules has greatly changed due to advances in NMR instrumentation, computer science, and the techniques of molecular biology, biochemistry and chemistry. In this lecture, recent NMR applications in structural biology and structural genomics will be placed in perspective relative to this historical background.

L040

LOOKING AT THE DYNAMICS OF PEPTIDE-RECEPTOR INTERACTION WITH TEMPERATURE DEPENDENT METHIONINE PROXIMITY ASSAY ON THE ANGIOTENSIN RECEPTOR AT1

J. Arsenault, M. Clément, M.-P. Lahaye-Renaud, M.-E. Beaulieu, R. Leduc, G. Guillemette, P. Lavigne, **E. Escher**

Department of Pharmacology, Faculté de Médecine et Sciences de la Santé, Université de Sherbrooke, QC, Canada

Rational drug design necessitates the knowledge of the structure of pharmacological targets in complex with their cognate ligands or therapeutic lead compounds. G-protein coupled receptors (GPCR) are a particular challenge since few instrumental analytical approaches have permitted to access directly to their native and liganded structures. Therefore mostly indirect methods are applied to gain structural information on free and liganded GPCR structures. We have extensively investigated the angiotensin II receptor AT1 by classical SAR, site-directed mutagenesis, homology modelling, various reactive ligand approaches and by studies on constitutive receptor mutants. Combination of those studies has allowed us to construct molecular structures of this receptor in complex with its natural ligand, angiotensin II. This structure of AT1 shows surprising similarities with the X-ray structure of bovine rhodopsin not only in the general GPCR structure but also in the ligand-receptor interaction: Many residues in contact with retinal are the same that contact AngII in AT1.

In the present contribution the structural mobility and the activation-related structural changes were assessed over a broad range of temperatures, including physiological temperatures. Methionine proximity assay studies performed at different temperatures on the AT1 receptor unveil a dynamic, temperature-dependent picture of the structure. Indeed, profound changes of particular peptide-protein interactions occur. Such changes are probably highly relevant to physiologically active receptor proteins and need to be considered for rational drug design.
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L041

NMR METHODS IN THE SEARCH OF COMPOUNDS ABLE TO DISRUPT THE BINDING OF VEGF TO ITS RECEPTOR

E. Giralt[1,2], R. Rodríguez-Mías[1], P. Tremmel[1], T. Tarragó[1]

[1]Biomedica Research Institute [2]Departament de QUÍMICA ORGÀNICA, Universitat de Barcelona, Spain

Vascular endothelial growth factor (VEGF) constitutes a very important therapeutic target due to its important role in promoting angiogenesis, especially in relation with tumour-growth and metastasis. Inhibition of the interaction of VEGF with its receptor constitutes a very promising strategy to fight again unwanted angiogenesis. We have used a variety of spectroscopic tools based on high-field NMR, to assist the design and evaluation of molecules able to interact with VEGF at the interface with its receptor. We have used E. coli expression of VEGF using both uniform and specific isotopic-labelling. We have found as the two more powerful approaches: i) 13C-filtered, 13C-decoupled, 1D-1H-NMR experiments using 13C-epsilon-Me-labelled samples that provide an entry to relatively high-throughput screening; and ii) differential chemical shift perturbation experiments using 1H-15N-HSQC uniformly-labelled VEGF. This last type of experiments allowed to circumvent the problems associated to the flexibility of the involved VEGF surface patch. Also from a methodological point of view, we have found that HSQC-monitored titration with a panel of organic solvents can be very useful to define the 'drugable' residues in the surface of a protein.

We have applied the NMR methods described above to screen a collection of aqueous extracts from plants used in traditional chinese medicine. We have been able to identify several active extracts. By chromatographic fractionation and spectroscopy we have identified some active molecules with flavone structure. We have synthesized a small collection of active derivatives that combine amino acid and peptides with the flavone moiety. Structure-activity relationship data will be reported.

L042

PLANT PHOTORECEPTOR PHYTOCHROME A AND B HAVE DIFFERENT DIMERIZATION MECHANISMS

T. Yamazaki[1], T. Kobayashi[2], M. Mishima[2], R. Tabata[2], R. Suzuki[1], M. Takano[3], C. Kojima[2]

[1]Biochemistry Department, National Institute of Agrobiological Sciences, Tsaukuba [2]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma [3]Plant Physiology Department, National Institute of Agrobiological Sciences, Tsukuba, Japan

Phytochrome is a dimeric chromoprotein that regulates plant growth and development through sensing the red and far-red light. The phytochrome molecule consists of an N-terminal photosensory domain with covalently linked chromophore and a C-terminal domain that contains signaling motifs such as a kinase and two PAS domains. The N- and C-domains are connected by a proteolytically-sensitive hinge region that includes phosphorylatable serine. The phytochrome function is regulated mainly by three molecular events: (1) photo-interconversion between two conformational states, (2) dimerization and (3) reversible phosphorylation within the hinge region. The C-terminal domain together with the hinge is responsible for the last two events. However, the precise mechanism that underlies phytochrome function is not clear because virtually no information about its 3D structure is available. Here we report comprehensive NMR studies of the PAS1 domains with the N-flanking hinge region of functionally distinct PhyA and PhyB, two major phytochromes out of three in rice. We found that the PhyA fragment exists as a monomer while the corresponding PhyB fragment forms a stable dimer. Importance of the N-flanking hinge region for the PhyB dimerization was clearly demonstrated because the deletion of the hinge resulted in the loss of the dimerization ability. A series of point mutation experiments suggest that positively charged residues within the hinge are crucial for the PhyB dimerization. The difference in the dimerization ability of the PAS1 domain with the N-flanking hinge region between PhyA and PhyB may participate in the difference of their physiological functions.

