INSECT OOSTATIC PEPTIDES CONTAINING CYCLIC AND ISOSTERIC STRUCTURES

Jan Hlaváček[a], Jan Mařík[a], Miloš Buděšínský[a], Václav Kašička[a], Blanka Bennettová[b], Richard Tykva[a]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences, 166 10 Prague, Czech Republic; [b]Institute of Entomology, Academy of Sciences, 370 05 České Budějovice, Czech Republic.

A shortening of the oostatic decapeptide **H-Tyr-Asp-Pro-Ala-(Pro)$_6$-OH** [1] from the C-terminus or insertion of CH_2O bond between Pro and Ala residues in the shortened molecules accelerated and enhanced the oostatic activity in the flesh fly *Sarcophaga (now Neobellieria) bullata* [2,3]. Now, we prepared a new series of the shortened analogs containing a cyclic structure: **c(Tyr-Asp-Pro-X); 1a,** X=Ala and **1b,** X=Ala-Pro or the CH_2O bond between the Tyr and Asp residues in the aminoterminus: **X-Pro-Ala-Y; 2a,** X=H-Tyr-ψ[CH_2O]Asp, Y=OH and **2b,** X= H-Tyr-ψ[CH_2O]Asp, Y=Pro-OH. The peptides **1a, 1b** were prepared on 2-chlorotrityl chloride resin using Fmoc/tBu protection, DIC/HOBt coupling in DMF and Fmoc deprotection by 20% piperidine in DMF. The peptides were split off the resin by AcOH-anisole in DCM, cyclized by TPTU in DMF and the side-chain tBu protecting groups were removed by TFA-anisole. The peptides **2a, 2b** were prepared by fragment condensation of the pseudodipeptide Boc-Tyr-ψ[CH_2O]Asp(OBzl)-OH with H-Pro-Ala-OtBu or H-Pro-Ala-Pro-OtBu in solution, followed by deprotection with TFA-anisole and H_2/Pd in MeOH-DMF. The key step of the pseudodipeptide synthesis consists in a stereospecific conversion of the (5S)-N-protected-5-substituted morpholin-3-one, prepared by coupling of aminoalcohol with 2-chloropropionic acid ethyl ester, to (2S,5S)-2,5-substituted morpholin-3-one which is hydrolyzed to the protected pseudodipeptide building block. CZ electrophoresis was used to study an effect of the peptides shape and peptides bond isosteric replacement on their electromigration. NMR spectroscopy was applied in a discussion on relationships between the peptides conformation and their oostatic activity, assessed in the flesh fly *Neobellieria bullata.*

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STRUCTURAL COMPARISONS OF α-CONOTOXINS: NICOTINIC ACETYLCHOLINE RECEPTOR BLOCKERS

Robert W. Janes

School of Biological Sciences, Queen Mary & Westfield College, University of London, Mile End Road, London, E1 4NS, UK.

There are two principal subtypes of nicotinic acetylcholine receptors (nAchRs) present in mammals, the neuromuscular and the neuronal. They function to transport sodium or potassium ions across the postsynaptic membrane, hence enabling neurotransmission. The neuromuscular nAchRs comprise of five subunits, $\alpha_2\beta\gamma\delta$, arranged as a pseudo-symmetric pentamer, with two pockets at the α/γ and α/δ subunit interfaces forming the acetylcholine binding sites. The neuronal receptors are also pentameric structures, but being comprised of different arrangements of subunits, the two subtypes are separated in regard to their susceptibility to blocking by polypeptide toxins. α-Conotoxins are a family of small polypeptides from marine snails of the genus *Conus*, which specifically block nAchRs. Within the family there is usually a high degree of selectivity towards either neuromuscular or neuronal nAchRs, but there is also a wide variance in the degree of block, and in the selectivity towards the two different pockets within the neuromuscular nAchRs. A number of structures now exist for members of the α-conotoxin family, principally for those targeted towards the neuromuscular receptors. Structures also exist for some toxins that act on the neuronal receptors. Comparisons of these polypeptide toxin conformations provide insight into the specific regions that guide their selectivity towards the nAchRs and the nature of the acetylcholine binding pockets within the nAchRs. These comparative studies present the possibility of producing highly selective peptomimetic pharmaceutical agents.

SEQUENCES OF STILBOFLAVINS B, PEPTAIBOL ANTIBIOTICS FROM THE MOLD *STILBELLA FLAVIPES*

Andreas Jaworski, Hans Brückner

Interdisziplinary Research Center, Department of Food Sciences, Heinrich-Buff-Ring 26-32, Justus-Liebig-University, 35392 Giessen, Germany

The mold *Stilbella flavipes* CBS 146.81 was cultured in Rhaulin-Thom medium (4.7 L). From the culture broth a mixture of polypeptides of the peptaibol group (i.e. peptides containing a high proportion of α-aminoisobutyric acid [Aib, U] and a *C*-terminal bonded amino alcohol) could be isolated. The crude peptides were isolated by XAD-2 and Sephadex LH-20 chromatography. Three groups of microheterogeneous peptides, named stilboflavins (SF) A, B, C could be separated by repetitive preparative thin-layer chromatography. The sequences of the peptides of SF-B could be determined by on-line high-performance liquid chromatography-electrospray ionization mass spectrometry performed in the positive and negative ion mode [1]. The peptides are of weak antibiotic action against *Bacillus subtilis* and *Staphylococcus aureus* and excert hemolytic activities on erythrocytes. The structures of the major components of SF B are shown.

2	Ac	U	P	U	A	U	A	Q	U	L	U	G	U	U	P	V	U	U	Q	Q	Vol
4[1)]	Ac	U	P	U	A	U	A	Q	U	L	U	G	U	U	P	V	U	U	Q	Q	Lol
5	Ac	U	P	U	A	U	U	Q	U	L	U	G	U	U	P	V	U	U	Q	Q	Vol
7[2)]	Ac	U	P	U	A	U	U	Q	U	L	U	G	U	U	P	V	U	U	Q	Q	Lol
8	Ac	U	P	U	A	U	U	Q	U	V	U	G	U	U	P	V	U	U	Q	Q	Lol

Ac, acetyl; Vol, valinol; Lol, leucinol; chiral amino acids and amino alcohols are of the L-configuration; identical to [1)] Hypelcin A II, [2)] Hypelcin A I [2].

Notably, we have recently also characterized peptaibols of the antiamoebin family from *Stilbella erythrocephala* ATCC 28144 and *Stilbella fimetaria* CBS 548.84 [3]. The results demonstrate that the genus *Stilbella*, in analogy to *Trichoderma*, is a rich source of bioactive Aib-peptides.

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PEPTIDYL ALDEHYDES BASED ON N-TERMINAL BINDING FRAGMENTS OF CYSTATINS AS CATHEPSINS INHIBITORS

Regina Kasprzykowska[a], Franciszek Kasprzykowski[a], Katarzyna Rutkowska[a], Anders Grubb[b],

[a] Faculty of Chemistry, University of Gdansk, Poland; [b] Department of Clinical Chemistry, University Hospital, Lund, Sweden.

It is well known that cathepsin K, a recently identified member of the papain superfamily of cysteine proteinases, is expressed selectively in osteoclasts and is the predominant thioproteinase in these cells. Cathepsin K has been proposed to play a key role in osteoclast–mediated bone resorption. We have shown previously that human cystatin C, the natural cysteine proteinase inhibitor synthesised in most tissues, strongly inhibits mineral mobilisation and bone matrix degradation. Additionally, we have found that the low-molecular peptidyl diazomethane, Z-Arg-Leu-Val-Gly-CHN_2, with peptidyl portion modelled on the cysteine proteinase interacting N-terminal segment of human cystatin C has a similar inhibitory effect on bone resorption. Unfortunately, the above mentioned diazomethane derivative posses some undesired features, e.g. poor selectivity, photolability, unstability in acidic conditions and strong toxicity. These imperfections disqualify diazoketones as candidates to be promising antiosteoporotic drugs. To investigate if any of low-molecular peptidyl derivatives based on the cysteine proteinase interacting segments of selected cystatins show the inhibitory activity against cathepsin K we evaluated a series of N-benzyloxycarbonyl-peptidyl aldehydes. This work demonstrates the syntheses of the above mentioned compounds and their proteinase inhibitory properties.

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INHIBITION OF SERINE PROTEASES AND NEURONAL CALCIUM CHANNELS BY LOCUST PEPTIDES

C. Kellenberger,[a] S. Kaneko,[b] and B. Luu [a]
[a] Laboratoire de Chimie Organique des Substances Naturelles, UMR 7509 CNRS/ULP, Strasbourg, France.
[b] Laboratory of Neuropharmacology, Kyoto University, Japan

In the past years, our laboratory has characterized a novel family of cysteine-rich peptides from the insect *Locusta migratoria*. These peptides, named PMP-C, PMP-D2 and HI, were chemically synthesized and their tertiary structures and functions were studied. We showed that PMP-C is a potent inhibitor of mammalian chymotrypsin while PMP-D2 and HI are converted into potent inhibitors by one single mutation (Kellenberger et al, *J. Biol. Chem.*,1995). In some preliminary experiments, we also showed that these peptides attenuate high voltage-activated calcium currents in rat neurones (Scott et al, *Neuropharmacology*, 1997)
In this report, we present a more detailed study of the inhibitory properties of PMP-D2 toward various trypsins (including locust trypsin) and point out some selectivity.
We also report (1) the effects of these peptides on N, L and P/Q calcium channels expressed in xenopus oocytes and (2) the activities of iodinated PMP peptides and some analogs, from binding experiments.

DECAPEPTIDE THAT CAN CURE PROSTATIC CANCER?

