P D61 - Peptides isolated from wheat bud chromatin inhibit cell cycle progression of HeLa cells

L. Mancinelli[1], P. De Angelis[2], A. F. Cruciani[1], G. L. Gianfranceschi[1], K. Elgjo[2]

1. Dep. Biologia Cellulare e Molecolare, University of Perugia - Italy
2. Inst. for Pathology, University of Oslo - Norway

A family of peptides of low molecular weight, tightly associated with the chromatin of eukaryotic and prokaryotic cells, has been reported to be a new class of factors involved in the control of cell growth and gene expression [1,2]. The peptide sequences and the biological mechanisms of action of these peptides are at present unknown. In this study we have investigated the effects of this family of chromatin peptides on Hela cell growth. The peptides were extracted from wheat bud chromatin as previously reported [3] and were tested on Hela cells at different concentrations and incubation times. The results show a 50-90% inhibition of cell proliferation that is dose-dependent in the range of 5-20 μg/ml, both after 24 and 48 hours of incubation. Treatment with 5 μg/ml peptide concentration resulted in a G2/M cell cycle block at 12 hr, 24 hr, and 48 hr., that was of interest to characterize further. The expression (presence or absence of) and the levels of expression of the G2/M-associated regulatory proteins cyclin B1 and cdc2 were investigated. Preliminary results demonstrate that while both the control and treated G2/M cells expressed cyclin B1, the treated cells at 12 and 24 hours had 1.5 and 1.3 times (higher) levels of cyclin B1 expression compared to the corresponding control G2 cells at the same timepoints. At 12 hr of treatment, a higher fraction of the treated G2 cells expressed cdc2 compared to the control G2 cells at the same timepoint (circa 50% versus 15%, respectively). Total cell counts at the 12 hr. timepoint (with peptide present) were reduced by 50%, and this cell loss was due to cell death by apoptosis, as assessed by the terminal deoxynucleotidyl transferase (TdT) assay and ELISA. Apoptotic cell death was highest in the treated G2 cells as compared to the control cells at the same timepoint. Future studies will focus on an investigation of whether the apoptosis seen is p53-dependent or –independent, and an investigation of the gene expression associated with peptide treatment, using cDNA microarrays.

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P D62 - Effects of β-amyloid peptide and its functional antagonists on G-protein activation

Z. Molnar[1], M. Zarandi[1], S. Benyhe[2], B. Penke[1], I. Lengyel[2]

1. Department of Medical Chemistry, University of Szeged - Hungary
2. Biological Research Centre, Szeged - Hungary

Alzheimer's disease (AD) is a neurodegenerative disorder that affects the cognitive function of the brain. Pathological changes in AD are characterized by the formation of amyloid plaques and neurofibrillary tangles as well as extensive neuronal loss. There are diffusible oligomers, low molecular weight intermediates, protofibrils and highly aggregated fibrils that are resistant against proteases. The toxicity of the aggregates seems to depend on the degree of aggregation. The molecular events underlying the toxic effects are largely unknown, but aggregated ß-amyloid (Aß(1-42)), and its shorter fragments, have been shown to stimulate GTPase activity in neurons. Although the connection between G-protein activation and toxicity is yet to be clarified, it was suggested that increasing levels of aggregation of Aß(1-42) may not only alter toxicity, but also the activation of G-proteins. In this study we determined the effects of different aggregated forms of synthetic Aß(1-42) and Aß(25-35) peptides on G-protein activation in crude rat brain membranes, and, using synthetic peptide "antagonists", we were trying to inhibit this activation. Diffusible aggregates were prepared from 10^{-3} M stock solutions in trifluoro-ethanol (TFE) by dilution in H_2O with or without lyophylisation (final concentration $5 \cdot 10^{-5}$ M) followed by one hour vigorous stirring. To obtain highly aggregated fibrils the 10^{-4} M solution was left undisturbed for one week than diluted to $5 \cdot 10^{-5}$ M final concentration. We found that diffusible aggregates of Aß(1-42) produced a concentration dependent, saturable activation of G-proteins with an ED_{50} of ~10^{-6} M. Highly aggregated Aß(1-42) fibrils only affected G-protein activity at concentrations above 10^{-5} M, but this stimulation was not saturable. The Aß(25-35) stimulated G-protein activity was similar to that of Aß(1-42) in the presence of 1% TFE. However, without 1% TFE present no GTPase activity could be detected till 10^{-5} M of Aß(25-35) and the stimulation observed at higher concentrations was not saturable. Inhibition of the actions of Aß(1-42) might have therapeutical relevance. BSB-D(42-38), a putative beta sheet breaker (BSB), did not inhibit G-protein activation by Aß(1-42), but rather acted as a weak stimulator on its own right. However, the functional agonist propionyl-Arg-Ile-Ile-Gly-Leu-NH_2 appeared to be a good inhibitor of Aß's toxicity. In our studies this peptide fully inhibited the Aß(1-42) stimulated GTP binding. In addition it had a profound inhibitory effect on basal G-protein activity (ED_{50}~10^{-5} M). Based on these observations this compound was designated functional-antagonist/inverse-agonist (FAIA). Replacement of propionyl with hexanoyl moiety further potentiated the inhibitory effect of the pentapeptide. Given that these compounds inhibit toxicity as well as G-protein activation, FAIAs might be useful in designing therapeutic agents.

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P D63 - Promotion of growth of colonic cancer cells by carboxymethyl gastrin through a non-CCK2 receptor

R. F. Murphy[1], B. Budai[1], S. Ahmed[1], S. Lovas[1]

1. Creighton University, Department of Biomedical Sciences, Omaha, NE - U.S.A

Gastrin gene products, which are not completely processed from C-terminally carboxmethylated to amidated forms, predominate in colonic cancers and in addition to gastrin, have been implicated in the promotion of growth of such cancers. Membrane preparations, however, from the human colonic cancer cell lines, DLD-1 and HT-29, have a specific binding site for carboxymethyl gastrin which does not bind the typical CCK_2 receptor ligands, CCK8 and gastrin [1].
Proliferation of DLD-1 and HT-29, respectively, cells in culture was significantly ($P<0.01$) increased up to about 30% by $10^{-9.5}$ to 10^{-7} and 10^{-10} to 10^{-8} M, carboxymethyl gastrin, as measured using the sulforhodamine B assay. At higher concentrations, up to 10^{-4} M, the peptide did not stimulate proliferation when, possibly, the receptor for carboxymethyl gastrin is either down-regulated or is a low affinity form, as apparently found by others [2,3] for the CCK_2 receptor, which does not signal proliferation.