L043

CONFORMATIONAL STUDY OF SELF-ASSEMBLING PEPTIDES

G. D'Auria[1], Ma. Vacatello[1], L. Falcigno[1], R. Oliva[1], R. Gambaretto[2], M. Dettin[2], C. Di Bello[2], L. Paolillo[1]

[1]Department of Chemistry, University of Naples "Federico II", Napoli [2]Department of Chemical Process Engineering, University of Padua, Italy

Understanding the biological processes of molecular assembling is fundamental in the perspective of mimicking biological functions for applications in many fields (nanotechnology, biosensors, tissue engineering). Bio-mimetic nano-structured surfaces may be developed by using self assembling oligopeptides sequences. The sequence named EAK-16,1 (Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys)2, has shown a strong propensity to adopt a beta-sheet structure and to spontaneously self-aggregate forming, in presence of salts, non toxic membranes. Here we focus on a series of EAK-16 analogues (16- and 8-mer peptides) designed to decode the role of charge and hydrophobic factors in modulating the self-assembling properties. The analyses were carried out in solution by spectroscopic techniques in order to correlate conformational properties and self-assembling propensity of the monomers in several environments. For the 16-mer peptides the NMR measurements reveals that random/beta-structure conformational equilibria are present in solution. However the equilibrium is i) very slow on the NMR time scale and ii) beta-strands form aggregates insoluble or too large to contribute to the NMR spectra (high nuclear relaxation rates). Also for the 8-mer peptides random/beta-structure conformational equilibria (in fast exchange on the chemical shift and NOE time scales) are present in solution. Some propensity for beta-structure is exhibited by quite all the analysed 8-mer sequences. These sequences mainly differ by the length of charged side chains. Our finding suggests that the electrostatic interactions play in the beta-structure stabilization a minor role compared to the hydrophobic ones. 1.Zhang S., Holmes T., Lockshin C. and Rich A., PNAS., 90, 3334– 3338, 1992.

L044

PEPTIDE DERIVATIZED LIPOSOMES AS TARGET SPECIFIC MRI CONTRAST AGENTS

A. Accardo[1], D. Tesauro[1], M. Vaccaro[3], L. Paduano[3], J. Texeira[4], E. Gianolio[2], G. Morelli[1], **E. Benedetti**[1]

[1]Department of Biological Sciences & CIRPeB University of Naples "Federico II", Naples [2]Department of Chemistry, IFM, University of Turin [3]Department of Chemistry, University of Naples "Federico II", Naples, Italy [4]Laboratoire Léon Brillouin (CEA-CNRS) CEA-Saclay, Gif-Sur-Yvette, France

MRI is one of the most powerful non-invasive diagnostic methods in medicine. It gives very resolved images but, due to its very low sensitivity, needs higher concentration (10-4 M) of contrast agents such as paramagnetic Gd(III) complexes. Gadolinium complexes containing supramolecular aggregates (micelles and liposomes), present two interesting properties: an enhanced ability to increase solvent proton relaxation rates; and an increased life time of the contrast agent in the circulating blood by avoiding the typical extravasation. The target specifity of the contrast agents could be obtained by labeling the supramolecular aggregates with bioactive peptides able to address them on the specific biological target overexpressed by cancerous cells.

We report on the relaxivity behaviour and on the structural characterization of liposomes obtained by mixing two amphiphilic monomers: one of them containing a gadolinium complex and the other the bioactive CCK8 peptide able to bind the cholecystokinin receptors, overexpressed by several human tumours. Monomers were synthesized according to SPPS, based on Fmoc strategy. The assembly of mixed liposomes in different molar ratio is achieved by sonication and extrusion procedures. Features of the aggregates have been obtained through the AFM and SANS techniques. Their average size is 660 ± 250 Å. The new peptide derivatized liposomes for the high relaxitivity value (17.2 mM-1s-1 at 20MHz and 298K) and for the presence of a surface exposed peptride are very promising candidates as target selective MRI contrast agents.

L045

DRUG DELIVERY BASED ON GNRH-III AS TARGETING MOIETY

G. Mező[1], A. Jakab[1], I. Szabó[1], S. Bősze[1], R. Szabó[1], K.B. Bai[1], B. Kapuvári[2], M. Boldizsár[2], B. Vincze[2], O. Csuka[2], F. Hudecz[1,3]

[1]Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University [2]National Institute of Oncology [3]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

The local chemotherapy approaches using drug delivery systems, and special strategies such as receptor-mediated targeting have opened a new way to enhance the efficacy of chemotherapy with fewer side effects. Specific targeting ligands for cancer cells usually includes three main components: an apoptosis-inducing anticancer drug, a targeting moiety, and a carrier. As carrier molecule tetratuftsin derivative [TKPKG]4 was applied in this study. GnRH-III (Pyr-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH2) having antiproliferative activity itself was used as targeting moiety recognized by GnRH receptors overexpressed on breast, prostate and/or colorectal cancer cells. Drug molecules (e.g. doxorubicin, methotrexate) were attached to the carrier via an enzyme labile spacer (GFLG). Several drug-conjugates with different number and conjugation sites of the GnRH peptide were prepared (e.g. GnRH-K(GnRH)-[TKPK(Drug-GFLG)G]4-NH2 or Drug-GFLG-K(Drug-GFLG)-[TKPK(GnRH)G]4-NH2). Cytotoxicity of the compounds was characterised by MTT assay. Receptor binding and cellular uptake of the conjugates on MCF-7 human breast cancer cells and C-26 mouse colon carcinama cell lines were studied by flow-cytometer.

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L046

N-ACYLATED AMINO ACID DERIVATIVES AS SEROTONINERGIC RECEPTOR LIGANDS

P. Zajdel[1,2], G. Subra[1], A.J. Bojarski[3], B. Duszynska[3], E. Tatarczynska[4], E. Chojnacka-Wójcik[4], M. Pawlowski[2], J. Martinez[1]

[1]Laboratoire des Aminoacides, Peptides et Proteines, UMR 5810, Universite Montpellier I Et II, Montpellier, France [2]Department of Medicinal Chemistry, Jagiellonian University Medical College [3]Department of Medicinal Chemistry, Institute of Pharmacology [4]Department of New Drug Research, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