Péter Kele[a], Awtar Krishan[b], Roger M. Leblanc[a*]
[a]University of Miami, Department of Chemistry, Coral Gables, Florida USA
[b]University of Miami, School of Medicine, Miami, Florida USA

Several endogenous proteins are involved in normal growth and functioning of human prostate. One of these proteins is the prostate inhibin, (also referred as prostate secretory protein PSP-94) a 94 amino acid, non-glycosylated protein, (1) has growth inhibitory effects on prostate tumor cells *in vitro* and *in vivo* (2). The action is believed to take place by down-regulating follicle-stimulating hormone (FSH) by binding to its receptor (3).
A synthetic analogue of the ^{85}Lys-Thr-Cys-Ser-Val-Ser-Glu-Trp-Ile-Ile^{94} sequence, in which ^{85}Lys was replaced by Tyr and ^{87}Cys was protected by acetamidomethyl group is the shortest peptide, reported so far, having inhibin like activity (4). The effect of this decapeptide was similar to that of the whole protein, which suggests the possibility to seek for analogues that are as or more potent in FSH suppressed cell regulation as PSP-94.
In recent studies, a series of decapeptides were synthesized in which Gly replaced each amino acid, respectively, in the ^{85}Tyr-Thr-Cys(Acm)-Ser-Val-Ser-Glu-Trp-Ile-Ile^{94} sequence. These peptides were tested on PC3 cells, a prostatic cancer cell line, to find out the positions that are responsible for the receptor binding. These positions are used then to make libraries, using the portioning-mixing method (5).

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SYNTHESIS OF SUBSTANCE P C-TERMINAL ANALOGS AND STUDIES OF THEIR ANTIPROLIFERATIVE ACTIVITY *in vitro*

George Korakakis[1], Vassiliki Magafa[2], Maria Liakopoulou-Kyriakides[3], Alekos Kortsaris[4] and George Stavropoulos[1]
Departments of [1]Chemistry and [2]Pharmacy, University of Patras, [3]Department of Chemical Engineering, University of Thessaloniki, [4]Theagenion Cancer Institute, Thessaloniki, Greece.

Small peptides currently are under investigation as possible anti-tumor agents. Thus tetrapeptides, analogs of AS-I toxin, like Cys-Val-Gly-Gln, showed significant antiproliferative activity[1] on the cancer cell lines HT29, HeLa and T47D.
Broad-spectrum neuropeptide antagonists could be used as therapeutic agents for small cell lung cancer (SCLC). The analogs [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]SP and [Arg^6,D-Trp^{7,9},MePhe^8]SP$_{6-11}$, which block the biological effects of a broad range of neuropeptides, also inhibit SCLC cell proliferation *in vitro* and *in vivo*[2]. The effects of SP or SP$_{4-11}$ analogs have recently been investigated on the constitutive and/or lipopolysaccharide (LPS)-induced expression of IL-10 and TNF-α. The results showed that SP and SP$_{4-11}$ C-terminal analogs increased TNF-α secretion, while they showed little effects on IL-10 secretion[3].
In the present study the analogs [Glp^6,Glu(Bu^t)^{11}]SP$_{6-11}$ **(I)**, [Glp^5,Glu(Bu^t)^{11}]SP$_{5-11}$ **(II)**, [Hyp^4,Glu(Bu^t)^{11}]SP$_{4-11}$ **(III)**, [Pro^4,Glu(Bu^t)^{11}]SP$_{4-11}$ **(IV)**, [Nip^4,Glu(Bu^t)^{11}]SP$_{4-11}$ **(V)**, [Inp^4,Glu(Bu^t)^{11}]SP$_{4-11}$ **(VI)**, [Nip^4,Glu(Bzl)^{11}]SP$_{4-11}$ **(VII)**, and [Inp^4,Glu(Bzl)^{11}]SP$_{4-11}$ **(VIII)** of the SP C-terminal fragments have been synthesized by stepwise synthesis or SPPS. The deprotected analogs were purified (HPLC), identified (FT-IR, ES-MS, ^{1}H-NMR) and tested for their antiproliferative activity in the cancer cell lines HT29, HeLa, and T47D, using the sulforhodamine B (SRB) colorimetric bioassay method, while for their cytotoxicity to normal cells the line L929 (mouse fibroplast) was used. The analogs **(I)** and **(II)**, with Glp as N-terminal amino acid and Glu(Bu^t) in place of Met^{11} of SP, showed significant inhibition in the HeLa, and T47D cancer cell lines proliferation. It is remarkable to mention that none of these analogs contains D-amino acids, incorporated in other peptides for antiproliferation studies.

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ENGINEERING OF BACTERIAL SURFACE FOR ENHANCED BIOACCUMULATION OF HEAVY METALS

Pavel Kotrba[a], Radka Saldova[a], Romana Bohuslavova[a], Michaela Rumlova[b], Lucie Doleckova[b], Tomas Ruml[a]
[a]Institute of Chemical Technology, Technicka 5, 166 10 Prague 6, Czech Republic; [b]Institute of Organic Chemistry and Biochemistry, 166 10 Prague 6, Czech Republic

In the last decade an increasing attention is being paid to the use of microorganisms for the recovery of heavy metals from the ecosystem. The low cost and higher efficiency represent the main advantages of the biotechnological processes in comparison to the physicochemical method for heavy metal removal. We initially selected two synthetic peptides based on their affinity to cadmium. The first one was Gly-His-His-Pro-His-Gly that is present as a triple repeat in human plasma membrane transporter known as the histidine rich glycoprotein. The second peptide of Gly-Cys-Gly-Cys-Pro-Cys-Gly-Cys-Gly was selected based on screening of position peptide library on a cotton support. Corresponding DNA sequences encoding selected peptides were designed and cloned as a fusion to the bacterial transmembrane protein to ensure the surface localization of the heavy metal binding domains. The hybrid surface proteins were efficiently expressed. The localization on the bacterial outer membrane was confirmed by Western blott analysis of isolated cell envelopes and by the susceptibility of the cell surface to release the bound metal by washing under stringent conditions. Resulting genetically modified bacteria exhibited significantly enhanced accumulation of cadmium. The selectivity for cadmium was tested using equimolar metal mixtures with zinc and copper.

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ANTIMICROBIAL ACTIVITY AND STRUCTURAL STUDY OF TWO CLASS II BACTERIOCINS : MESENTEROCIN 52B AND LEUCOCIN B-33A.

François Krier[a], Catherine Corbier[b], Bernard Vitoux[b], Anne-Marie Revol[a].
[a] Laboratoire de Fermentations et Bioconversions Industrielles, ENSAIA- INPL, 2, avenue de la Forêt de Haye, 54 505 Vandoeuvre-lès-Nancy Cédex France. [b]Lcm3b. ESA7036. UHP-Nancy I. BP239. 54506 Vandoeuvre-lès-Nancy Cédex France.

Lactic acid bacteria produce various antimicrobial compounds such as bacteriocins. These molecules are proteins or protein complexes that are active against closely related bacterial species. They constitute a large family of polypeptides, which can be subdivided into different classes based on their mode of action and their structure [1].
This study focus on two class II bacteriocins, mesenterocin 52B (*Ln. mesenteroides* subsp. *mesenteroides* FR52) and leucocinB-TA33a (*Ln. mesenteroides* TA33a). These two bacteriocins share 62% sequence identity, do not possess the YGNGV consensus sequence and exhibit a narrow spectrum of activity limited to *Leuconostoc* and *Weissella* genus, whereas the other bacteriocins produced by *Leuconostoc* sp. strains display a wide spectrum of activity.
Because of their potential for use as antimicrobial additives, most studies focus on lantibiotics and class IIa bacteriocins. They have been the subject of extensive biochemical and genetic characterisation, including their mode of action and structural studies. In opposite, few is known concerning the structure-activity relationships of bacteriocins having a very narrow activity spectrum. In order to investigate the activity and the structure of mesenterocin 52B and leucocin B-TA33a, synthetic peptides of both bacteriocins have been prepared. These peptides display the same antibacterial activities than the natural purified peptides [2].
This poster presents the characterisation of the biological activity of both peptides as well as the results of circular dichroism experiments carried out either in aqueous or in micellar media. It comes out that despite a strong sequence identity and a similar behaviour in membrane mimicking environments, the two peptides display very different antibacterial activities, dependent of the indicator strain.

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INHIBITION OF HUMAN MULTIDRUG RESISTANCE P-GLYCOPROTEIN 1 BY ANALOGUES OF THE POTENT δ-OPIOID ANTAGONIST, DMT-TIC

Lawrence H. Lazarus,[a] Tara Lovekamp,[a] Peter S. Cooper,[b] Jenny Hardison,[a] Sharon D. Bryant[a], Remo Guerrini,[c] Gianfranco Balboni,[d] and Severo Salvadori[c]
[a]Peptide Neurochemistry, LCBRA, NIEHS, Research Triangle Park, NC 27709; [b]NCBI, NLM, Bethesda, MD 20894; [c]Department of Pharmaceutical Science and Biotechnology Center, University of Ferrara, I-44100 Ferrara, Italy; [d]Department of Toxicology, University of Cagliary, I-09126 Cagliary, Italy

Human multidrug resistance glycoprotein 1 (hMDR1) is expressed in a variety of normal human tissues and plays an important physiological role in maintenance of the blood-brain barrier (BBB) in addition to a role in drug resistance by pumping drugs out from cells. In an effort to design δ-opioid antagonists of Dmt-Tic (2',6'-dimethyl-L-tyrosine-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) that would more efficiently cross the BBB, we focused on the addition of hydrophobic substituents at the N- and C-termini. Hydrophobic analogues inhibited hMDR1 P-GP expressed in a G-185 fibroblast cell line in a dose dependent manner. Inhibition was comparable to the standard verapamil. The Dmt-Tic analogues containing C-terminal 1-adamantyl amide or *tert*-butyl amide were the most efficacious in the order of *N,N*(Me)$_2$-Dmt-Tic-NH-1-adamantane,H-Dmt-Tic-NH-1-adamantane>*N,N*(Me)$_2$-Dmt-Tic-NH-*t*But > H-Dmt-Tic-Ala-NH-1-adamantane. Other inhibitory analogues included *N,N*(Et)$_2$-Dmt-Tic-OH > *cyclo*(Dmt-Tic) > H-Dmt-Tic-Ala-NH-*tert*-butyl amide. Inactive opioid peptides included deltorphin, dermorphin and hydrophobic analogues thereof, DPDPE, DAGO, DAMME, non-peptide opiates (naltrexone, naltrindole) in addition to numerous other opioids. Whereas *N,N*(Me)$_2$-Dmt-Tic-NH-1-adamantane exhibits high δ- and μ-receptorbinding properties as well as bifunctional δ antagonism and μ agonism *in vitro*, H-Dmt-Tic-NH-1-adamantane was devoid of bioactivty in spite of high receptor affinities. Interestingly, *N*-alkylation by dimethyl groups enhances biological properties of the Dmt-Tic by 20-fold, yet the free acid derivative was inactive to inhibit hMDR1. Our results demonstrate that enhanced hydrophobic and lipophilic derivatives of Dmt-Tic conferred an inhibitory activity toward hMDR1. These compounds could have a potential role as chemosensitizing agents in a "two-pronged strategy" in combination with a chemotoxic agent for combating cancers containing hMDR1. In particular, H-Dmt-Tic-NH-1-adamantane could be an ideal synergistic drug of choice due to its lack of bioactivity.