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P D64 - Delta sleep inducing peptide (DSIP) and cell membranes

I. A. Prudchenko[1], I. I. Mikhaleva[1], G. T. Richireva[2], I. N. Golubev[3]

1. Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences - Russian Federation
2. Semenov Institute of Chemical Physics Russian Academy of Sciences - Russian Federation
3. State Research Institute of Organic Chemistry and Technology - Russian Federation

DSIP (TrpAlaGlyGlyAspAlaSerGlyGlu) is known as a neuromodulator with strong and unique stress protective action. A wide range of evidences confirmed beneficial effects of this peptide under experimental stress models. Being introduced in a small dose prior to stressful manipulations it completely prevents or considerably suppresses stress induced metabolic disturbances helping to retain stress responses within the physiological range. Based upon these findings DSIP-related drug "Deltaran"™ was developed and registered in 1998 in Russia as intranasal preparation. Placebo controlled randomized clinical trials confirmed substantial stress protective efficiency of this new peptide drug. Since the primary molecular mechanism of DSIP activity remains unclear, we have undertaken the study on DSIP interaction with cellular membranes. Earlier [1] we investigated DSIP effects on mouse synaptosomes and erythrocyte membrane of human blood by means of paramagnetic probes with different immersion depths of the nitroxyl radical into a membrane. It was found from ESR spectra that DSIP at 10^{-6} and 10^{-9} M concentration causes the maximal decrease of an order parameter (S) at the depth of 4-6 Å and 20-22 Å within the membrane. Investigating the effect of DSIP on thermal transitions in human erythrocyte membranes we found a remarkable decrease of the S parameter and also smoothing action of the peptide on the dynamics of temperature-induced transitions of main lipid fractions undergoing such transitions.
We also compared dose-dependent influence of DSIP and its biologically inactive analogue TrpAspAlaSerGlyGlu on the dynamic structure of human erythrocyte membranes. The analogue only slightly affected the dynamic properties of the membrane. DSIP interaction with thrombocyte membranes from human blood by the same technique was studied. The peptide caused a significant decrease of S parameter within the concentration range 10^{-15} – 10^{-4} M. The maximal lost in order parameter (24%) was registered at 5 x 10^{-9} M peptide concentration at the depth of 20-22 Å.
Thus, the experimental data demonstrate the significant impact of DSIP on the dynamic structure of cell membranes related both to lipid-lipid and protein-lipid interactions.

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P D65 - Conformational studies of chimeric cell penetrating peptides in membrane mimicking environment

P. Ruzza[1], S. Elardo[1], A. Calderan[1], A. Donella-Deana[2], M. Crisma[1], A. M. Brunati[2], M. L. Massimino[2], L. A. Pinna[2], G. Borin[1]

1. Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova - Italy
2. Department of Biological Chemistry, University of Padova - Italy

An essential mechanism in cellular regulation is the generation of protein-protein interactions that are involved in signal transduction. A class of these interactions is represented by the Src homology 2 (SH2) domains that bind ligand containing phosphotyrosine residues (pY), whose affinity binding is specifically dictated by the three residues carboxy terminal to pY.
The use of phosphopeptides as intracellular modulators of SH2-mediated signaling is hampered by the poor cellular uptake and membrane permeation of these peptides. To overcome this obstacle, we have previously synthesized a chimeric peptide **RQIKIWFQNRR-Nle-KWKIK*PEGDpYEEVLE*** (peptide **A**) composed of a SH2 interacting peptide (the [392-401]pTyr^{396} fragment of HS1) at its C-terminal side and a membrane translocating peptide (the third helix of Antennapedia) at its amino-terminal segment as a carrier peptide [1].
To correlate the conformational properties of carrier peptides in membrane mimicking environment and their translocation capability inside living cells, we have synthesized a new chimeric peptide **AAVALLPAVLLALLA*PEGDpYEEVLE*** (peptide **B**) replacing the Antennapedia carrier peptide with the hydrophobic region of the signal sequence derived from the Kaposi Fibroblast Growth Factor (kFGF). In addition a short cationic sequence of six-arginine residues was bound to the N-terminal residue of the phosphorylated HS1 fragment as carrier peptide (**RRRRRR*PEGDpYEEVLE***, peptide **C**).
The conformational properties of the three chimeric peptides (**A**, **B** and **C**) have been studied by circular dichroism, ATR FT-IR in aqueous buffer and in membrane mimicking environment. The conformation of peptides is largely random in water. On the contrary, the CD spectra obtained in SDS micelles as well as in TFE/Tris-HCl buffer (9:1, by vol.) indicate that these media induce -helix structure in peptides **A** and **B**. The presence of phospholipid vesicles affect in different way the conformation of peptides **A** and **B**. The Antennapedia analogue (peptide **A**) adopts mainly a β-sheet structure in the presence of anionic DMPG vesicles, whereas in the presence of zwitterionic DMPC vesicles the conformation of peptides is largely random. On the contrary, the kFGF derivative (peptide **B**) adopts mainly a α-helix structure in presence of the both phospholipids. The poly-Arg chimeric peptide (peptide **C**) is not characterized by any classical CD spectra in both aqueous or membrane mimicking environment.
Fluorescence spectroscopy permits to determine the lipid binding affinity of 5-carboxyfluorescein-labeled peptides and also the membrane permeabilizing activity of chimeric peptides **A**, **B** and **C** by means of calcein release from dye-entrapped LUVs.
The ability of phosphorylated chimeric peptides **A**, **B** and **C** to penetrate inside living cell was assayed by fluorescence microscopy using 5-carboxyfluorescein labeled peptide.