At a time of identification and determination of biological function of 5-HT1A and 5-HT2A receptors, the development of their novel ligands is still of considerable interest. Perhaps the most thoroughly studies class of ligands are long-chain arylpiperazines, which were shown to posses diversified ago/antagonistic pharmacological profile.1 Continuing our research in that field, we have developed new class of arylpiperazine derivatives containing N-acylated amino acid residues (aspartic acid, glutamic acid, asparagine, proline) in the amide fragment. In first stage a 132 member library was synthesized according to sort-and-combine approach on BAL linker SynPhaseTM Lanterns and 5-HT1A, 5-HT2A receptors affinity was estimated in the preliminary screening protocol.2 QSAR within the library were analyzed with Fujita-Ban method. Subsequently, the most active compounds were re-synthesized in solution, evaluated in full radioligand in vitro assays for 5-HT1A, 5-HT2A, D2 receptors, and their intrinsic activity at 5-HT1A and 5-HT2A was assessed in in vivo functional tests. The most potent 3-N-Cyclohexanoyl-amino-1-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-pyrrolidine-2,5-dione, showing 5-HT1A pre- and postsynaptic agonistic and 5-HT2A antagonistic activity, seemed to be particularly promising regarding its anxiolytic and antidepressant effect.

This study was partially supported by KBN Grant No. 2PO5F 042-26.

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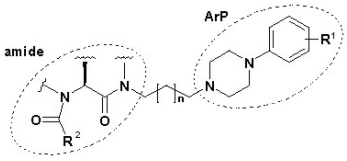

L047

NOVOKININ, AN ANGIOTENSIN AT2 AGONIST PEPTIDE, DECREASES FOOD INTAKE IN MICE

K. Ohinata, Y. Fujiwara, M. Yoshikawa

Graduate School of Agriculture, Kyoto University, Uji, Kyoto, Japan

Novokinin (RPLKPW) is an angiotensin AT2 receptor agonist, which has been designed by replacing 4 amino acids in a vasorelaxing hexapeptide ovokinin(2-7) (RADHPF) derived form ovalbumin. We previously reported that novokinin relaxed blood vessel and decreased blood pressure after oral administration through AT2 receptor. In this study, we found a novel function of novokinin on food intake.

Novokinin decreased food intake after intracerebroventricular and oral administration in fasted mice. The anorexigenic effect of centrally administered novokinin was blocked by a selective AT2 antagonist PD123319. In addition, angiotensin III, an endogenous AT2 agonist, also decreased food intake after central administration. Thus, AT2 signaling might play an important role in food intake regulation in the central nervous system.

Next, we investigated mechanism downstream of AT2 receptor in anorexigenic action of novokinin. The anorexigenic effect of novokinin was blocked by an antagonist for EP4 receptor among four receptor subtypes (EP1-4) for prostaglandin (PG) E2. We also found that an EP4 receptor agonist suppressed food intake in mice. Taken together, novokinin decreases food intake via AT2 receptor followed by PGE2 secretion and EP4 receptor activation.

L048

EXPLORING PROTEIN-LIGAND-INTERACTIONS THROUGH SYNTHETIC MIMICRY OF PROTEIN BINDING SITES

R. Franke, U. Strijowski, E. Sudarman, T. Hirsch, **J. Eichler**

German Research Centre for Biotechnology, Braunschweig, Germany

Protein-mediated biological processes are initiated by specific interactions between proteins and their ligands. The design and generation of molecules capable of mimicking the binding and/or functional sites of proteins, represents therefore a promising strategy for the exploration and modulation of protein function through controlled interference with the underlying binding events. In addition to their basic significance, such proteinmimetics are also useful tools for a range of biomedical applications, in particular the inhibition of protein-ligand interactions.

The binding sites of proteins are often not localized in short, continuous stretches of the amino acid sequence, but rather in sequentially distant fragments of the molecule, which are brought into spatial proximity by protein folding. Synthetic molecules aimed at mimicking such discontinuous protein binding sites should therefore also be conformationally constrained and/or sequentially discontinuous (1).

The synthetic basis of this concept are scaffolded and assembled peptides, in which protein-derived peptide fragments are presented through a molecular scaffold in a non-linear, discontinuous fashion. Recently, we have introduced strategies for the generation of structurally diverse scaffold molecules (2,3). This lecture will present the utilization of these strategies for the synthetic mimicry of discontinuous binding sites of a range of proteins, including interaction domains, cytokines, as well as viral proteins.

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L049

INTRA- AND INTERMOLECULAR FLEXIBILITY: A NEW VIEW ON PROTEIN-PROTEIN INTERACTIONS

A. Perczel

Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary

Biomolecular processes take place via the formation and dissociation of intramolecular complexes. Recently, increasing number of NMR and theoretical stdudies yield evidence that these complexes, either tight or loose, have considerable internal flexibility. The emerging new view on biomolecular interactions stresses the importance of the dynamical features of the partners which is often neglected during ligand or drug design. Peptides and proteins are excellent candidates to study and demonstrate the role of flexibility in biomolecular interactions. Not only alteration of the flexibility occurs upon complex formation, but, apparently, internal dynamics of the free components itself is tuned for the interaction1. Functionally disordered proteins represent an extreme case where unfolded-folded transition is required for target protein binding and it is presumed that conformational preferences characteristic of the bound form are already present in the unfolded state. Significance of the study of such systems reaches as far as understanding conformational diseases like prions and Alzheimer's. Examples to be presented include detailed NMR study of serine protease inhibitors involved in locust invasions and their exceptionally tight (Kd~10⁻¹²) complexes with proteases, Complement Control Proteins participating in immune response as well as partially or fully disordered proteins, such as dUTPase, a promising new target in cancer treatment and calpastatin, a protease inhibitor essential for storing and recalling memories in the human brain.