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QSAR ANALYSIS OF TRYPANOSOMAL CYSTEINE PROTEASES: INVESTIGATION OF THE S2 SUBSITE THROUGH THE USE OF PHE ANALOGS

Fabien Lecaille[1], Sylvie Mavel[2], Thierry Moreau[1], Carole Serveau[1], Claire Desmazes[1], Francis Gauthier[1] and Gilles Lalmanach[1].
[1] Proteases and Vectorisation, INSERM EMI-U 00-10, Laboratory of Enzymology and Protein Chemistry, Faculty of Medicine, 2 bis Bd Tonnellé, 37032 Tours cedex, France. tel : + (33) 2 47 36 61 51 – email : lecaille@yahoo.com
[2]Laboratory of Organic Chemistry, Faculty of Pharmaceutical Sciences, Tours.

Evidence has accumulated over the past few years showing that trypanosomal cysteine proteases (cruzain, congopain) play a critical role in invasion of the host and alteration of the immune response. They represent attractive targets to define new rational antiparasite drugs. However the main drawback of this strategy is that these enzymes have a substrate specificity similar to that of their mammalian homologues (lysosomal cathepsins L and B) thus representing an obstacle in the design of specific inhibitors. In the present work, we have introduced non-proteogenic structural analogs of Phe into fluorogenic substrates of the series dansyl-Xaa-Arg-Ala-Pro-Trp (Xaa = P2 residue) to investigate the S2 subsite (the major determinant of specificity) of parasite cysteine proteases and their host enzymes. Kinetic constants (Km, kcat/Km) were improved for congopain and cruzain by mono-, di-chlorination or nitration of Phe, by contrast with structurally constrained Phe analogs. Furthermore, the loss of aromaticity is not critical for interaction, since presence of a saturated cycle (i.e. cyclohexylalanine Cha) significantly improved the Km. LUDI link scores of modelled Phe analogs were in good agreement with enzymatic affinity constants. A linear relationship might be established with logP, suggesting that fitting into the S2 subsite increase with the hydrophobicity of Phe analogs.

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ANALOGUES OF OXYTOCIN CONFORMATIONALLY RESTRICTED IN THE N-TERMINAL PART OF THE MOLECULE

Elżbieta Łempicka[1], Izabela Derdowska[1], Bernard Lammek[1], Jirina Slaninova [2].
[1] Faculty of Chemistry, University of Gdańsk, 80-952 Gdańsk, Poland.
[2] Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Prague, Czech Republic.

We described the synthesis and some pharmacological properties of four new oxytocin (OT) analogues. Three of them contain dipeptide D-Phe-D-Phe with ethylene bridge spanning two subsequent peptide nitrogens. We decided to check how such type of modification, which previously resulted in interesting arginine vasopressin analogues, will change the pharmacological properties of oxytocin derivatives. We also synthesized [(N-Me-D-Phe)^{2,3}]OT. This compound may be considered to be an analogue of [(D-Phe-D-Phe)^{2,3}]OT in which the ethylene bridge is replaced by two N-methyl groups. Such modification should preserve the chemical character of the peptide, but remove its additional conformational constraints.

Some pharmacological properties of the analogues were determined by the following tests: uterotonic test *in vitro* in the absence of magnesium ions, the vasopressor test using phenoxybenzamine-treated male rats and the antidiuretic test on conscious rats. The results obtained in the present studies demonstrated that the modification proposed resulted in either the removal or a great drop of all activities checked. However, it is interesting that although our modification introduced into the known oxytocin antagonist [1-mercaptocyclohexaneacetic acid)^1]OT resulted in a compound with approximately two orders of magnitude lower antidiuretic activity, this peptide is very selective. As regards [(N-Me-D-Phe)^{2,3}]OT, although its antioxytocic activity is again not very high, this peptide is interesting, especially when one realizes that it may be considered as an analogue of [(D-Phe-D-Phe)^{2,3}]OT in which the ethylene bridge is replaced by two methyl groups. Such modification i.e. cutting the ethylene bridge, converted the inactive peptide into a very selective blocker of oxytocin receptors *in vitro*.

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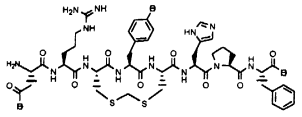
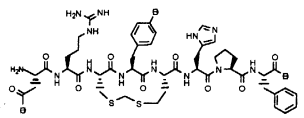
S-CH_2-S CYCLIZED ANALOGUES OF ANGIOTENSIN II

Susanna Lindman, Gunnar Lindeberg, Anders Karlén and Anders Hallberg.
Dept. of Organic Pharmaceutical Chemistry, BMC, P.O 574, Uppsala University, SE-751 23 Uppsala, SWEDEN

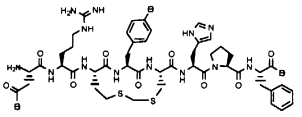
Conformational restriction of a flexible linear peptide by cyclization is a general method to study its bioactive conformation. Spear[1] and we[2] have previously applied this strategy on the hypertensive octapeptide angiotensin II (Ang II, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). The cyclic peptides c[$Hcy^{3,5}$]-Ang II, c[$Cys^{3,5}$]-Ang II and c[$Pen^{3,5}$]-Ang II showed significant activity at Ang II receptors. Methylenedithioether cyclization constitutes a complement to the above disulfide-based cyclization procedure. Recently a new method for S-CH_2-S bridging was reported.[3] We have applied this simple procedure to synthesize the Ang II analogues **1-4** as a comparison to the disulfide analogues. Their binding affinities to Ang II receptors will be reported.



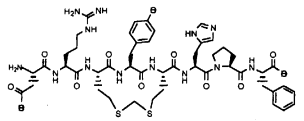
cyclo(S-CH_2-S)[$Cys^{3,5}$]Ang II (1)



cyclo(S-CH_2-S)[Cys^3Hcy^5]Ang II (2)



cyclo(S-CH_2-S)[Hcy^3Cys^5]Ang II (3)



cyclo(S-CH_2-S)[$Hcy^{3,5}$]Ang II (4)

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BIOLOGICALLY ACTIVE PEPTIDE FROM HUMAN LEUKEMIA DIFFERENTIATION FACTOR. IDENTIFICATION AND PROPERTIES.

V.M. Lipkin, I.A. Kostanyan, M.V. Astapova, A.P. Bogachuk, S. M. Dranitsyna, I.M. Molotkovskaja, T.N. Lepekhova[a], E.V. Navolotskaja[a], I.L. Rodionov[a], G.B. Telegin[a]
Shemyakin&Ovchinnikov Institute of Bioorganic Chemistry RAN Moscow, Russia; [a]FIBCh RAN, Moscow region, Russia,

Human Leukemia Differentiation Factor (HLDF), isolated from culture medium of human promyelocytic HL-60 line cells, treated with retinoic acid, has been studied. During investigation the HLDF six-membered peptide (HLDF6) was identified, which kept the ability of the whole factor to cause differentiation and to stop proliferation of HL-60 cells. The HLDF6 peptide was synthesized by solid-phase method. We failed to detect the receptors to HLDF6 peptide on the surface of HL-60 cells. HLDF6 was shown to influence on binding of IL-1β, cytokine participating in proliferation processes. The antitumour activity of peptide was revealed on the animal model of reinjected NSO myeloma.

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IDENTIFICATION OF AN HEXAPEPTIDE WITH SPECIFIC ACTIVITY AGAINST PHYTOPATHOGENIC FUNGI.

Belén López-García[a,b], Luis González-Candelas[b], Jose F. Marcos[b], Enrique Pérez-Payá[a]
[a]Unitat d'Enginyeria de Pèptids i Proteïnes. Departament de Bioquímica i Biología Molecular, Universitat de Valencia, Spain; [b]Departamento de Ciencia de los Alimentos, Instituto de Agroquímica y Tecnología de Alimentos (IATA)-CSIC, Valencia, Spain.

Fungicide applications are a usual practice to fight against plant diseases caused by filamentous fungi. However, there are some disadvantages to the current use of fungicides, and therefore it is important to search for alternative disease management strategies and/or safer antifungal agents, which could substitute them.
Our groups are interested in the utilisation of combinatorial peptide libraries to identify peptides with antifungal activity. An hexapeptide of amino acid sequence Ac-Arg-Lys-Thr-Trp-Phe-Trp-NH_2 was demonstrated to have activity against fungi causing postharvest decay in fruits. The peptide synthesized with either all D- or all L- amino acids inhibited the *in vitro* growth of strains of *Penicillium digitatum*, *Penicillium italicum* and *Botrytis cinerea*, with minimum inhibitory concentrations (MIC) of 60-80 μM and IC_{50} of 30-40 μM, depending of fungus. The hexapeptide retarded mould diseases of fruits under controlled inoculation conditions, thus providing evidence for the feasibility of using very short peptides in plant protection. The inhibitory activity of the peptide was both sequence- and fungus- specific since: (i) sequence-related peptides (including one with five amino acid residues identical to the active sequence) did not show activity, and (ii) other filamentous fungi (including some of the genus *Penicillium*) were found which were insensitive to its fungitoxic action. Moreover, the peptide was not inhibitory to other microorganisms, including bacteria and yeasts, showing that it is very specific to selected fungi, as opposed to previously described short cationic antimicrobial peptides. The activity was dependent of ionic strength, suggesting that the inhibitory effect was driven by electrostatic interaction. Conidial germination is particularly sensitive to inhibition although mycelial growth was also affected. Ours and previous studies with related peptides indicated some degree of peptide amino acid sequence and structure conservation associated with the antimicrobial activity, and suggested a general sequence layout for short antifungal peptides, consisting in one or two positively charged residues combined with aromatic amino acid residues. In order to gain knowledge on the structure/function relationships, we have conducted structural studies (circular dichroism and fluorescence measurements) on the active peptide and also on the one residue-substituted inactive analogue. To the same goal, additional experiments consisting on the synthesis and analysis of a complete set of six Ala substitution analogues will be undertaken.