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P D66 - Orientation of GPCR fragments (loops and transmembrane helices) in lipid membranes

S. Schreier[1], C. S. Shida[1], R. K. Salinas[1], M. T. Grijalba[1], A. C. Paiva[2], C. R. Nakaie[2]

1. Department of Biochemistry, Institute of Chemistry, University of São Paulo - Brazil
2. Department of Biophysics, Federal University of São Paulo - Brazil

The study of the conformation and dynamics of membrane proteins is hampered by the difficulties in applying high resolution techniques (X-ray crystallography and NMR) to these molecules. For this reason, researchers have resorted to investigating protein fragments. We have been examining the conformational properties of peptides corresponding to extra- and intra-cellular loops (EL and IL, respectively) and transmembrane (TM) domains of G protein coupled receptors (GPCR), both in solution and in the presence of model membranes (phospholipid bilayers and detergent micelles) making use of fluorescence, circular dichroism (CD) and NMR. In most cases, it was found that the peptides conformation is modulated by the conditions of the medium and that they have the propensity to acquire their predicted conformation in the whole receptor. In this context, several peptides from the angiotensin II AT_{1A} receptor were found to form amphiphilic α-helices: IL2, the N-terminal portion of IL3 (NIL3), and the N-terminal portion of the receptor C-terminus (fCT) in TFE-water and upon binding to model membranes. Moreover, a hydrophobic peptide corresponding to TM2 of the bradykinin B2 receptor was also found to be highly α-helical in these media. Here we present studies of the membrane location of these peptides by means of oriented circular dichroism (OCD) and fluorescence differential quenching by spin-labeled phospholipids (PCSL). OCD experiments were performed with oriented phospholipid films, while fluorescence quenching was done on large unilamellar vesicles doped with PCSL carrying the doxyl moiety at C-5, C-12, and C-16 of the *sn*-2 acyl chain (5-PCSL, 12-PCSL, and 16-PCSL, respectively). The differential fluorescence quenching by nitroxide groups located at different depths in the bilayer provides information about the depth of peptide insertion. The OCD results indicate that IL2, NIL3, and fCT are predominantly oriented parallel to the membrane surface, while TM2 is preferentially oriented parallel to the bilayer normal. The fluorescence quenching data corroborate these results. 5-PCSL and 12-PCSL were more effective at quenching the fluorescence of IL2, NIL3, and fCT at all pHs studied, indicating that the peptides must be inserted in the membrane polar head group region, in agreement with its expected location in the whole receptor. In contrast, the fluorescence of TM2 was most efficiently quenched by 12- and 16-PCSL indicating that the peptide is inserted in the hydrocarbon region of the membrane, again as expected. The results show that the peptide fragments are able not only to acquire their predicted conformation in the whole protein, but also that their membrane topography corresponds to that expected for these receptor domains. Moreover, the data provide additional support for the validity and usefulness of studies of peptide fragments in order understand the structure and dynamics of membrane proteins.

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P D67 - Neurotoxicity of aggregating peptides (amyloids, amylin and prion sequences) in MTT assay.

K. Soos[1], Z. Datki[1], M. Zarandi[1], G. K. Toth[1], B. Penke[1]

1. Medical Chemistry - Hungary

Alzheimer's disease (AD) are the most common forms of age-related neurodegenerative disorder. *In vitro* and *in vivo* experiments showed that accumulation of β-amyloid peptides (Aβ) in different region of the human brain can be one of the factors which cause formation of senile plaques and neuronal loss in Alzheimer's disease. It was hypothesized that the neurotoxic effects are due to the aggregation of antiparalell β-sheet structure of different peptides e.g. Aβ, prion, amylin etc.
Recent studies from neuropathology genetics and biology show that abnormal protein aggregation characterizes many, not just AD, but also Parkinson's disease, Creutzfeldt-Jakob disease, Huntington's disease and familial amyloid polyneuropathy (FAB). Amyloid deposits contain extremly insoluble protein fibrils that share similar morphological features but contain many different proteins with no sequence similarity.
The aim of our work was to receive information and compare the toxicity level of different amyloidogenic type peptide, which share the phenomenom of β-sheet formation. We synthetized all of the peptides were used in our study, with solid phase methode. The toxicity level of the sequence of prion (106-126), amylin (1-37) and β-amyloid peptide (1-42) and its analogues were evaluated by MTT assay at $2x10^{-5}$ M of the peptide concentration.
All of the investigated peptides showed toxicity, but the highest one was 45% compare to the absolute control, shown by the β-amyloid peptide (1-42). Prion (106-126) and amylin (1-37) showed 25% and 30% toxicity comparing to absolute control, respectively.

P D68 - Structural studies of glycopeptide-membrane association

L. Stella[1], M. Venanzi[1], A. Palleschi[1], A. Canini[2], M. Carafa[3], G. Zanotti[4], B. Pispisa[1]

1. Dip. di Scienze e Tecnologie Chimiche, Univ. di Roma Tor Vergata, 00133 Roma - Italy.
2. Dip. di Biologia, Univ. di Roma Tor Vergata, 00133 Roma - Italy.
3. Dip. Chimica delle Sostanze Biologicamente Attive, Univ. Roma La Sapienza, 00185 Roma - Italy.
4. Centro di Chimica del Farmaco, C.N.R., c/o Università di Roma La Sapienza, 00185 Roma - Italy.

The interaction of proteins and peptides with phospholipid membranes is a fundamental step in many biological processes; membrane association is particularly relevant for glycoproteins, since almost all integral membrane proteins are glycosylated. However, the experimental techniques normally used for structural determination of biomolecules (X-ray crystallography and NMR) are of difficult application in the presence of membranes, and alternative approaches are needed. We show here that relatively simple optical spectroscopic techniques in combination with molecular mechanics calculations allow us to determine the most relevant structural features of peptides in solution, their mode of association by a model membrane and their effect on membrane properties. To this end we have studied several glycopeptides, one of which is reported here. The sequence of the model octapeptide is shown in Fig. 1: the threonine side chain was O-glycosylated with a glucose moiety (G), and the glycopeptide was marked with two covalently bound fluorescent probes, dansyl (D) and naphthalene (N). CD studies suggest that in methanol solution the backbone chain attains an ordered, intramolecularly H-bonded structure. Fluorescence energy transfer (FRET) measurements show that the peptide populates only two conformations, with significantly different fluorophore arrangements. The most probable conformations were built up by molecular mechanics calculations, whose molecular parameters agree quantitatively with those experimentally determined by FRET. Association studies with model membranes (small unilamellar vesicles) show that both fluorophores are inserted into the membrane phase, but close to its surface. Since the two probes are located at the extremes of the glycopeptide, the peptide chain must lie at the membrane-water interface, approximately parallel to it. Surprisingly, the peptide examined displays a stabilizing, rather than a destabilizing effect on the membrane. It increases vesicles' size from 500±100 to 800±200Å according to electron microscopy experiments and their resistance to rupture by a membrane destabilizing solvent, such as methanol, while leaving the phospholipid phase transition temperature unperturbed.