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L050

DEVELOPMENT OF NOVEL APAF-1 LIGANDS: CHEMICAL INHIBITORS OF CELLULAR APOPTOSIS

M.J. Vicent[1], L. Mondragon[1], M. Orzaez[1], G. Sanclimens[2], A. Messeguer[2], E. Perez-Paya[1,3]

[1]Department Medicinal Chemistry, Centro de Investigacion Principe Felipe, Valencia [2]Iiqab - Csic, Barcelona [3]Instituto de Biomedicina de Valencia, CSIC, Valencia, Spain

Apoptosis is an important process in a wide variety of biological systems, including normal cell turnover, the immune system, embryonic development and metamorphosis. Inappropriate apoptosis is involved in human pathology, including neurodegenerative diseases such as Alzheimer's and Huntington's and ischemia. From the screening of a diversity-oriented chemical library, we have identified a family of peptoids that inhibit the activity of the apoptosome, a macromolecular complex that activates mitochondrial-dependent apoptosis pathways. The active compounds rescued from the library were chemically optimized to obtain molecules that bind to both recombinant and human endogenous Apaf-1 in a cytochrome c-non competitive mechanism that inhibits the recruitment of procaspase-9 by the apoptosome. Furthermore, the analysis of cell compatible delivery systems of such peptoid inhibitors is presented. To obtain more cell permeable derivatives, firstly, the active peptoid was fused to cell penetrating peptides such as penetratin (PEN-peptoid) and HIV-1 TAT (TAT-peptoid). PEN-peptoid showed an enhanced cell viability and as a consequence better efficiency as apoptosis inhibitor than the TAT-peptoid. Then, a polymeric derivative, poly-(L-glutamic acid)-peptoid conjugate (PGA-peptoid), was also synthesised. In the mitochondrial-mediated models of cellular apoptosis analysed this new polymeric analogue has shown the best activity as inhibitor of apoptosis phenotype. In conclusion, our results contribute to reinforce the observation that the selection of an appropriate carrier is a key issue having an important influence on the therapeutic efficiency of an identified drug candidate.

L051

PROTEIN-PROTEIN INTERACTIONS OF 53BP2: REGULATION OF P53-MEDIATED APOPTOSIS

C. Katz[1], H. Benyamini[1], M. Lebendiker[2], S. Rüdiger[3], S. Rotem[1], **A. Friedler**[1]

[1]Department of Organic Chemistry [2]The Wolfson Center for Applied Structural Biology, The Hebrew University of Jerusalem, Israel [3]Cellular Protein Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, The Netherlands

The tumor suppressor protein p53 maintains genome integrity and prevents cancer in cells. Following oncogenic stress, p53 halts the malignant transformation by either promoting cell cycle arrest and DNA repair or by sending the cells with the potential to develop cancer to programmed cell death (apoptosis). The p53-binding protein 53BP2 stimulates the apoptotic p53 response. 53BP2 is a part of a larger protein called ASPP2, and is by itself a target for tight regulation. This is mediated by interacting with other proteins, among them are the anti-apoptotic protein Bcl-2, the NF-kappa-B and protein phosphatase 1. These interactions are poorly understood at the structural and molecular level. The research aim was to elucidate the role of the 53BP2 binding proteins in regulation of the protein, as a basis for understanding the mechanism of p53-mediated apoptosis. We have used a combination of experimental and computational methods, utilizing biochemical and biophysical techniques to identify the binding sites and the mechanism of interaction of 53BP2 with its partner proteins., we have used peptide mapping to identify the 53BP2-binding sites in the partner proteins. We have designed a membrane-bound peptide library, consisting of overlapping peptides derived from the proteins that are known to bind 53BP2. The peptide library was screened for binding 53BP2 in order to locate the binding sites for 53BP2 on these proteins. Binding peptides were found to match biologically important surfaces of the examined proteins. Based on our findings, possible mechanisms of action of 53BP2 in will be discussed.

L052

MOLECULAR APPROACHES FOR IMMUNO-THERAPY AND –DIAGNOSIS OF ALZHEIMER'S DISEASE BASED ON EPITOPE-SPECIFIC ANTI-ß-AMYLOID ANTIBODIES

M. Przybylski[1], R. Stefanescu[1], M. Bacher[2], M. Manea[1], A. Moise[1], I. Perdivara[1], A. Marquardt[1], R.C. Dodel[2]

[1]Department of Chemistry, Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, University of Konstanz [2]Departments of Neurology and Neuro-Psychiatry, University of Bonn, Germany

The accumulation of extracellular plaques containing the neurotoxic ß-amyloid peptide (Aß) of Aß precursor protein (APP) is characteristic of Alzheimer's disease (AD), but the (patho)physiological degradation pathways of APP are still unclear. Although immuno-therapeutic approaches for AD are currently finding much interest, an initial clinical trial in AD patients with Aß-specific antibodies - produced by immunisation with Aß(1-42) - that disaggregate Aß plaques was discontinued due to extensive neurotoxicity. Using proteolytic excision of the immobilised Aß antigen-immune complex in combination with high resolution FTICR-mass spectrometry, the Aß-plaque-specific epitope was identified at the N-terminal residues Aß(4 10), which are accessible in Aß(1-42) and in oligomeric Aß-protofibrils [1]. Most recently, Aß-autoantibodies in serum capable of eliciting a physiological, "protective" response inhibiting Aß-plaque formation have gained high interest. We have identified the epitope recognised by Aß-autoantibodies using high resolution-MS and epitope-excision. The epitope structure determinations of Aß-specific antibodies from healthy individuals and AD patients provide a breakthrough in (i), the development of new immuno-therapeutic approaches by immunisation with Aß-specific IgG, and (ii) the development of new molecular diagnostic tools for AD with absolute specificity [2]. Results of initial clinical studies with AD patients treated with serum IgG will be reported. Furthermore, our results point to a new, hitherto undefined, role of AD as an age-related autoimmune disease.

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[2] Przybylski, M., R.C. Dodel et al. (2006), Nature, submitted.

L053

HIGHLY SPECIFIC ANTIBODIES FOR USE IN SANDWICH-TYPE ANTIBODY MICROARRAY ANALYSES OF COMPLEX BIOLOGICAL SAMPLES

R. Rübenhagen, **R. Frank**

Department of Chemical Biology, German Research Centre for Biotechnology, Braunschweig, Germany

Antibody microarrays are the protein complement of DNA microarrays for proteome research and medical protein diagnostics. Antibodies represent ideal protein capture molecules due to their high robustness as well as their availability in almost unlimited diversity. However, a high specificity against the protein antigen is a prerequisite for use in antibody microarrays. Even subtle cross-reactivities to other proteins may severely limit the performance of this analytical tool, particularly when complex biological samples are to be analysed. This is even more eminent when two antibodies for capture and detection of the same analyte protein are required in a sandwich-type assay. To address this need and also to open new sources for highly specific antibodies we decided to exploit the comprehensive and diverse antibody pools available from polyclonal sera of immunized animals.