ISOLATION AND PARTIAL CHARACTERIZATION OF BRADYKININ-POTENTIATING PEPTIDES FROM *BOTHROPS ERYTHROMELAS* SNAKE VENOM

LUNA, K. P. O.[1]; MAIA, M. B. S.[2]; AIRD, S. D.[3] and GUARNIERI, M. C. G[1].
[1]Laboratório de Animais Peçonhentos e Toxinas, Departamento de Zoologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil; [2]Laboratório de Fisiologia, Departamento de Fisiologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil; [3]Departamento de Fisiologia, Universidade Estadual do Ceará, Fortaleza, Ceará, Brazil.

Bothrops envenomings are characterised by haemorrhage, mionecrosis, edema, coagulation disturbances, and hypotension. The latter results principally from the action of peptides in the venom that potentiate the action of bradykinin. *Bothrops erythromelas* is probably responsible for the majority of the ophidian accidents registered in the state of Pernambuco. The objective of this work was to purify peptides present in *B. erythromelas* venom and to characterize those with bradykinin potentiating activity. When fractioned on Superdex-200, *B. erythromelas* venom (750 mg) resolved into eight fractions. The last one was pooled and applied to a Source-RPC column, yielding eight new fractions. These were subsequently subfractionated by a series of ion exchange steps, using Source S or Source Q, and reverse phase steps in order to desalt the peptides prior to biological testing. Among 25 fractions of low molecular weight, partially or completely purified from *B. erythromelas* venom, only eight (renamed Pe1 to Pe8) were tested on guinea pig ileum (3,2 μg) to evaluate their capacity to induce contractions in presence or absence of histamine (10 mg/ml) or bradykinin (10 ng/ml). All fractions, except Pe1, contracted ileum. Fractions Pe2, Pe3, Pe4, Pe5 e Pe7 did not alter contractions elicited by histamine or bradykinin. Pe6 partially inhibited contraction caused by histamine, probably due the inhibition of its receptors, but this fraction did not present any potentiating activity on bradykinin. Fractions Pe1 and Pe8 did not present inhibitory activity in presence of histamine, but they did potentiate contraction induced by bradykinin *in vitro*. These results indicate that Pe1 and Pe8 are bradykinin potentiating peptides. This study is important to understand the mechanism of action of some agents in hypertension.

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PROTEIN-BASED DETECTION OF ANTI-FILAGGRIN ANTIBODIES IN REUMATOID ARTHRITIS

Anna Magyar, Katalin Uray, József Szakonyi[a], Márta Brozik[a], Bernadette Rojkovich[b], Péter Gergely[a], Ferenc Hudecz
Research Group of Peptide Chemistry Hungarian Academy of Science, Eötvös Loránd University, PO BOX 32 Budapest112, 1518 Hungary
[a]Central Laboratory of Immunology, Semmelweis University, Faculty of Medicine Budapest, Hungary
[b]National Institute of Rheumatology, Budapest, Hungary

Since modern treatment of rheumatoid arthritis (RA) is shifting towards aggressive antirheumatic therapy diagnosis in an early phase with high specificity is desirable. Anti-filaggrin antibodies (AFA) directed against the 37-40 kD epidermal protein belong to the most specific markers of RA and include the anti-keratin antibodies (AKA) and anti-perinuclear factor (APF). It has been shown that AFA appear in a very early stage of the disease and its level correlates with the severity and the progression of RA. Epitopes, containing deiminated arginine (citrulline) within the sequence of filaggrin, have been recently identified as major antigenetic sites recognised by AFA .
The aim of our study is to determine the minimal size epitope(s) with optimal antibody recognition properties. For this 19-mer peptides, representing the six most immunogenic sequences of filaggrin (Schellekens et al. J.Clin.Invest. 1998) and their N- or C-terminal truncated peptide analogues were prepared using the "multipin" solid phase synthesis method. In ELISA experiments the presence of AFA was determined using serum samples of RA patients and healthy blood donors. AKA and APF were also studied by indirect immunofluorescence.
Results show that the deimination of arginine in position 312 plays a major role in the antigenecity of filaggrin, but removal of some amino acid residues from the N-terminal have had no significant effect on antibody recognition. These shortened epitopes might be useful for diagnosis and prediction of disease outcome.

MOLECULAR ASPECTS OF THROMBIN-INDUCED ANGIOGENESIS

John Matsoukas[a], Michael E. Maragoudakis[b], Demetrios Vlahakos, Panagiotis Fatseas[c], Ilze Mutule, Ilga Mutule, Tatiana Keivish, and Ludmila Polevaya[d]
[a]Department of Chemistry, University of Patras, 26500 Patras, Greece; [b]Department of Pharmacology, University of Patras, Medical School, 26110 Rio, Patras, Greece;
[c]Onassis Cardiac Surgery Center, 17674 Athens, Greece; [d]Department of Peptide Chemistry, Latvian Institute of Organic Synthesis, 1006 Riga, Latvia

Angiogenesis, the development of new blood vessels, is a highly regulated process that is considered a key event in tumor growth and metastasis. Among the many factors reported to promote angiogenesis, VEGF is the most important angiogenic factor and specific endothelial cell mitogen. More recently it has been reported that thrombin strong potentates VEGF-induced mitogenesis and that this synergic effect is accompanied by activation expression of the mRNAs for VEGF receptors (Fit-1 and KDR) [Maragoudakis, 1999]. The up-regulation of mRNA for VEGF receptors was also mimicked by the thrombin receptor activating peptide TRAP, that suggest a correlation between thrombin receptor activation and the angiogenesis process. It is shown that integrin $\alpha v\beta 3$ is also involved in the mechanism of thrombin angiogenic action. The question as to how thrombin might affect some of the distinct step in the angiogenic cascade, including such as intracellular signaling via thrombin receptor activation and the initiation of matrix remodeling has arisen. In this study, to identify the structural requirements responsible for angiogenic activity in thrombin-thrombin receptors system, we attempted to analyze interplay of various angiogenic factors, including inhibitors of angiogenesis, and corresponding receptors. Earlier we have reported about potential role of the thrombin receptor (PAR-1) ectodomain in the promoting of specificity for thrombin recognition in binding mode similar to F2 kringle of prothrombin [Polevaya and Matsoukas, 1998]. Kringle-like folding of PAR1 ectodomain seems to be important for thrombin-induced angiogenesis. Indeed, kringles are essential for biological activity and control of hepatocyte growth factor and prothrombin; angiostatin, a proteolytic fragment of plasminogen (kringles 1-4), is potent inhibitor of angiogenesis. We propose that a stretch of ten residues from thrombin, EKIYIHPRYN 85-94, might be one of the essential sites for thrombin- kringle interaction. For supporting this idea, we have synthesized new peptides structures of which and bioassays will be presented and discussed. Further studies along these lines may provide ways to develop effective therapeutic agents for angiogenesis-dependent diseases.

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SYNTHESIS OF C-TERMINAL DOMAIN SEQUENCES OF TISSUE INHIBITOR OF METALLOPROTEINASE-1 FOR STRUCTURE BIOLOGICAL ACTIVITY RELATIONSHIP INVESTIGATIONS

Nikolett Mihala, József Bódi, Helga Süli-Vargha
Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös University, Budapest, Hungary

Tissue inhibitors of metalloproteinases (TIMP family) specifically inhibit matrix metalloproteinases (MMP family) responsible for the degradation of extracellular matrix components.
All TIMP family members contain 12 cysteines, which form six disulfide bonds and consequently six loops in the molecule. Both the N- and the C-terminal domains consist of three loops. Enzyme inhibition, which is caused by the binding of the N-terminal domain of TIMP to the active centre of MMP plays an important role in pathologic conditions caused by MMP overexpression, like tumour metastasis, rheumathoid arthritis, periodontosis, etc.
However, TIMP-1 exerts biological activities, such as growth promoting activity, erythroid potentiation, apoptosis inhibition, etc., which are supposed to be independent of the enzyme inhibiting potency. As the enzyme inhibiting potency is associated with the N-terminal domain, and this region behaves even alone as an inhibitor, the aim of our present work was to investigate the biological role of the C-terminal part of TIMP-1. To our knowledge the C-terminal domain has not been prepared yet separately either by gene technology, or by chemical synthesis. The observation that, while chemical reduction and alkylation of TIMP-1 completely demolishes its enzyme inhibitory effect, but does not influence its antiapoptotic property, suggests that continuous sequences in the TIMP-1 C-terminal region may be responsible for the biological effects, which are not connected with MMP inhibition. Therefore as a first approach we cut this 59 amino acid containing domain into seven parts and synthesised the linear fragments - 126- 133, 134-142, 143-154, 155-164, 165-172, 173-181, 182-184 - for structure biological activity relationship investigations and for completion of the whole domain, too. The smallest loop in this domain containig the 132-137 sequence was synthesised separately, as well.

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STUDIES ON INTERACTION OF DELTA SLEEP INDUCING PEPTIDE WITH BIOLOGICAL MEMBRANES *IN VITRO*

Inessa Mikhaleva[1], Igor Prudchenko[1], Galina Richireva[2], Sergei Kopylovsky[2] and Igor Golubev[3]
[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, 117871 Russia, [2]N.N.Semenov Institute of Chemical Physics Russian Academy of Sciences, ul. Kosygina 4, Moscow, 117977 Russia, [3]State Institute of Organic Chemistry and Technology, sh. Entuziastov 23, Moscow, 111024 Russia.