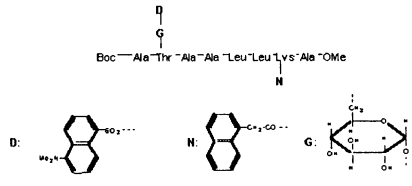

Fig. 1 - Schematic representation of the glycopeptide studied

P D69 - Structural requirements for cellular uptake of cathelicidin peptides by mammalian cells

L. Tomasinsig[1], B. Giabbai[2], R. Circo[1], A. Sgorbissa[2], B. Skerlavaj[1], M. Zanetti[3]

1. Dept. Sciences and Biomedical Technologies, University of Udine, ple Kolbe 4, 33100 Udine - Italy
2. Natl. Lab. CIB, AREA Science Park, 43012 Padriciano-Trieste - Italy
3. Dept. Sciences and Biomedical Technologies, University of Udine, ple Kolbe 4, 33100 Udine and Natl. Lab. CIB, AREA Science Park, 43012 Padriciano-Trieste - Italy

Cathelicidin-derived peptides are cationic peptides stored in the neutrophil granules and released on neutrophil activation. These molecules are components of the first-line defenses of mammals against microorganisms. They are remarkably diverse by size and sequence, and include amphipatic α-helical (LL37, BMAPs), Pro-rich (Bac7, PR39), Trp-rich (indolicidin), and disulphide bridged (PG1) peptides. Despite marked structural differences, all these peptides exhibit considerable antimicrobial activity against a variety of microorganisms. Some of them have been shown to exert other host-protective effects in addition to a direct microbial killing. LL37 is chemotactic for blood cells, BMAP28 causes apoptosis of lymphocytes, and PR39 is involved in wound repair. These effects suggest that these peptides interact with target host cells to trigger specific cellular responses. Little is known on early events in these responses, and in most instances, it has not been established if interaction with the cell surface is sufficient, or cellular uptake is required for the peptide to trigger the cellular response. We addressed this problem and performed a confocal analysis to investigate the ability of chemically synthesized and fluorescein-conjugated LL37, BMAP28, Bac7(1-35) to interact with mammalian cells. The peptides were found to rapidly translocate through the plasma membrane of human (HeLa, IMR90) and murine (3T3) cell lines. LL37 and BMAP28 showed a cytoplasmic localization, whereas Bac7(1-35) associated with the nucleoli, suggesting the presence of different intracellular targets. Unlike BMAP28, that showed some toxicity at 3 μM, the internalization of up to 50 μM LL37 and Bac7(1-35) did not cause membrane permeabilization, as no uptake of propidium iodide and no significant release of the cytoplasmic enzyme LDH were detected after peptide addition to the cells. The process does not involve an endocytic pathway, as the uptake was not inhibited at 4°C, and is not energy-dependent, as it was not prevented by depletion of cellular ATP. The all-D enantiomers were internalized at the same extent as the L-peptides, ruling out the need of stereospecific recognition of surface molecules. These peptides possibly use an internalization mechanism common to Arg-rich peptides, which is not explained by a typical endocytosis. Preliminary studies have also been performed to identify structural requirements for the uptake of Bac7(1-35), using synthetic fragments truncated at the C- or the N- termini. Bac7(5-35), lacking the N-terminal RRIR residues was found associated to the cell surface, but not intracellularly. The C-terminally truncated peptide Bac7(1-16), but not Bac7(1-15) was internalized and showed a nucleolar localization. The data suggest that the N-terminal cluster of cationic residues and a length of at least 15 residues from the N-terminus are important requirements for internalization of the peptide.

P D70 - Neurotrophic action of ACTH4-7 analog SEMAKS on regeneration of peripheral axones tested *in vivo* and *in vitro*

I. V. Vardya[1], O. P. Balezina[1], S. V. Popov[2]

1. Moscow State University, Biological faculty, Dept.Human&Animal phisiology - Russian Federation
2. University Illiniis Chicago, Dept.Phisiology - Russian Federation

Influence of synthetic ACTH4-7 analog SEMAKS on functional properties of outgrowing axones were studied under conditions of chronic intranasal peptide injections in mice or direct peptide application to cultured embryonal spinal neurones of frog (Xenopus laevis). It is found, that by chronic SEMAKS injections *in vivo* in doses 50, 100 200μg/kg/daily it facilitates the conduction of compound action potential (CAP) of regenerating axones: there takes place the statistically significant, dose-dependent increase of CAP amplitude for 1,20, 1,76 and 1,78 times and conduction velocity for 1,53, 1,7 and 1,8 times correspondingly. Injections of 200)μg/kg/daily of SEMAKS causes the accelerated reestablishment if CAP time course for 32,3%. Experiments *in vitro*, on embryonal spinal frog neurons in culture did not reveal any significant changes of neurites elongation velocity by direct peptide application to neurons for 14 houres. Nevertheless, a new property of semaks was discovered - to increase the level of ionized calcium for 128,5% ($p<0,05$) in growth cones of outgrowing axones.

P D71 - Refinement of the experimentally-based model of parathyroid hormone-receptor interactions by photoaffinity-crosslinking of residues in the mid-region of PTH(1-34)

A. Wittelsberger[1], M. Corich[1], A. Barazza[1], R. Yacobi[1], D. F. Mierke[1], M. Rosenblatt[1], J. M. Alexander[1], M. Chorev[1]

1. Division of Bone and Mineral Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02115 - U.S.A
2. Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, Rhode Island 02912 - U.S.A.

The parathyroid hormone (PTH) is the major hormone responsible for the regulation of calcium levels in blood and of bone remodeling. These activities are mediated through its cognate G-protein coupled receptor, PTH1 Rc, a member of the class II family. Understanding the molecular mechanisms of ligand recognition and signal transduction by the PTH1 Rc may lead to the design of novel hormone analogs as drugs for the treatment of diseases such as osteoporosis, hypercalcemia of malignancy, and hyperparathyroidism. Using the benzophenone-based photoaffinity-crosslinking scanning approach, several contact points between positions 1, 13, and 27 of PTH(1-34) and the receptor have been identified and used to generate an experimentally-based model of the ligand-receptor bimolecular interface [1-4]. In this report, we present the design, biological characterization, and photoaffinity crosslinking studies of a series of novel PTH analogs containing the photoreactive p-benzoylphenylalanine (Bpa) residue in the mid-region of the hormone sequence, i.e. positions 11, 15, 18, and 21 (see Table). These positions are located in proximity to the putative hinge sites (positions 12 and 19) connecting the N-terminal and the C-terminal helical segments of the molecule. The identification of novel contact sites on the receptor for the Bpa-substituted positions in the mid-region of PTH allowed us to enhance and refine the currently available model of the PTH--PTH1 Rc interaction.