The concept takes advantage of our experience in peptide-based epitope mapping. A series of overlapping antigen-derived peptide fragments are synthesized in a macroarray format on a cellulose membrane (SPOT synthesis). Antibodies from a polyclonal serum bind their specific linear epitopes (peptide fragment) and thereby separate into epitope specific antibody pools. Bound antibodies are eluted from the peptide spots and then systematically evaluated under real assay conditions by screening the respective pools as detection antibodies on a microarray of capture antibodies together with the protein sample to be analysed. The most suitable pool/s then are selected for purification in larger quantities by peptide affinity chromatography. The subsequent generation of anti-epitope monoclonal antibodies will also be exemplified.

L054

HUMORAL RESPONSE AGAINST MYCOBACTERIAL HEPARIN-BINDING HEMAGGLUTININ : THE RÔLE OF METHYLATION STUDIED USING PEPTIDE MICROARRAYS

O. Melnyk[1], R. Desmet[1], E. Diesis[1], H. Drobecq[1], C. Rouanet[2], K. Chemlal[2], A.-S. Debrie[2], C. Locht[2], F. Mascart[3]

[1]UMR 8161 Biological Institute of Lille [2]U629, Pasteur and Biological Institute of Lille, France
[3]Erasme Hôspital, Brussels, Belgium

Infection by the members of the /Mycobacterium tuberculosis/ complex is an important cause of morbidity and mortality worldwide (3 million annual deaths, 8-10 million new cases yearly). /M. tuberculosis/ expresses on its surface a 28 kD adhesion called heparin binding hemagglutinin (HBHA).

This adhesin seems to be an important virulence factor since HBHA is involved in the interaction with non phagocytic cells and in extrapulmonary dissemination.

HBHA undergoes a post translational methylation resulting in the presence of mono and dimethylated lysine residues all located in the C-terminal part of the protein. The methylation was shown to play a key role in the immunogenicity of the protein. In humans antibodies against HBHA were detected both in patients with latent and active tuberculosis. However, only patients with latent TB displayed an antibody response directed against the C-terminal methylated domain of HBHA.

In search for a peptide that could be useful in the diagnosis of latent infections by /M. tuberculosis/, we have studied in a microarray format the interaction between mouse antibodies produced by immunization with rHBHA (/E. coli/ recombinant protein, non methylated), native HBHA from /BCG / (methylated) or infection with /M. Tuberculosis/ and a collection of peptides derived from the C-terminal Lys-rich region of HBHA and presenting different methylation motifs. A peptide that allowed the efficient capture of antibodies could be identified and will be evaluated for its interest in the diagnosis of latent tuberculosis.

K. Pethe et al. PNAS 2002, 99, 10759-10764.

L055

A PEPTIDE VACCINE AGAINST FOOT-AND-MOUTH DISEASE VIRUS

B.G. De la Torre[1], J. Bárcena[2], C. Cubillos[2], E. Borràs[1], A. Jakab[1], F. Sobrino[2,3], E. Blanco[2], **D. Andreu**[1]

[1]Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona [2]Animal Health Research Center (CISA-INIA), Valdeolmos [3]Severo Ochoa Center for Molecular Biology, Madrid, Spain

The immunogenicity and protection conferred to natural hosts of foot-and-mouth disease virus (FMDV) by a peptide vaccine containing immunorelevant FMDV epitopes will be described. Four test animals were immunized with the vaccine and 20 days after the last immunization were infected with the homologous virus. Three animals were fully protected against clinical disease; one developed a small mouth lesion at 4 d.p.i. that disappeared the next day. B- and T-cell responses were examined after vaccination as well as after challenge infection. Strong antibody response specific against FMDV was found, with neutralization titres around 2.3 log. The presence of virus-specific T-lymphocytes in peripheral blood mononuclear cells was also studied. All animals showed a strong T-cell response against FMDV and the peptide vaccine. Interestingly, no transmission from challenged animals was observed, as two naïve pigs, kept in the same box as contact controls, did not develop clinical signs of FMDV infection even after 10 days of contact. Yet at 14 days post-contact, these two animals were challenged and developed typical clinical FMD lesions at 3 d.p.i.

L056

MODULAR SYNTHESIS AND IMMUNOLOGICAL EVALUATION OF MUC-DERIVED GLYCOPROTEINE ANTIGENS

G.-A. Cremer[1], N. Bureaud[1], D. Lelievre[1], V. Piller[1], H. Kunz[2], F. Piller[1], **A.F. Delmas**[1]

[1]Cbm, Cnrs Upr4301, Orléans, France [2]Institut für Organische Chemie, Mainz, Germany

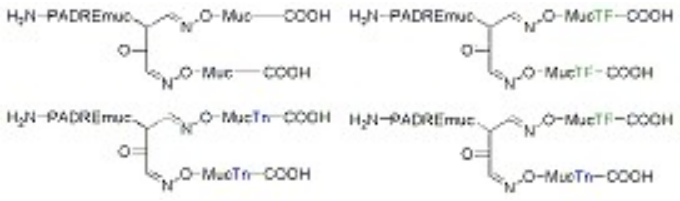
Our goal was to develop highly immunogenic mimics of MUC1, which should be able to induce an efficient immune response against the tumor-associated form of MUC1, but should be sufficiently different from the natural antigen to bypass the immunologic tolerance in humans. For this purpose, we synthesized analogues of the MUC1 protein composed of two MUC1 repeat units of inverse orientation and a universal T-helper epitope. The immunologically dominant MUC1 sequence PDTRP was glycosylated and modularly coupled by chemical ligation based on oxime chemistry to give a branched construct.