The interaction of Delta Sleep Inducing Peptide (DSIP) with cell membranes was investigated by means of nitroxide spin label and membrane spin probes. As a result of incubation of the spin labeled DSIP derivative with human erythrocyte suspension in phosphate buffer (pH 7.4) at 37°C we observed in EPR spectra an increase in the rotational correlation time and in the molecular order parameter. Using spin probes 5-, 12- and 16-doxylstearic acids and 3-doxylandrostanol we revealed the remarkable increase in the mobility of the hydrophobic moiety of erythrocyte membrane bilayer on the depth 20-22 Å and also in its surface layer (4-6 Å) in the presence of DSIP. The maximal disordering of the membrane lipids was found at 10^{-9} and 10^{-6} M peptide concentration. We have elucidated DSIP effects on phase transitions in human erythrocyte membranes within the temperature range 10- 40 ^{0}C using two spin probes. The data obtained show the significant influence of DSIP on the dynamic membrane structure that touches upon the field of lipid-lipid and protein-lipid interactions.

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DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL ANALOGUES OF THE FRAGMENT 227-237 OF GLYCOPROTEIN IIb MEMBRANE

Mihail L. Mondeshki[1], Nevena Pencheva[2] and Ljubomir T. Vezenkov[1]*
[1]University of Chemical Technology and Metallurgy, Organic Chemistry Department, 1756 Sofia, Bulgaria
[2]Institute of Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Fragment 227-237 of the glycoprotein (GP) IIb membrane molecule (Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu) inhibits the binding of fibrinogen to the platelet surface receptor complex GP IIb/IIIa thus inhibiting platelet aggregation. The presence of Asp-Gly-Arg (DGR) sequence probably leads to the ligand-binding properties of the fragment.
The aim of the present study was the synthesis of the short analogues of fragment 227-237 of the GP IIb molecule: Arg-Gly-Asp-Gly-Arg (1), Val-Asn-Arg-Gly-Asp-Gly-Arg (2) and Val-Phe-Arg-Gly-Asp-Gly-Arg (3). These analogues include also the antithrombotic active sequence Arg-Gly-Asp (RGD), which we designed by incorporating the Arg residue to the Gly residue in 231 position. The combined influence of the sequences RGD and DGR and the influence of the amino acid asparagine on the platelet aggregation response were under investigation.
The protected peptides Z-Arg(NO_2)-Gly-Asp(OBzl)-Gly-Arg(NO_2)-OBzl and Z-Val-Asn-Arg(NO_2)-Gly-Asp(OBzl)-Gly-Arg(NO_2)-OBzl were synthesized by the consecutive coupling of the N-protected amino acids to the C-terminal arginyl residue by DCC/HOBt, TBTU and activated esters methods, and the peptide Z-Val-Phe-Arg(NO_2)-Gly-Asp(OBzl)-Gly-Arg(NO_2)-OBzl was obtained by the fragment coupling 2+5 by the azide method. The protecting groups were removed by treatment with boric trifluoroacetate and the obtained peptides were purified on Sephadex G-15 using 0.2 N or 1 N acetic acid. The inhibition of adenosine diphosphate (ADP) - induced aggregation response by the newly synthesized analogues using citrated platelet-rich plasma (PRP) and platelet poor plasma (PPP) was investigated.

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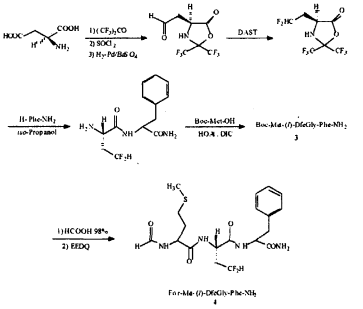
ANALOGUES OF CHEMOTACTIC PEPTIDES CONTAINING FLUORINATED AMINO ACIDS

Maurizio Moroni, Beate Koksch and Klaus Burger
Institut für Organische Chemie, Universität Leipzig; Johannisallee 29, 04103 Leipzig, Germany

A new approach to the rational design of biologically active peptides is the modification of the peptide backbone by incorporation of C^{α}- trifluoromethyl substituted amino acids (α-Tfm amino acids)[1]. In this contribution we incorporated α-Tfm amino acids into fMLF peptides and studied the effects on the receptor mediated reactions.
The native peptide For-Met-Leu-Phe-OH is well known as a strong chemo attractant to granulocytes. Incorporation of aminoisobutyric acid (Aib) in position 2 improved biological activity. To study the recognition and binding process more closely we introduced a chiral Aib-surrogate, namely α-Tfm-alanine.
Biological activity was tested by luminol amplified chemiluminescence (LCL) which is a convenient method for studying the respiratory burst in stimulated neutrophils[2].
First test results are discussed for For-Met-(α-Tfm)Ala-Phe-NH_2, H-(α-Tfm)Xaa-Leu-Phe-OMe (Xaa = Leu, Phe) and For-Met-(*l*)-(α-Dfe)Gly-Phe-NH_2.



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DESIGN AND SYNTHESIS OF AN ESSENTIAL PHOSPHORYLATION-SITE DOMAIN OF HUMAN CDC25C WHICH INTERACTS WITH BOTH 14-3-3 AND CYCLINS

May Catherine Morris[a], Jean Mery[b], Annie Heitz[c], Frederic Heitz[b], Gilles Divita[b]
[a]The Scripps Research Institute, Department of Molecular Biology, La Jolla, USA, [b]CRBM, CNRS-UPR 1086, 34293 Montpellier Cedex 5, France. [c]CBS, Faculté de Pharmacie, 34060 Montpellier Cedex, France.

Human cdc25C is a dual-specificity phosphatase involved in the regulation of cell cycle progression in both unperturbed cells and cells subject to DNA-damage or replication checkpoints. We have designed, synthesized and purified a 51 amino acid peptide derived from an essential domain of human cdc25C phosphatase which interacts with 14-3-3 proteins. *In vivo*, differential phosphorylation of this domain regulates either the induction of mitotic processes, or the checkpoint arrest of eukaryotic cells in response to DNA damage.

Peptide synthesis was achieved using the stepwise Fmoc strategy and resulted in an important yield of highly pure peptide. The final peptide was identified by amino acid analysis, electrospray mass spectrometry and nuclear magnetic resonance, which revealed that one of the two methionines within the peptide was oxidized into its sulphoxide derivative

We have demonstrated that this domain is a bi-functional interactive motif which interacts with cyclins and 14-3-3. Characterization of the structural features of this domain by circular dichroism and NMR reveals an elbow-shaped structure composed of two α-helices interconnected by a loop carrying the 14-3-3 binding site. Combining our structural and biochemical data, we propose a detailed model of the molecular mechanism of cdc25C regulation by 14-3-3 and cyclins.

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EFFECTS OF MONO- AND BICYCLIC ANALOGUES OF HUMAN PARATHYROID HORMONE ON BONE GROWTH AND MECHANICAL STRENGTH IN RAT

Paul Morley[a], James F. Whitfield[a], Gordon E. Willick[a], Virginia Ross[a], Susanne MacLean[a], Jean-René Barbier[a], Troels T. Andreassen[b]
[a]Institute for Biological Sciences, National Research Council, Ottawa, ON, Canada, K1A 0R6; [b]Department of Connective Tissue Biology, Institute of Anatomy, University of Aarhus, DK-8000 Aarhus C, Denmark

Parathyroid hormone (PTH), given in intermittent doses, is a powerful osteogenic agent. PTH analog [L^{27}]cyclo(E^{22}-K^{26})hPTH(1-31)NH_2 (**1**) stimulates adenylyl cyclase (AC) with an effective dose for 50% maximal stimulation (ED_{50}) 6x greater than that of the natural sequence analog hPTH(1-31)NH_2 (**2**), and is a candidate for the treatment of osteoporosis. A similar increase in AC ED_{50} activity was observed with [$E^{17}L^{27}$]cyclo(K^{13}-E^{17})hPTH(1-31)NH_2. We synthesized the bicyclic analog, [E^{17},L^{27}]c(K^{13}-E^{17},E^{22}-K^{26})hPTH(1-31)NH_2 (**3**), using Fmoc chemistry and allyl – alloc protection of the groups forming the lactams. We compared the secondary structure and bioactivities of analog **3** to the monocyclic **1**. PTH has a helix-turn-helix stucture, with α-helices from residues 3-11 and 17-29. A lactam formed between residues 22 and 26 in **1** stabilizes the second helix. Introduction of a second cyclic did not result in additional α-helix, as measured by circular dichroism spectroscopy, suggesting a negative synergy between the N-terminal and C-terminal helices of **3**. The AC activity of **3** was about one-half that of either monocyclic analog. The bicyclic analog **3** increased trabecular volume and thickness and bone mechanical strength in femurs in an ovariectomized rat model for osteoporosis, with an activity approximately equivalent to that of the single cyclic analog **1**.

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2D-NMR AND MD STUDIES OF COMPLEMENTARY PEPTIDES TO B AND T-CELL EPITOPES OF THE La/SSB AUTOANTIGEN FOR IMMUNOREGULATION IN SJOGREN'S SYNDROME (pSS).