PTH analogs*	Binding affinity IC50 (nM)	Adenyl cyclase activity E C50 (nM)
Bpa^{11}-PTH	50	7
Bpa^{15}-PTH	20-25	3.0-3.5
Bpa^{18}-PTH	300	25
Bpa^{21}-PTH	100	4-5
PTH(1-34)	18-20	2

*The sequence of the PTH analogs is based on the [$Nle^{8,18}$,$Arg^{11,13}$,Nal^{1},Tyr^{34}]bPTH(1-34)NH_2 scaffold.

Table 1 - Biological characterization of the PTH analogs.

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P D72 - Synthesis of trimeric collagen peptides with artificial and natural cystine knots

D. Barth[1], H. Musiol[1], C. Renner[1], S. Frank[2], J. Engel[2], L. Moroder[1]

1. Max-Planck-Institut für Biochemie, AG Bioorganische Chemie - Germany
2. Biozentrum, University of Basel - Switzerland

Natural homo- and heterotrimeric collagen molecules are often crosslinked by complex cystine knots. Since oxidative refolding of such collagen molecules generally does not produce the native disulfide connectivities, for the synthesis of heterotrimeric collagenous peptides containing natural collagen epitopes we made use of an artificial cystine knot to assemble the chains in the correct raster [1]. To generate in regioselective manner the cysteine connectivities the chemistry based on activation of single cysteine residues as 2-nitropyridylsulfenyl derivatives followed by thiol/disulfide exchange reactions with a second cysteine residue in defined order was adopted [2]. Whilst this approach was successful for selective disulfide crosslinking of collagenous peptides containing natural sequences of lower triple-helix propensity, it proved to be exceedingly troublesome when peptide chains consisting of exclusively $(Gly\text{-}Pro\text{-}Hyp)_n$ repeats were assembled into trimers. The high tendency of such chains to selfassociate into homotrimers proved to be the main cause of failure of the well established regioselective cysteine chemistry [3]. More recently, it was observed that the cysteine-rich region of collagen type III, i.e. Gly-Pro-Pro-Gly-Pro-Cys-Cys-Gly-Gly-Gly, when added as C-terminus to (Gly-Pro-Pro)n sequences allowed for expression of trimeric collagen molecules crosslinked by a cystine knot of yet undefined disulfide connectivities [4]. These results prompted us to explore the efficiency of this foldon sequence to assemble synthetically $(Gly\text{-}Pro\text{-}Hyp)_n$ peptides into homotrimers. By using the GSSG/GSH redox system to allow for the thermodynamically most favored species to be formed, mainly disulfide-crosslinked trimers are generated. Thus from the synthetic point of view the use of this foldon appears to be the most simple and efficient synthetic strategy for the production of at least homotrimeric collagenous model peptides. The effect of this native cystine knot in comparison to the artificial one on the triple-helix stability as well as related NMR structural investigations will be discussed

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P D73 - X-Ray study of the glutaredoxin from *Poplar trichorpa* in complex with glutathione or peroxiredoxin

K. D'Ambrosio[1,3], C. Corbier[1], N. Rouhier[2], J. Jacquot[2], E. Benedetti[3], A. Aubry[4]

1. LCM3B, Faculté des Science, UHP, 54506 Vandoeuvre-les-Nancy Cedex - France
2. Interactions Arbres Microorganismes UMR 1136 INRA, Faculté des Science, UHP, 54506 Vandoeuvre-les-Nancy Cedex - France
3. Istituto di Biostrutture e Bioimmagini, C.N.R.c/o Department of Biological Chemistry,Università di Napoli "Federico II",Via Mezzocannone 6,80134 Napoli - Italy
4. LCM3B, Université Henri Poincaré Nancy I, UMR 7036 BP 239, 54506 Vandoeuvre lès Nancy Cedex - France

Glutaredoxins (GRX) belong to the thiol-disulphide oxydo-reductase family and are involved in the adaptative response during oxidative stress. In all known organisms, glutaredoxins are reduced via a cascade that involves NADPH, a glutathione reductase and the tripeptide glutathione and they are the preferred electrons and protons donor to ribonucleotide reductase. Glutaredoxins are rather well known in bacteria (such as *E. coli*), yeast and mammalian systems, but the informations about plant glutaredoxins are more scarce. The questioning of protein data banks indicates that there are multiple genes in plants with rather large variations at the active site (from the canonic YCPYC to the less frequent YCPFC). Quite recently, glutaredoxin has been cloned and overproduced from poplar (Rouhier et al., Prot. Expr. Purif. 2002, in press). It has been shown to be very effective in the dehydroascorbate reduction, but also to be a good proton donor to a new cytosolic peroxiredoxin (PRX, a reductase without haem, that catalyzes the reduction of hydrogen peroxide to water and of alkyl hydroperoxides to the corresponding alcohols) isolated from the same source (Rouhier et al., Plant Physiol. 127, 1-11, 2001). This constitutes up to now an unique example since the common proton donor of this class of peroxiredoxin is usually thioredoxin. A stable complex (mixed disulfide intermediate) has been namely isolated between PRX and GRX, using a monocysteinic mutant of the latter. The aim of our study is to solve the X-Ray structure of both enzymes, alone and in complex. We have obtained crystals for a monocysteinic mutant ($C_{30}S$) of the GRX (that one required to obtain the stable complex with PRX, see above) in complex with glutathione, using the vapour diffusion technique in the presence of polyethylene glycol 4000 as precipitating agent. The crystal we have tested diffracted to a resolution limit of 1.8 Å in cryogenic conditions (LURE, DW32). Since there, crystals of better shape and thickness have been obtained that might possess better diffraction power. In order to obtain the phases, molecular replacement is currently in progress, although the GRX structures available in the PDB possess a very low sequence identity with our protein. If molecular replacement fails, we envisage to use the anomalous signal of sulphur (3 sulphur atoms are present), or a Se-Met derivative.
Concerning PRX, 3 crystal forms have been obtained for the wild-type protein (triclinic, monoclinic and orthorhombic). The crystals diffracted to a resolution limit of 2.3, 2.5 and 1.6 Å. The corresponding data set have been collected at the LURE in cryogenic conditions (DW32). The phases have been obtained by molecular replacement, using the structure of human peroxiredoxin (Pdb code:1HD2) as molecular model. Refinement of the structure is currently underway. Finally, crystallisation trials of the two proteins in complex are in progress.