The tri-branched non-glycosylated peptide was synthesized first. The key intermediate was the stem peptide bearing two masked aldehydes, which can be successively unmasked by oxidation and acidolysis to yield the branched oxime-linked conjugate in a very satisfactory yield1. To synthesize small glycosylated proteins, two aminooxy peptides containing GalNAc (Tn) and Gal-GalNAc (TF) epitopes were synthesized on solid support according to Kunz.2 The deprotected aminooxy-containing glycopeptides were then used as building blocks for the synthesis of small glycoproteins. Three glycoproteins containing two GalNAc or two Gal-GalNAc epitopes or one GalNAc and one GalGalNAc epitopes were synthesized.

The multiantigenic constructs described here were highly immunogenic in mice eliciting high titer antibodies. Only the glycopeptide constructs induced antibodies able to recognize native tumor-associated MUC1 present on a human breast cancer cell line.

(1) G-A. Cremer et al 2004. Chem. Eur. J. 10: 6353

(2) H. Kunz. 2003. J. Peptide Sci. 9: 563



L057

TOWARDS THE DESIGN OF A PEPTIDE MIMETIC HIV VACCINE TARGETING THE VIRAL FUSION MACHINERY

A. Pessi[1], E. Bianchi[1], R. Geleziunas[2], S. Lennard[3], M. Luftig[1], P. Ingallinella[1], M. Finotto[1], R. Hrin[2], J. Joyce[2], X. Liang[2], M. Citron[2], R. Cortese[1], G. Barbato[1], A. Carfi[1], P.S. Kim[2], D. Hazuda[2], J. Shiver[2], M.D. Miller[2]

[1]IRBM P. Angeletti, Pomezia, Italy [2]Merck Research Laboratories, West Point, PA, USA [3]Cambridge Antibody Technology, Cambridge, UK

Because of the continuously shifting antigenic surface of the HIV-1 envelope glycoprotein, eliciting a broadly neutralizing humoral immune response has proven elusive. We focused our efforts on the most conserved part of the glycoprotein, the fusogenic subunit gp41. gp41 undergoes major conformational changes during fusion, culminating in the formation of a 6-helix bundle, where 3 alpha-helices of the heptad repeat region 2 (HR2) pack in antiparallel manner against a central trimeric coiled coil formed by the heptad repeat region 1 (HR1). During this process, HR1 and HR2 are transiently exposed. HR1 is highly conserved, and is the target of enfuvirtide, a peptide derived from HR2 used in the treatment of therapy-experienced HIV patients, which is believed to act by interfering with the assembly of the 6-helix structure.
To validate HR1 as a vaccine target, we used designed HR1 peptide mimetics as selectors from a phage antibody library, and identified a human antibody, D5, which blocks viral infection in vivo with an enfuvirtide-like mechanism [1]. It follows that the HR1 mimetics used in the selection are a promising starting point for the design of fusion-inhibiting vaccines, since they must at least partially mimic the true structures transiently exposed by the virus. Using a variety of biochemical assays and X-ray crystallography, we have now obtained detailed information on the D5-HR1 peptide interaction, which is being used to refine the design of D5-like antibody eliciting synthetic vaccines.

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L058

NEUROPEPTIDE ?CONVERSION TO BIOACTIVE FRAGMENTS - AN IMPORTANT PATHWAY IN ?NEUROMODULATION

F. Nyberg

Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

Previous and current research has revealed that a majority of neuroactive peptides induce their actions on cellular systems through specific receptors located on the cell surface. These receptors are known as G-protein coupled receptors, which mediate effects through interaction with ion channels or enzymes located within the cell membrane. Following binding and stimulation of their receptors to induce their cellular response the peptides are inactivated by enzymatic degradation. However, in many cases the active neuropeptides are enzymatically converted to products with retained bioactivity. These bioactive fragments may mimic but also counteract the action of the parent peptide. Thus, the released fragment may serve as a modulator of the response of the original compound. This phenomenon has been found to occur in a number of peptide systems, including the opioid peptides, tachykinins, as well as peptides belonging to the renin-angiotensin system, such as angiotensin II. In some cases the conversion product interact with the same receptor as the native compound but sometimes it appears that the released fragment interacts with receptors or binding sites distinct from those of the original peptide. This presentation deals with the formation and effects of peptide fragment released from opioid related peptides and substance P that have been shown to modulate the action of their parent compounds. For instance, the kappa receptor selective opioid peptide, dynorphin A, recognized for its ability to produce dysphoria when administrated into brain areas related to reward, is converted to the delta receptor agonist Leu-enkephalin, with euphoric properties. The tachykinins, typified by substance P (SP), which may be converted to the bioactive fragment SP(1-7), a heptapeptide mimicking some but opposes other effects of the parent peptide represents another peptide system of relevance in this context. The bioactive angiotensin II, known to bind to and stimulate the AT-1 and AT-2 receptor, is converted to angiotensin IV (i.e. angiotensin 3-8) with preference for the AT-4 receptor or to angiotensin (1-7), not recognized by any of these receptors. Thus, it seems that the activity of many neuroactive peptides is modulated by bioactive fragments, which are formed by the action of a variety of conversion reactions. This phenomenon appears to represent an important regulatory mechanism that occurs in many peptidergic systems in the CNS but is generally not acknowledged. The functional relevance of this type of neuropeptide modulation at many levels in the CNS will be discussed.

L059

SYNTHESIS AND CONFORMATIONAL STUDIES OF GLYCOSYLATED β *β* -PEPTIDES

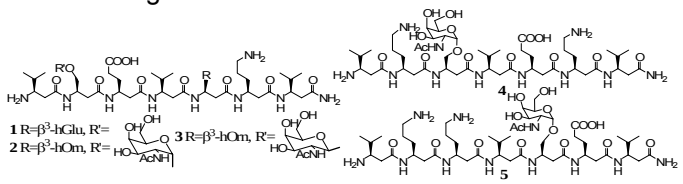
A.S. Norgren, P.I. Arvidsson

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

Oligomers of β-amino acids, so-called β-peptides, represent a new and exciting type of peptidomimetic foldamers. The β-amino acids differ from natural α-amino acids by the extra methylene group incorporated into their backbone. This leads to the formation of unnatural peptides, which possess more stable secondary structures than their natural analogues. In addition, the backbone modification makes these molecules stable towards proteolytic degradation.