Avrilia Moulia[a], Christine Hemmerlin[b], Vassilios Tsikaris[a], Maria Sakarellos-Daitsiotis[a], Constantinos Sakarellos[a], Athanasios G. Tzioufas[c], Haralampos M. Moutsopoulos[c] and Manh-Thong Cung[b]

[a] Department of Chemistry, University of Ioannina, 45110 Greece; [b] LCPM, UMR 7568 CNRS-INPL, Nancy, France; [c] Department of Pathophysiology, National University of Athens, Greece

It has been reported that vaccination with complementary peptides, encoded by complementary RNA, induces the production of anti-idiotypic and anti-clonotypic Abs. The combining sites of the latter are complementary to and therefore reactive with Ag receptors on disease epitope-specific B and T cells. Recently, we have demonstrated that anti-La/SSB antibodies derived from pSS patients sera are directed towards four linear epitopes spanning the regions 145-164, 289-308, 301-320 and 349-368. Predictive methods have shown that the epitope 289-308 is also a T-cell epitope. Two peptides, encoded by complementary RNA (termed cpl(289-308) La/SSB and cpl (349-368) La/SSB were used in immunization experiments with the aim to manipulate the immune network in pSS. In particular, these peptides were coupled, separately, in duplicate to the Lys-$N^{\varepsilon}H_2$ groups of the carrier Ac-(Lys-Aib-Gly)$_4$, (SOC_4), and then used in animal immunizations together with SOC_4-(289-308)$_4$ and SOC_4-(349-368)$_4$ respectively.
The conformational properties of the two 20-residue peptide corresponding respectively to epitope 289-308 and of the complementary peptide cpl(289-308) were studied by using 2D-NMR experiments (COSY, TOCSY, ROESY and NOESY) and the short interproton distances estimated from the NOE correlations have been introduced as conformational constraints in molecular dynamics and minimization procedures to obtain the folded conformation of these two peptides.

ANALYSIS OF ANTIMICROBIAL PEPTIDE–MEMBRANE INTERACTIONS USING BIOSENSOR TECHNOLOGY WITH MONOLAYER AND BILAYER MODEL MEMBRANES

Henriette Mozsolits and Marie-Isabel Aguilar
Department of Biochemistry & Molecular Biology, Monash University, P.O Box 13D, Victoria, 3800. Australia

Peptide-lipid interactions play a critical role in the regulation of several biological phenomena. A common feature of these interactions is the induction of secondary structure following binding of the peptides to the membrane surface. In order to investigate these membrane-mediated processes at the molecular level, a series of immobilised model membranes has been prepared and used to study the lipid-binding properties of the antimicrobial peptide, melittin, using surface plasmon resonance. In particular, the hydrophobic association (HPA) and pioneer sensor chip L1 was used with the BIAcore instrument. The hydrophobic association (HPA) sensor chip is composed of long-chain alkanethiol molecules upon which liposomes adsorb spontaneously to create a flat, lipid monolayer membrane surface. The surface of the Pioneer sensor chip L1 consists of dextran modified with lipophilic compounds that capture lipid membrane vesicles such as liposomes. This means that while the HPA chip is suitable for modelling monolayer studies, L1 gives us the opportunity to work with bilayer systems for the study of peptide-lipid interactions. The ability of these surfaces to act as a stable and sensitive biomimetic systems was analysed and the interactive behaviour of melittin with these biomimetic membrane surfaces has been studied. Multiple sets of binding curves with different peptide concentrations were generated and zwitterionic and anionic phospholipids were used to assess the role of lipid structure on peptide binding. Linearisation analysis and curve fitting using numerical integration analysis were performed to derive estimates for the association (K_a) and dissociation (K_d) constants.

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SOLID PHASE SYNTHESIS OF THE IMMUNOMODULANT MARINE CYCLOPEPTIDE HYMENAMIDE C.

Assunta Napolitano, Ines Bruno, Paolo Rovero, Luigi Gomez-Paloma, Raffaele Riccio, from Dipartimento di Scienze Farmaceutiche, Università di Salerno, I-84084, Fisciano, Italy

Hymenamide C is a member of a new class of natural homodetic cyclopeptides which have interesting bioactivities and remarkable structural similarity. In particular they are all hepta- or octapeptides and they can be defined "proline rich" because in their aminoacidic sequence at least one of the residues is a proline.
Hymenamide C was isolated from the marine sponge *Axinella carteri*, collected off the Vanuatu Islands and has received our attention on the basis of its immunomodulant properties.
The heptapeptide hymenamide C is an head-to-tail cyclic peptide, in which the ring is closed between the amino and the carboxyl groups of N- and C-termini. To conveniently carry out the synthesis of hymenamide C, we decided to take advantage of the *pseudo-diluition* phenomenon and performing the hymenamide C cyclization with the peptide anchored to the solid support. This is obtained using the ω-carboxyl functions of glutamic acid for the attachment to the solid support. According to a three-dimensional orthogonal protection scheme, fully compatible with the Fmoc/tBu chemistry, we are using a Fmoc (α-NH_2)/allyl (α-COOH)/tBu (side-chain) protections.
In this communication, we will present hymenamide C solid phase synthesis and pharmacological properties. The optimization of this synthetic procedure can be of importance when considering the potential use of solid phase peptide synthesis for obtaining a library of hymenamide analogues.

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FACTOR Xa INHIBITORS, ANALOGUES OF TICK ANTICOAGULANT PEPTIDES

Emilia Naydenova[1], Ljubomir Vezenkov[1], Nevena Pencheva[2]
[1]University of Chemical Technology and Metallurgy, Organic Chemistry Department, 1756 Sofia, Bulgaria
[2]Institute of Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Tick anticoagulant peptide (TAP) isolated from extracts of the soft tick *Ornithodoros mubata* is a slow tight-binding inhibitor specific for factor Xa. The inhibitor also acts as an anticoagulant in human plasma clotting assays. A peptide corresponding to residues 1-9 of the TAP molecule is a competitive inhibitor of factor Xa, interacting with the active-site region of this serine protease. On the other hand D-Phe-Pro-Arg and its new type analogues inhibit thrombin as well as some other clothing enzymes involved in thrombin generation. Thus the peptide sequences combining both 1-9 residues of TAP and D-Phe-Pro-Arg derivatives have been prepared by adding D-Phe to Pro in position 8. We incorporated the peptides into disulfide ring by adding Mrp to Tyr in position 1. The preparation of the protected undecapeptide Mrp(PAM)-Tyr-Asn-Arg(NO_2)-Leu-Cys(PAM)-Ile-Lys(Pac)-D-Phe-Pro-Arg(NO_2)-OBzl was carried out and by means of an azide condensation of the corresponding fragments (2+9). The protected nonapeptide ester was obtained by consecutive attachment of Boc-amino acids to H-Arg(NO_2)-OBzl by the DCC/HOBt method and HBTU/DIEA method. The used approach allows us to synthesize the protected tetrapeptide, pentapeptide, hexapeptide, heptapeptide and octapeptide, respectively, which contain the active group D-Phe-Pro-Arg. The protective groups of the undecapeptide and short fragments were removed by treatment with boric trifluoroacetat.
The several human plasma clotting assays activated partial prothrombin time (APTT), prothrombin time (PT) and thrombin time (TT) and ADP-induced platelet aggregation have been used to evaluate the activity of the new peptides. The structure-activity relationships obtained allow to discuss the approaches used for obtaining potent and selective inhibitors of factor Xa and thrombin.

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MACROPHAGE CHEMOTAXIS INDUCED BY ELASTIN-DERIVED HEXAPEPTIDES AND ITS SIGNAL TRANSDUCTION MECHANISM

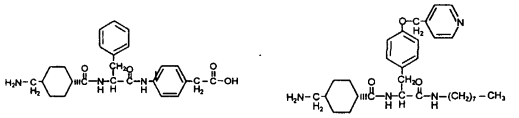
Kouji Okamoto[a], Maria Portia P. Briones[a], Satsuki Kamisato[a], Iori Maeda[a], Noboru Takami[b],
[a]Department of Biochemical Engineering and Science, Kyushu Institute of Technology, Iizuka, Fukuoka, Japan; [b]RI Center, Fukuoka University School of Medicine, Fukuoka, Japan

Elastin, an insoluble extracellular matrix which is the core protein of the elastic fiber, confers elasticity to extensible tissues such as arterial walls, lungs, skin and ligaments. Its degraded fragments (elastin peptides) interact with a variety of cell types to modulate cellular behavior and are implicated to play roles in several diseases such as atherosclerosis, emphysema, and metastasis. For example, elastin digests and elastin derived peptides has been shown to be chemotactic for monocytes, fibroblasts, endothelial cells, smooth muscle cells, macrophages, and tumor cells. VGVAPG, a hexapeptide sequence repeated several times in human, bovine, and porcine elastin molecules has been also found as a chemoattractant. However, there are six considerable hexapeptides in the elastin molecule. It is the aim of this study to elucidate the chemotactic responsiveness of macrophages induced by elastin-derived hexapeptides and its signal transduction. Macrophage chemotactic response was elicited by four hexapeptide permutation peptides: VGVAPG, GVAPGV, VAPGVG and GVGVAP. Exposure of macrophages with lactose or laminin peptide LGTIPG completely abolished chemotaxis. The cGMP level was enhanced in macrophages stimulated by elastin-derived hexapeptides, whereas cAMP level was not. There was no increase in NO_2^- production observed in macrophages treated by elastin-derived hexapeptides. An inhibitor specific for cGMP dependent protein kinase (PKG), KT5823, inhibited the chemotactic response to elastin-derived hexapeptides. From these results, we propose that elastin-derived hexapeptides bind on the cell surface through a receptor, amplify the signals, and direct chemotaxis through cGMP and PKG.

PLASMIN INHIBITOR, YO-2, INDUCES APOPTOSIS OF TUMOR CELL LINES THROUGH CASPASE-3 ACTIVATION

Yoshio Okada[a], Yuko Tsuda[a], Keiko Wanaka[b], Utako Okamoto[b], Shosuke Okamoto[b], Gyorgy Keri[c], Bela Szende[c]
[a]Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan; [b]Kobe Research Project on Thrombosis and Haemostasis, 3-15-18, Asahigaoka, Tarumi-ku, Kobe 655-0033, Japan; [c]Semmelweis University of Medicine, Budapest, Hungary H-1088

We are developing active center-directed inhibitors of plasmin (PL) and plasma kallikrein (PK) and studying the structure-activity relationships. *Trans*-4-aminomethylcyclohexanecarbonyl-Phe-4-carboxymethylanilide (**YO-1**)[1] is a plasma kallikrein selective inhibitor and *trans*-4-aminomethylcyclohexanecarbonyl-Tyr(*O*-picolyl)-octylamide (**YO-2**) is a plasmin selective inhibitor. This presentation deals with the relationship between plasmin and plasma kallikrein selective inhibitors and their apoptosis induction activity on the tumor cell lines.
YO-2 and other plasmin selective inhibitors potently induced apoptosis of M1/9 (melanoma) cell line and HT 29 colon cell line and **YO-2** did not exhibit cytotoxicity, while **YO-1** and other plasma kallikrein selective inhibitors did not induce apoptosis of above cell lines. However, the mechanism whereby **YO-2** induces apoptosis of the above tumor cell lines remains incompletely understood. We have investigated the role of **YO-2** in signal transduction by using HT 29 colon cell line. It was revealed that **YO-2** activated caspase-3, resulting in induction of apoptosis of HT 29 colon cell line. The relationship of plasmin inhibitory activity of **YO-2** and caspase-3 activation activity of **YO-2** is now under investigation. The effect of **YO-2** on the angiogenesis is also discussed.