P D74 - Hydrophobic clusters control protein folding and stability

R. S. Hodges[1], C. T. Mant[1], S. C. Kwok[1]

1. Department of Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, Denver, Colorado - U.S.A

We have designed a series of four coiled-coils of identical chain length (8 heptads), identical amino acid composition, and identical sequence except for four "a" positions in the hydrophobic core (the heptad repeat is denoted "gabcdef" where positions "a" and "d" form the hydrophobic core). The four peptides each contain nine large hydrophobes (Leu or Ile) and seven small hydrophobes (Ala) in positions "a" and "d". Thus, by shuffling the Ile and Ala residues in the four "a" proteins, we can create coiled-coils with one, two, and three hydrophobic clusters, where each cluster contains three large hydrophobes (Leu, Ile, Leu at positions "d", "a", "d"). It is important to note that all peptides have identical inherent hydrophobicity in the hydrophobic core "a" and "d" positions.
The folding and stability was determined by CD studies using temperature and urea denaturation and temperature profiling in reversed-phase HPLC of both the disulfide-bridged and reduced coiled-coils.
The results show that an increase of a single cluster has an enormous effect on folding and stability (>15°C increase in T_m and ~2M increase in $urea_{½}$ value) even though the overall inherent hydrophobicities of the different coiled-coils are identical.
The discovery of the quantitative significance of hydrophobic clusters is a major breakthrough in our understanding of folding and stability of coiled-coils and ultimately will allow us to predict folding and stability from amino acid sequence conformation. Hydrophobic clustering has major implications in understanding conformational change in coiled-coils and explains why native coiled-coils with large numbers of destabilizing residues in the hydrophobic core can still fold with reasonable stabilities.

P D75 - Conformational stability of a uniquely-folded 20-residue miniprotein

G. V. Nikiforovich[1], N. H. Andersen[2], R. M. Fesinmeyer[2]

1. Department of Biochemistry and Molecular Biophysics, Washington University, St. Louis, MO 63110 - U.S.A
2. Department of Chemistry, University of Washington, Seattle , WA 98195 - U.S.A.

Spectroscopic data have shown that the linear peptide with the sequence NLYIQWLKDGGPSSGRPPPS (**TC5b**) is uniquely folded in water forming a "Trp-cage" [1]. In this structure, the indole ring of Trp^6 is shielded from the solvent by aliphatic side chains of Pro^{18} and Pro^{12}, as well as by the aromatic ring of Tyr^3. In some mutants, the fold remains stable even at temperatures as high as 60°C. The NOE connectivities for **TC5b** yielded a well-converged 39-structure ensemble with no NOE violation greater than 0.15Å. This classically-derived ensemble assumed that a single conformer could rationalize all NOEs. Here we present an alternative structure generation procedure [2] that does not make this assumption by first calculating low energy structures and then comparing their interatomic distances to the NMR restraints. Energy calculations using the ECEPP force field were performed for the Trp-cage backbone identifying 7,288 conformers with relative energies less than 10 kcal/mol. The side chains of **TC5b** were added and new energy calculations found 324 low-energy backbone conformers, each with optimal spatial arrangement of all side chains. To check the importance of the $\chi 1$ of Trp^6 for each of those conformers, energy calculations starting from three different (g^-, t, g^+) rotamers of Trp^6 were performed. These calculations yielded 270 different low-energy conformers. They were compared to the ensemble of conformers obtained from the NOE data. Four of the structures selected based on calculated stability were within an rms < 2.0 Å (C^α atoms residues 2-19, $1C^\alpha$ and $20C^\alpha$ were disordered in the NOE-derived ensemble) *versus* all members of the NOE-derived ensemble. For each of these four, all combinations of rotamers for side chains of Tyr^3, Trp^6, Asp^9 and Arg^{16} were considered for additional energy minimization. Finally, inter-residue backbone distances (74, in all) corresponding to the experimentally measured NMR restraints were calculated for the three low-energy conformers most similar to the NMR ensemble. Excluding the distances where NMR had defined only a lower boundary distance, the calculated distances averaged over the three low-energy conformers were essentially the same as those deduced directly from NMR measurements. The only difference was the $9H_N/11H_N$ distance, which was 3.7 Å as measured by NMR, and 6.2 Å as calculated over the low-energy conformers. This is in line with observation that the $12H_\alpha/15H_N$ distances are different in the three conformers from energy calculations (3.4 Å, 3.4 Å and 7.9 Å). Given the agreement observed for other NOE distances this suggests that the possibility of increased conformational averaging within the Gly-rich fragment 9-15 (DGGPSSG) of **TC5b** needs to be examined. Generally, independent energy calculations for the **TC5b** showed remarkable consistency with the NMR data, and these may be a basis for the design of more stable analogs utilizing substitutions of Gly residues by Ala or D-Ala.

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P D76 - Conformational model for early stages of β-protein folding

G. V. Nikiforovich[1], C. Frieden[1]

1. Department of Biochemistry and Molecular Biophysics, Washington University, St. Louis, MO 63110 - U.S.A

Despite significant progress in predicting 3D structures of proteins starting from their sequences (see, e.g., the results of the CASP-4 meeting, December 2000), the mechanism of protein folding is still very difficult to deduce using available experimental data or applying molecular dynamics studies. This presentation discusses a novel approach to the problem based on systematic molecular modeling studies of oligopeptide fragments comprising the protein sequence. Basic assumptions of the approach are as follows: ***(i)*** only local interactions are really important in the totally unfolded conformational state of a protein; ***(ii)*** a convenient unit to study local interactions is the hexapeptide, the fragment with length sufficient to reproduce and differentiate all possible types of nucleation centers, i.e., α-helix, β-strand and the nascent β-hairpin (β-turn); and, ***(iii)*** at the early steps of folding, near-neighboring interactions are still predominant (up to about 12-residue fragments that corresponds to two hexapeptide "legs" in a β-hairpin). Energy calculations were performed for all hexapeptides in the 131-residue intestinal fatty acid binding protein (IFABP) that consists of ten β-strand fragments and two small α–helical fragments packed in a general β-sheet 3D structure. Low-energy conformers found for the hexapeptides were compared to the "template" 3D structures of a six-residue β–turn, α–helix and β–strand to reveal relative propensities for the particular hexapeptide to adopt a specific type of 3D structure. This allowed determining which protein fragments possess highest probability to induce the nascent β-hairpins or α-helical fragments at the early stages of IFABP folding (6 out of 8 hairpins and 1 out of two helical fragments in the X-ray structure of IFABP), and which are to be restructured at the later stages of folding. The data obtained are consistent with HSQC NMR data collected at different denaturant concentrations [1]. Energy calculations performed for 8-, 10- and 12-residue fragments obtained by residue-by-residue elongation of the initial β-hairpin and α-helical fragments found the larger IFABP fragments that had a high probability to adopt 3D structures corresponding to the X-ray structure of IFABP. These fragments can be considered as segments acquiring autonomous stability at the next steps of IFABP folding. The above approach was applied also to the 127-residue ileal lipid binding protein (ILBP) that is very similar to IFABP in 3D structure, but not in sequence or experimentally suggested folding intermediates [2]. Some differences in folding process between the two proteins suggested by experimental observations [2] are consistent with findings by our approach. Also, the results of our approach are consistent with experimental observations related to folding of the model β-hairpins [3,4]. Therefore, one can expect that our modeling approach presents fairly accurate picture of the early stages of the β-protein folding.