The majority of proteins in nature are post-translationally modified, and the most common modifications involve attachment of carbohydrates, lipids, or phosphoryl residues to the protein. By studying these modifications, the localization and function of the proteins can be better understood. The most abundant post-translational modification is the protein glycosylation, which introduces wide structural variety to proteins. Glycoproteins have an important role in the biological recognition processes, such as immunodifferentiation, cell adhesion, cell differentiation and regulation of cell growth.

By solid-phase synthesis, five glycosylated β3-heptapeptides (1-5) were synthesized parallel to one referencing unglycosylated β3-peptide analogue to sequence 1. Investigation by CD measurements, NMR experiments, and theoretical Monte Carlo conformational search were made in the search for the sequence giving the most stable 314-helical secondary structure. Additionally, the interactions between these peptides and carbohydrate binding Lectins have been investigated.



L060

BISAMINOPHOSPHONATES AND THEIR PEPTIDIC DERIVATIVES AS POTENTIAL RECEPTORS FOR AMINO ACIDS.

P. Młynarz, S. Śliwińska, B. Bańkowski, A. Czernicka, P. Kafarski

Faculty of Chemistry, WrocłAw University of Technology, Wrocław, Poland

The host-guest interaction studies were performed for molecules having cationic Lys and Arg amino acids residues in the sequence. This high affinity of studied receptors is due to their strong selective binding for guanidino- and amino- group [1]. The use of phosphonate function gives the possibility of regulation of the molecule charge. This should directly influence electrostatic interactions between guest and host molecule as well as formation of networks of hydrogen bonds. Simultaneous use of the protonated amino group gives a possibility of hydrogen binding donor and positively charged moiety for electrostatic attraction for negatively charged parts of guest molecules.
In this work investigation of aminophosphonates compounds and their peptidic derivatives as amino acids binding molecules was performed. The receptor built on aryl skeletons and possessing aminophosphonic units in 1,4 position. Introduction of additional amino acids residues to starting entities efficiently enhanced the molecular recognition properties of the built receptors for Arg and Lys in methanol solution. Aminophosphonic receptors having estrified all phosphonate group by polyglycol derivatives has been found to be effective binders of cationic amino acids. Moreover, the receptors having the aminophosphonate units in the 1,4 positions should be only meso form regarding □-carbons bearing aminophosphonic groups [2], thus together with coupled amino acid residues may exhibit except molecular also the chiral recognition towards certain amino acids.

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L061

DIRECT ANTIPROLIFERATIVE EFFECT ON BREAST CANCER CELLS OF NEW LEUPROLIDE ANALOGUES WITH MODIFICATIONS IN POSITION 3 & 6

A.A. Zompra[1], E. Athanasopoulou[1], A. Thomopoulou[1], A. Nikolopoulou[2], B. Nock[2], Th. Maina[2], V. Magafa[1], F.N. Lamari[1], G.A. Spyroulias[1], N.K. Karamanos[3], P. Cordopatis[1]

[1]Department of Pharmacy, University of Patras, Patras [2]Institute of Radioisotopes-Radiodiagnostic Products, NCSR "Demokritos", Athens [3]Department of Chemistry, University of Patras, Greece

Analogues of GnRH have been widely used in oncology and gynaecology to induce reversible chemical castration. In addition to the classic hypophysiotropic action of GnRH, it has been shown that many malignant cells, such as breast cancer cells, secrete GnRH and express the GnRH receptor/s. Leuprolide is a synthetic analogue with agonistic properties and Active Pharmaceutical Ingredient (API) of peptide drugs. Furthermore, it has been shown that Leuprolide inhibits directly the proliferation of breast cancer cells.

In a continuation of our previous work and in order to study the effect of modifications in positions 3 and 6 of Leuprolide on breast cancer cell proliferation, we synthesized fifteen new conformationally restricted analogues. D-Leu6 of Leuprolide was substituted by a,a-dialkyl amino acids (Aib: α-aminoisobutyric acid, Deg: diethylglycine), D-Gly(tBu) and β-cycloexyl-D-alanine (D-Cha). Trp3 by D–Trp, D- and L-1,2,3,4,-tetrahydro-isoquinoline-3-carboxylic acid (Tic). Results show that the inhibitory effect on the proliferation of human breast cancer cells greatly depends on the nature of the substituted amino acid in position 6 and is usually increased by substitutions of Trp3 with non natural aromatic amino acids. This structure-activity relationship study for GnRH analogues contributes to the on-going research of GnRH binding requirements of breast cancer cells and provides new perspectives for the design of effective GnRH analogues.

L062

DESIGNER SELF-ASSEMBLING PEPTIDE MATERIALS

S. Zhang

Center for Biomedical Engineering, Center for Bits & Atoms
Massachusetts Institute of Technology
Cambridge, MA, USA

There are two complementary technologies that can be used in the fabricaction of new biological materials for nanomedical technology. In the 'top-down' approach, biomaterials are produced by polymerizing homogeneous monomers into covalently linked microfibers, sheet, coatings and other structures. This contrasts sharply from bottom up approach, where biological materials are assembled heterogeneous population of molecules to produce supra-structures and diverse architectures. The latter approach will become an integral part of biological materials production in the coming years. This approach requires our deep understanding of individual molecular building blocks, their structures, assembling properties and dynamic behaviors. Two key elements in molecular nanobiological material production are chemical complementarity and structural compatibility, both of which require the weak and noncovalent interactions that bring building blocks together during self-assembly. Significant advances have been made at the interface of materials chemistry and biology, including the design of helical ribbons, peptide nanofiber scaffolds for three-dimensional cell cultures, regeneration medicine, and molecular ink peptides for arbitrary printing and coating surfaces. These designer self-assembling peptides have far reaching implications in broad spectrum of applications in biology, medicine and nanobiomedical technology, some of which are beyond our current imaginations. Zhang is a member of American Association of Advancement for Science, American Society of Biochemistry and molecular Biology, the Human Genome Organization Americas, the Protein Society, New York Academy of Sciences, International Society for the Study of Origin of Life, and Sigma Xi. He is a 2003-2004 Fellow of Japan Society for Promotion of Science (JSPS fellow) and a 2005 Fellow of Japan Advancement for Medical Instrument. His work on designer peptide scaffold won 2004 R&D100 award. His and his colleagues' work on biosolar energy was selected to be the Top 100 Science Stories in 2004 by *Discover* Magazine. His work on bio-solar energy was selected to be one of 10 finalists of the *2005 Saatchi & Saatchi Award for World Changing Ideas.* He is one of the 2006 John Simon Guggenheim Fellows and 2006 recipient of the Wilhelm Exner Medal of Vienna, Austria.