YO-1 **YO-2**

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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW PLASMA AND TISSUE KALLIKREIN INHIBITORS

Christophe Olivier[a], Muriel Amblard[a], Gilbert Bergé[a], Pierre Dodey[b], Jean Martinez[a]
[a]Laboratoire des Aminoacides Peptides et Protéines, UMR 5810, CNRS-UM1-UM2, Faculté de Pharmacie, 15 av. C. Flahault, 34060 Montpellier Cedex 2.

The kallikrein-kinin system is involved in many pathophysiological processes such as hypertension and inflammation. This system comprises the serine protease kallikreins (plasma and tissue) which release the vasoactive kinins (bradykinin and kallidin) from the precursor kininogen.[1] During the last decades, a large number of kinin antagonists have been described but only few investigations have been directed toward the kallikrein inhibition. However, as kallicreins are probably implicated in a variety of disease states, we were interested in the design of new non-peptidic kallicrein inhibitors of low-molecular weight. The structure of our inhibitors were based on the cleavage site I of the kininogen and on potent pseudopeptide inhibitors[2,3] derived from the minimal binding sequence H-Pro-Phe-Arg-Ser-NH_2. The starting point for their design was the use of a -Pro-Phe- dipeptide mimetic. Introduction at the "P_2-P_1" position of 5-carboxymethyl-2,3-dihydro-1,5-benzothiazepin-4-one (DBT) moiety which was successfully used in the design of potent bradykinin agonists[4] and antagonists allowed us to obtain small molecules inhibiting both the human plasma and tissue kallikreins in the micromolar range. The synthesis and pharmacological profile of some inhibitors will be reported as well as the result of a molecular dynamic study realized on a tissue kallikrein active site model.

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INVESTIGATION OF MONOCLONAL ANTIBODY POLYSPECIFICITY WITH SYNTHETIC PEPTIDE LIBRARIES, PHAGE DISPLAY AND CRYSTALLOGRAPHIC ANALYSES

Livia Otte[a], Claudia Ivascu[a], Achim Kramer[a,d], Barbara Schuwirth[a], Liying Dong[a], Thomas Keitel[b], Wolfgang Hoehne[b] and Jens Schneider-Mergener[a,c]
[a]Institut für Medizinische Immunologie, [b]Institut für Medizinische Biochemie, Universitätsklinikum Charité, Humboldt-Universität zu Berlin, Schumannstr. 20-21, 10098 Berlin, Germany; [c]Jerini Bio Tools GmbH, Rudower Chaussee 29, 12489 Berlin, Germany; [d]present address: Department of Neurobiology, Harvard Medical School, 220 Longwood Ave, Boston MA 02115, USA

We have investigated the binding specificities of two different monoclonal antibodies CB4-1 (Kramer *et al.*, 1997) and TE33. CB4-1 was raised against the capsid protein p24 of HIV-1 and TE33 against cholera toxin. Earlier epitope mapping studies (Höhne *et al.*, 1993; Anglister *et al.*, 1988) revealed that both antibodies recognize short linear sequences on their respective antigens. However, when we screened synthetic or phage display peptide libraries with these antibodies, several peptides which had no sequence similarities to the original epitopes were identified binding the two antibodies at their hypervariable regions.
Here, we report on the different types of synthetically or biologically generated peptide libraries applied to analyze the binding specificity of CB4-1 and TE33. Binding analysis of complete sets of substitution analogs of the library-derived peptides gave insight into the molecular basis of the antibody-peptide recognition processes. Furthermore, structural aspects of the interaction of unrelated peptides with the same antibody molecule were investigated by X-ray structure analysis.

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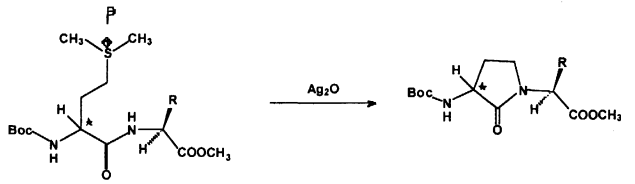
SILVER(I)-OXIDE ASSISTANCE IN THE FORMATION OF GAMMA LACTAMS

Ferenc Ötvös[a], Géza Tóth[a], György Dombi[b]
[a]Institute of Biochemistry, Biological Research Center, Szeged, Hungary; [b]Department of Pharmacological Analysis, Albert Szent-Györgyi University, Szeged, Hungary.

γ-Lactams (II), inserted in a peptide chain, are conformational constraints which stabilise β-turns type II or type II' depending on the configuration of C(3) of the lactam ring. In a known reaction path of their synthesis the ring closure on the starting methylsulfonium iodide salt of a protected methionyl peptide fragment (I) is initiated by a strong base such as NaH. Replacing the base with silver(I)-oxide in the reaction leads to the formation of the same reaction product:



The advantage of silver(I)-oxide over strong bases is that it is compatible with most protecting groups in peptide chemistry, allows open air conditions and the use of common reagent grade solvents. The amount of side products is negligible.
The role of silver(I)-oxide seems to be unique in the reaction, because neither other oxides, nor soluble silver salts exhibited the same effect. The choice of solvent is also important.

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SYNTHESIS AND EFFECTS OF COMPOUNDS STRUCTURALLY RELATED TO MELANOCYTE-INHIBITING FACTOR ON NOCICEPTION

Svetlana Pancheva[a], Tamara Pajpanova[a], Adriana Bocheva[b], Maria Lazarova-Bakarova[b]

[a]Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; [b]Institute of Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.

The problem of the efficient therapy of pain is important not only from clinical but from social and economic point of view. The great achievements in medicine are connected with the research on the development of antinociceptive drugs.
Melanocyte-inhibiting factor (MIF) is a tripeptide (Pro-Leu-Gly-NH_2) that was discovered in hypotalamus. Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) is representative of the MIF's family of endogenous peptides. It has been isolated from bovine hypotalamus and human parietal cortex. They exhibit a range of behavioral and pharmacological effects after central and peripheral administration and alter opiate actions in different systems of the organism. The synthesis of non-protein amino acids and their incorporation into biologically active peptides might become a powerful method for the design and development of modified analogues of natural peptides. Having in mind these data we synthezied a number of new MIF and Tyr-MIF-analogues, containing unnatural amino acids such as Cav, sLys, sLeu, sIle and sNle and *in vivo* experiments were performed to study their action on the nociception. The changes in nociceptive effects were examined in male Wistar rats by the Randall-Seitto paw-pressure test. The preliminary results show that the newly sinthesized analogues exert an antinociceptive effects, antagonized by naloxone.

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BIOLOGICALLY ACTIVE LIPOPOLYSACCHARIDE BINDING PEPTIDES DERIVED FROM A CONFORMATIONALLY DEFINED HAIRPIN-BASED PEPTIDE LIBRARY

Mayte Pastor, Herminia González-Navarro and Enrique Pérez-Payá
Departament de Bioquímica i Biologia Molecular, Universitat de València, 46100 Burjassot, València, Spain.

Combinatorial approaches using in vitro procedures have challenged traditional methods for the generation of new lead compounds. The combinatorial approaches have primarily been focused on the generation os small molecule diversities (i.e., short peptides , peptidomimetics, or organic compounds). The generation of molecular diversities based on defined structural motifs can be expected to broaden the use of combinatorial libraries for those applications requiring the presence of a well defined secondary and/or tertiary structure. Novel binding proteins originating from alternative frameworks should have diagnostic and bioseparation potential. These scaffolds preferably should be based on secondary structure elements that are monomeric and small, soluble, easily engineered, and effectively produced in large scale using low-cost expression systems. Simultaneous randomization of several residues of such elements would potentially result in large repertoires of novel complementarily surfaces grafted onto a common constrained scaffold. We used conformationally defined peptide libraries to select novel peptides that bind to and inhibit bacterial endotoxins. In humans, septic shock and the severe pathological changes associated with it is responsible for thousands of deaths throughout Europe annually, and no specific drugs are as yet available. Septic shock arises from a cascade of molecular and cellular events following infection by micro-organisms, predominantly Gram-negative bacteria. The onset of shock is due to the interaction of bacterial endotoxins (lipopolysaccharide; LPS) with membrane-bound receptors on macrophages and blood monocytes. Recent approaches to develop molecules that neutralize endotoxin have concentrated on lipid A-binding regions from LPS-binding proteins. In particular the potential LPS-binding site of LALF (*Limulus* anti-LPS factor) has been described as an extended β-conformation. We have designed a conformationally defined monomeric β-hairpin library by randomizing four positions in a 17-amino acid-long peptide. In an initial step, the structural nature of this library was used to study the structural allowance of the design. Furthermore, a set of peptides was identified as having the ability to neutralizes LPS *in vitro* as tested by the chromogenic *Limulus* amebocyte lysate assay.