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P D77 - Monitoring of high pressure protein denaturation by hydrogen/deuterium exchange and electrospray ionization mass spectrometry.

I. Petry[(1)], Z. Szewczuk[(1)]

1. University of Wroclaw, Faculty of Chemistry - Poland

It is known, that a high pressure can induce reversible denaturation of some globular proteins. A high-pressure treatment (HPT) of food can be used as an alternative for a heat treatment (cooking, frying) to create products with minimal effect on flavour, colour, and nutritional value. High pressure also can be used for pascalisation at low temperatures and in some countries it is widely applied. An influence of pressure on proteins and a comparison of thermal and pressure denaturation are subjects of scientific interest of many biochemists, physicists, biologists, and nutrition chemists. However, their studies require costly and advanced high-pressure analytical techniques. Therefore, there is a need for monitoring of the high-pressure folding kinetics by standard apparatus, after releasing the pressure.
Measurements of a hydrogen-deuterium (H/D) exchange between peptide backbone amides and solvent provide considerable insight into protein folding. The H/D exchange rate is closely related to its local environment. In general, a protein in a tightly folded conformation has fewer protons available for deuteration, than the same protein in an unfolded conformation. Since the deuteration increases the molecular mass of a protein, an electrospray mass spectrometer (ESI-MS) is often used to monitor the H/D exchange. We attempted to use the mass spectrometry to study the H/D exchange at different values of pressure, on selected model proteins, which are known to undergo a reversible HPT denaturation. To characterize the denaturation degree as a function of pressure we denatured model proteins (lysozyme, α-lactalbumin and β-lactoglobulin) in deuterated solvent by HPT and after the high pressure was released, we dissolved them in water and monitored the D/H exchange kinetics by ESI-MS. The degree of deuteration was pressure dependent. The native proteins treated with D_2O at pressure below the value which causes denaturation, re-exchanged their deuterons with protons very fast after decompression, resulting in negligible increase of their molecular weight. In contrast, when the proteins were denaturated in D_2O at higher pressure, the renaturated conformation protected 10-20 deuterons even after two hours of exchange. A series of experiments performed at various pressures from 1 to 14000 bars at a fixed temperature from 4°C to 25°C allow us to point a pressure of folding equilibrium and to find a number of deuterons trapped in the protein refolded structure as a function of pressure. Our results obtained for the model proteins were consistent with those previously observed directly at high pressure by NMR and fluorescence spectroscopy, what confirmed the usefulness of our method. We also monitored a two-state unfolding of lysozyme and obtained results identical to those described in literature.

P D78 - Crystal state structure of a linear trichogin dodecapeptide analogue.

M. Saviano[(1)], R. Improta[(1)], E. Benedetti[(1)], B. Carrozzini[(2)], G. L. Cascarano[(2)], C. Toniolo[(3)], M. Crisma[(3)]

1. Istituto di Biostrutture e Bioimmagini, CNR, 80134, Napoli - Italy
2. Istituto di Cristallografia, CNR, Dip. Geomineralogico, Campus Universitario, 70125 Bari - Italy
3. Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova - Italy

Recently, we have studied the intramolecular quenching of photoexcited triplet states by free radicals linked to peptide templates by time-resolved EPR with pulsed laser excitation [1]. The systems investigated are 3_{10}- and mixed 3_{10}/α-helix forming peptides, having in the amino acid sequence the stable free radical 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) and a triplet precursor (Bpa) incorporated at different relative positions. Upon interaction with the excited triplet, the TOAC nitroxide radical spin sublevel populations assume values that differ from the Boltzmann equilibrium values. This spin polarization effect produces EPR lines in emission whose time evolution reflects the triplet quenching rate. In particular, in a series of short peptides labelled with Bpa and TOAC at selected positions in the 3_{10}-helix, a radical-triplet interaction was observed in all cases. Interestingly, the EPR signal of the terminally protected peptide Boc-Bpa-Aib-Gly-Leu-Aib-$(Gly)_2$-Leu-TOAC-Gly-Ile-Leu-OMe, where the Bpa and TOAC residues are separated by a large number of amino acids, exhibits the same features as those shown by the short peptides investigated, though of significantly lower intensity. This peptide is also the [Boc-Bpa^0, TOAC^8, Leu^{11}-OMe] analogue of the membrane-active lipopeptaibol trichogin GA IV (Fig.1).
In this Communication we describe the 3D-structure of this trichogin analogue as obtained by an X-ray diffraction analysis. The peptide crystallizes with two independent molecules in the asymmetric unit. The structure solved using the latest version of Sir2000-N [2], a direct method program designed for 'ab initio' solution of macromolecular crystal structures. The two independent molecules present non regular helical conformations, with distortions at the level of to the Bpa side chains. We report also 'ab initio' calculation to give some insights into the dodecapeptide conformational behaviour.

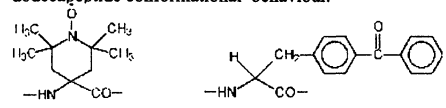

Fig. 1 Schematic representation of TOAC and BPA residues –

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P D79 - Peptide 4-imidazolidinones as drug delivery systems

M. J. Araújo[(1)], R. Castanheiro[(1)], R. Ferraz[(1)], H. Pinto[(1)], R. Moreira[(2)], P. Gomes[(1)]

1.Centro de Investigação em Química da Universidade do Porto - Portugal
2. Centro de Estudos de Ciências Farmacêuticas - Faculdade de Farmácia de Lisboa - Portugal