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L063

DIRECTIONAL ELECTRON TRANSFER IN CONFORMATIONALLY CONSTRAINED, 3-10-HELICAL, OLIGOPEPTIDES

F. Formaggio[1], I. Guryanov[1], C Toniolo[1], Q.B. Broxterman[2], B. Kaptein[2], E. Gatto[3], A. Palleschi[3], L. Stella[3], M. Venanzi[3]

[1]Department of Chemistry, University of Padova, Padova, Italy [2]DSM Research, Life Sciences, Advanced Synthesis and Catalysis, Geleen, The Netherlands
[3]Department of Chemical Sciences and Technologies, University of Tor Vergata, Rome, Italy

Addressing and control of electron transfer (ET) processes are the main intermediate steps for the realization of molecular electronic devices. Peptide scaffolds functionalized by ET donor-acceptor (D/A) pairs show peculiar electron conductive properties, depending on the peptide secondary structure and side-chain arrangements. Specifically, the electric macrodipole associated to α-helical peptides has been previously shown to produce an electrostatic field oriented from the N-(δ+) to the C-(δ-) terminus that affects the efficiency and the rate of the ET process. For the present study we synthesized the following Nα-protected 8-peptide amides:
Z-Aib-Api(Pyr)-AMN-Aib-AMN-AMN-Aib-Api(Boc)-NHtBu (P2);
Z-Aib-Api(Pyr)-AMN-Aib-AMN-AMN-Aib-Api(Azu)-NHtBu (P2A8);
Z-Aib-Api(Azu)-AMN-Aib-AMN-AMN-Aib-Api(Pyr)-NhtBu (A2P8)
[Aib, α-aminoisobutyric acid; Api, 4-aminopiperidine-4-carboxylic acid; AMN, Cα-methylnorvaline; Pyr, 1-pyrenyl-carbonyl; Azu, 1-azulenyl-carbonyl]. P2A8 and A2P8 differ by the position of the D(Pyr) /A(Azu) pair in the chain and generate charge transfer states oriented along and opposite to the electric field direction, respectively. FTIR absorption, NMR, and CD spectra indicate that the peptides investigated overwhelmingly populate a 3-10-helix conformation, as expected from a sequence based exclusively on Cα-tetrasubstituted α-amino acids. Time-resolved fluorescence experiments show that the ET rate constant for A2P8 (kET=1.39·10exp8 1/s) is larger by a factor 2 than that measured for P2A8 (kET=0.67·10exp8 1/s). These results extend the effect of the peptide electrostatic field on the stability of the charge transfer pair generated by the photoinduced ET process to the 3-10-helix. Through-bond and through-space contributions were evaluated by studying the solvent dependence of the ET process. A theoretical conformational analysis was also carried out to gain information on the 3D-structure/ET efficiency relationship.

L064

CHEMICAL ENGINEERING OF PROTEINS USING SMALL COFACTORS

W.W. Li, W. Haehnel

Institute of Biology II/Biochemistry, Albert-Ludwigs-University Freiburg, Germany

Redox cofactors including quinone, heme, and flavin are ubiquitous and essential for a large number of biological complexes and redox enzymes. Novel protein mimetics have been created through chemical engineering with these small cofactors. Via chemical modifications, different natural quinone analogues have been introduced to yeast iso-1 cytochrome c (cyt c) 1 and human hemoglobin via thioether bond formation. Using de novo protein design, template assembled four-helix bundle protein scaffold2 was chosen as a host. A single ubiquinone-0 or menaquinone-0 molecule was introduced to create model quinoproteins.3 Heme was later introduced by non-covalently binding through coordination with His.2,4 Flavin was covalently incorporated through the synthesis of different Fmoc-protected flavin-containing amino acids.5 A number of four-helix bundle proteins of 11-14 kDa with one, two, and/or three cofactors have been chemically synthesized and biophysically characterized. In combination with these approaches, de novo peptides containing flavin were ligated to cyt c to yield flavocytochrome c. These constructs are useful to study the interactions between cofactors in redox proteins.

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L065

BACKBONE-MODIFIED AMYLIN DERIVATIVES: IMPLICATIONS FOR AMYLOID INHIBITOR DESIGN AND AS TEMPLATE FOR SELF-ASSEMBLED BIONANOMATERIALS

D.T.S. Rijkers, R.C. Elgersma, R.M.J. Liskamp

Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Utrecht, The Netherlands

Uncontrolled aggregation of proteins or polypeptides can be detrimental for normal cellular processes. Proteins/polypeptides which form these amyloid deposits differ in their primary sequence but share a common structural motif: a (anti)parallel beta-sheet. A well accepted approach to interfere with beta-sheet formation is the design of soluble beta-sheet peptides to disrupt the hydrogen bonding network leading to the disassembly of the aggregates or amyloid fibrils. We have synthesized amylin derivatives in which three amide bonds at alternate positions in the amyloidogenic sequence (20-29; SNNFGAILSS) have been modified by N-butylation or by incorporation of peptoid- or ester bond moieties. We found that these amylin derivatives were not able to form amyloid fibrils and were able to inhibit fibril growth of native amylin(20-29). Unexpectedly, these amylin derivatives were able to form large supramolecular assemblies like helical ribbons and peptide nanotubes in which beta-sheet formation was absent. Apparently, the increased hydrophobicity and the presence of essential amino acid side chains of the newly designed amylin(20-29) derivatives were found to be the driving force of self-assembly into helical ribbons and peptide nanotubes. The described amylin derivatives open up promising possibilities to further study the process of aggregation as well as to carry out SAR-studies in order to design bionanomaterials based on self-assembly of amyloid-derived peptides.