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SYNTHESIS OF SOME OLIGOPEPTIDES FOR LIGAND – RECEPTOR POSSIBILITY OF INTERACTIONS INVESTIGATION

M Pawlowski[1], P Zajdel[1], J-A Fehrentz[2], J Martinez[2*]
[1]Katedra Chemii Farmaceutycznej Collegium Medicum UJ, Kraków, Polska
[2]Laboratoire des Amino-acides, Peptides et Protéines (LAPP), CNRS – Faculté de Pharmacie, Universités Montpellier, France

The effects caused by 5-hydroxytryptamine (5-HT) via serotonin receptors seem to play an important role in pathology of many psychiatric disorders such as anxiety and depression. 5-HT_{1A}, 5-HT_{1C}, 5-HT_2 receptors are the best known subtypes of the serotonin's receptors family. The research carried out on the clonned receptors confirm that the aspartyl residue is responsible for creating the bioactive complex with the ligand. Based on the sequence 1

1 H_2N – Leu – Asp – Val – Leu - COOH

as the basic fragment of the transmembrane segment III of the 5-HT_{1A} receptor, the synthesis of

Boc - Leu - Asp(OH) – Val – Leu – OH
Boc - Ala - Asp(OH) – Val – Leu – OH
Boc – Ala - Leu - Asp(OH) – Val – Leu – OH
Boc – Ile - Ala - Asp(OH) – Val – Leu – OH
Boc –Ile - Ala - Leu - Asp(OH) – Val – Leu – OH
Boc –Ala - Ile - Ala - Asp(OH) – Val – Leu – OH

was done.
The synthesis was carried out in solution according to the general procedue of the peptide synthesis by the stepwise chain building method. The obtained N-protected tetra-, penta- and hexa- peptides were hydrogenated giving products which have free carboxylic function of aspartic acid.
The structure of componds were confirmed by spectral (^{1}H-NMR, MS) and elemental analysis.
The obtained peptides are subjected of pharmacological investigation giving evaluation their possibility of creation complex with the specific receptor's ligands.

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STRUCTURE-FUNCTION RELATIONSHIP STUDIES ON ANALOGS OF THE 1-34 FRAGMENT OF PARATHYROID HORMONE (PTH) CONTAINING β-ALANINE RESIDUES.

Evaristo Peggion[1], Stefano Mammi[1], Elisabetta Schievano[1], Laura Silvestri[1], Michael Chorev[2], Lukas Schiebler [2]and Michael Rosenblatt[2]
[1]Department of Organic Chemistry, University of Padova, Via Marzolo 1, 35131 Padova Italy. [2] Beth Israel Deaconess Medical Center, Division of Bone and Mineral Metabolism, Harvard Medical School, 330 Brookline Ave, Boston, MA, 02215 USA.

The parathyroid hormone (PTH) is an 84 residue peptide which regulates extracellular calcium homeostasis. It has been shown that the N-terminal fragment retains the calciotropic activity of the intact hormone. Previous studies on a series of active and inactive analogs of PTH(1-34) lead to the hypothesis that the structural elements essential for biological activity are two N-terminal and C-terminal α-helical segments and flexibility or hinges around residues 11-12 and 19. In particular, we suggested that the flexible points could play a very important role, allowing the correct orientation of the two helical segments in the receptor-bound state. In order to substantiate this hypothesis in the present work we synthesized and characterized the following analogs of PTH(1-34) containing β-Ala residues at positions 11-12 and 12-13.
1) [Nle^{8,18}, β-Ala^{11,12},Nal^{23},Tyr^{34}]bPTH(1-34)NH_2
2) [Nle^{8,18}, β-Ala^{12,13},Nal^{23},Tyr^{34}]bPTH(1-34)NH_2.
Both analogs resulted to be biologically inactive. The peptides were studied in aqueous solution containing dodecylphosphocholine (DPC) micelles by circular dichroism, 2D-NMR and molecular dynamics (MD) calculations. These studies indicate that both analogs partially fold into the α-helical conformation in the presence of DPC micelles, with a maximum helix content of ~40%. The introduction of β-Ala residues has a remarkable effect on the conformation of the N-terminal sequence. In both peptides there is an increment of the flexibility of the helical structure in this portion of the sequence, more evident in peptide 2. The reduced stability of the N-terminal helix is presumably at the origin of the of the lack of biological activity. The results are consistent with the hypothesis that the presence of N- and C-terminal helices and of flexible hinges at specific locations of the sequence are essential for biological activity of PTH(1-34) analogs.

ANTIMICROBIAL POLYPEPTIDES STRUCTURALLY BASED ON THE SEQUENCE OF THE BACTERICIDAL DOMAIN P_{18-39} OF APROTININ

Antonio Pellegrini, Ursula Thomas, Roland von Fellenberg Institute of Veterinary Physiology, Division of Applied Physiology, University of Zürich, CH 8057 Zürich, Switzerland

Digestion of aprotinin, a proteinase inhibitor, by clostripain, yielded three bactericidal polypeptide fragments with the amino acid sequences **RPDFCLEPPYTGPCK** (P_{1-15}), **IIRYFYNAKAGLCQTFVYGGCR** (P_{18-39}) and **AKRNNFKSAEDCMRTCGGA** (P_{40-58}). The bactericidal domain P_{18-39} was the most potent with a high bactericidal activity against Gram-positive and Gram-negative bacteria. It possesses the structural feature of two antiparallel β-sheet connected with a short turn. In order to understand the structural requirements for antibacterial activity, the sequence of the domain P_{18-39} was taken as the base of the investigation. Several shorter peptides derived from the sequence P_{18-39} were synthesized and their bactericidal properties investigated. Two peptides with the sequence each of them corresponding to a single β-sheet structure were examined for bactericidal activity. The peptide P_{18-28}, corresponding to the N-terminal antiparallel β-sheet structure with the sequence **IIRYFYNAKAG** was active against almost all the bacteria strains investigated, whereas the peptide **LCQTFVYGGCR** corresponding to the C-terminal B-sheet structure was only poorly active. The truncated peptide P_{18-26} (**IIRYFYNAK**) partially retains the bactericidal activity of P_{18-28}, however replacement of lysine 26 with arginine in P_{18-26} (**IIRYFYNAR**) increased the bactericidal capacity of **IIRYFYNAK**. The retropeptide **RANYFYRII** retained the bactericidal activity of **IIRYFYNAR** and the random peptide **IANRIYRYF** was as bactericidal as **IIRYFYNAR**. Elimination of the N- hydrophobic terminal Ile-Ile from **IIRYFYNAK** caused almost the loss of the bactericidal activity of the peptide. Moreover the random peptide possessed in contrast to **IIRYFYNAR** a strong antifungal activity against *Candida albicans*. The insertion of the hydrophobic peptide FFVAP into the C-terminal of P_{18-26} (**IIRYFYNAKFFVAP**) increased the bactericidal potency of the peptide considerably. It was concluded that the order of the amino acids in the sequence of the bactericidal peptides is not a critical feature for bactericidal activity. Hydrophobic interaction between peptides and bacterial membrane is probably the most important feature for bactericidal activity.

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SYNTHETIC APPROACH TO DELINEATE A FUNCTIONAL REGION IN FASCICULIN

Clara Peña, German G. Gornalusse, Roberto J. Falkenstein. Departamento de Química Biológica-IQUIFIB, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113-Buenos Aires, Argentina.

Fasciculins are toxins isolated from the venom of *Dendroaspis angusticeps* (green mamba) known to be non-competitive nanomolar inhibitors of acetylcholinesterase. The crystal structure of Fasciculin-2 disclosed that this peptide is structured into a core region with three protruding loops. Residues 22-27, 34-39 of loop II and 48-53 of loop III form a triple stranded antiparallel β sheet. To evaluate the functional role of this highly conserved structural motif two peptides encompassing such region were synthesized and characterized chemically, structurally and functionally. Standard Boc-chemistry was adopted to synthesize fasciculin sequence 17-52 (Fas-F), while a 28-residues chimera (Fas-C) was prepared applying the Fmoc-strategy. Each of these peptides contains two disulfide bridges which were formed sequentially protecting Cys side chain with *p*-methylbenzyl and acetamidomethyl groups during the synthesis of the parent fragment and with tert-butyl and trityl groups to make the chimerical construction. SDS-PAGE patterns, mass spectrometry analysis and enzymatic digestion were used to check the purity of RP-HPLC separated peptides. Circular dichroism indicated β-sheet and β-turn content compatible with that present in the native toxin. Both fasciculin fragments although not reaching the affinity level of the parent native toxin (K_i: 0.3 nM), displayed inhibition on eel acetylcholinesterase in the μmolar range, supporting our previous results (Falkenstein & Peña, (1997) Biochem. Biophys. Acta 1340:143-151) which indicate that residues from loop II play the key role in binding to the peripheral anionic site of acetylcholinesterase. Other regions of the fasciculin molecule are thought to contribute to the conformational stability of the toxin-enzyme complex.

NEW BRADYKININ ANALOGUES IN CONTRACTION OF RAT UTERUS

Adam Prahl[a], Bernard Lammek[a], Henryk I. Trzeciak[b],
[a] Faculty of Chemistry, University of Gdańsk, 80-952 Gdańsk, Poland
[b] Department of Pharmacology, Silesian Medical University, 40-752 Katowice, Poland

In the present studies we synthesised twenty of our previously designed bradykinin (BK) analogues and evaluated them on the contractility of isolated rat uterus by Holton's procedure with minor modifications. We used [Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7]BK, the B_2 antagonist of Vavrek and Stewart as a model, when designing our analogues. In most cases, the N-terminus of our peptides is acylated with bulky substituent, additionally some analogues, contain in their molecules fragments which imposed conformational constraints. From the results presented, it is clear that the effects of acylation with a bulky group on the contractility of isolated rat uterus, depend substantially on the chemical character of the acyl group. We observed that this modification may change the range of antagonism or even transform it into agonism. It seems that either the positive or negative charge on the N-terminal acyl group is responsible for the transformation of activity. The results obtained in the present study often correspond poorly with those obtained previously in blood pressure tests. We also found that in the case of analogues conformationally restricted in the C-terminal part, acylation may not improve, as we expected, antagonistic properties of new compounds. One can assume that such interaction between analogue and receptor, which took place with many B_2 antagonists acylated with a bulky substituent is, in the case of analogues containing a sterically restricted fragment, disturbed because of the more rigid structure of their C-terminal part. Summing up, besides an improved characterisation of BK analogues, our studies have provided new information on the structure-activity relationships. The results presented also appear to support the hypothesis of others about the presence of different subtypes of B_2 receptors in rat uterus and blood vessels.

Acknowledgements
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