Malaria is the major public health problem in tropical and sub-tropical areas, with an annual incidence of more than 110 million clinical cases and up to 1 million deaths. Global heating and high mobility of the populations are critical factors in bringing up this disease to a worldwide-scale, whereas the rapid emergence and spread of chloroquine-resistant Plasmodium falciparum is currently the primary technical factor affecting malaria control efforts. Primaquine (PQ, 1) is the only antimalarial drug known to break the reproductive cycle of Plasmodia parasites in humans, thus blocking disease transmission from host to the mosquito vector. Despite its high gametocytocidal activity, PQ is rapidly inactivated in vivo by monoamino oxidase (MAO) and cytochrome P450. Further, PQ is haemolytic for humans with a congenital deficiency in glucose-6-phosphate dehydrogenase (6-GPD). In view of this, development of chemical delivery systems (prodrugs) of primaquine and other currently available antimalarials is an useful approach to novel antimalarial therapies. We present here the synthesis and study of PQ potential prodrugs and pro-prodrugs, based on PQ N-acyl derivatives containing amino acids or dipeptides (2), and on their corresponding 4-imidazolidinones (3). Our synthetic approach involves three main steps (Fig. 1) and final products are obtained in reasonable yields. Target compounds were satisfactorily characterised and identified by NMR, MALDI-TOF MS and elementary analysis. Pharamacokinetics of the 4-imidazolidinones prepared, in both PBS (pH 7.4) and human plasma at 37 °C, are now under study and we hope results to be available by the time when this communication will be presented.

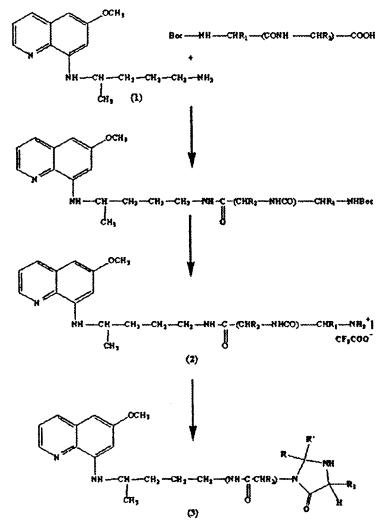


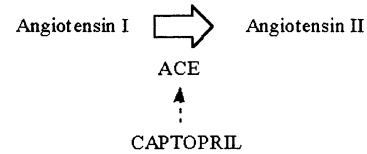
Fig. 1 - Synthesis of peptide 4-imidazolidinones as primaquine delivery systems

P D80 - A new lipophilic captopril analogue

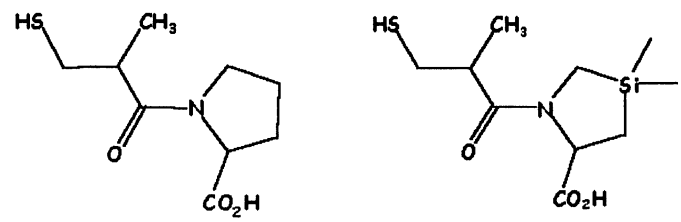
F. Cavelier[(1)], D. Damien Marchand[(1)], J. Galleyrand[(1)], J. Martinez[(1)]

1. Laboratoire des Aminoacides, Peptides et Protéines, UMR-CNRS 5810, Université Montpellier I et II, 34095 Montpellier cedex 05 - France

Captopril, an orally active hypertensive agent, is a competitive inhibitor of angiotensine I-converting enzyme (ACE). This enzyme plays a fundamental role in blood pressure regulation by converting the inactive decapeptide angiotensin I to the potent vasopressor octapeptide angiotensin II.



This paper presents the synthesis of a new analogue of captopril in which proline is replaced with silaproline, with the aim of increasing lipophilicity while conserving the inhibitory activity considering structural similarity.



Captopril *N-R-3-mercapto-2-methylpropanoyl-L-silaproline*

P D81 - Enhanced antitumor activity of bovine lactoferricin derivatives by introducing non-coded aromatic amino acids

L. T. Eliassen[1], B. E. Haug[2], G. Berge[1], Ø. Rekdal[1]

1. Department of Biochemistry, Faculty of Medicine - Norway
2. Department of Chemistry, Faculty of Science, University of Tromsø, N-9037 Tromsø - Norway

The lactoferricin derivative, LFB 17-31 has been subjected to structure-antibacterial activity relationship studies showing that two Trp residues were important for antibacterial activity [1]. Further studies concerning the significans of size, shape and aromatic character of the side chains revealed that substitution of the Trp residues with bulky non-coded aromatic amino acids (Dip, Bip, Tbt, Tpc) increased the antibacterial activity of the 15-residue LfcinB 17-31 [2], indicating that the size of Trp is more important than its amphipaticity or hydrogen bonding ability. Some lactoferricin derivatives have also been reported to possess antitumor activities [3,4]. In the current study we have tested the antitumor activity of LFB 17-31 derivatives where Trp residues were replaced with larger non coded amino acids (Dip, Bip, Tbt and Tpc), or modified with large and bulky groups at the N-terminus. The effect against three cancer cell lines and normal endothelial and fibroblast cells were investigated. It was found that the antitumor effect activity was enhanced by several of the modified peptides relative to the LFB 17-31 peptide. Based on the information gained during the screening of the modified peptides, some additional LFB-peptides were designed to explore whether a selective activity against tumor cells versus normal cells could be improved.

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P D82 - Structure-activity relationship of human urotensin II

P. Labarrère[1], D. Chatenet[2], C. Marionneau[3], J. Leprince[2], A. Chavanieu[1], E. Scalbert[4], B. Pfeiffer[5], P. Renard[5], P. Pacaud[3], H. Vaudry[2], B. Calas[1]

1. Centre de Biochimie Structurale Montpellier - France
2. Laboratoire de Neuroendocrinologie Cellulaire et Moléculaire Mont Saint Aignan - France
3. Inserm U533 Nantes - France
4. IRIS - France
5. ADIR - France

The cyclic peptide urotensin II (UII) has been first isolated from the caudal neurosecretory system of the teleost fish *Gillichthys mirabilis* [1]. The sequence of UII has been characterised in different vertebrate species. All isoforms of UII possess a hydrophobic cyclic core totally conserved among species (Cys-Phe-Trp-Lys-Tyr-Cys) whereas the N-ter sequence is highly variable. Human UII, discovered in 1998 [2], is the undecapeptide H-Glu-Thr-Pro-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH and was reported as the most potent vasoconstrictor peptide identified so far [3]. In order to study the structure-activity relationships of human UII, we have synthesised several sets of peptides corresponding to an Ala-scan, a D-scan, a step-by-step deletion and amino acid mutations. The biological activity of the peptides was tested on endothelium-denuded rings of rat thoracic aorta. The results indicated a relative tolerance for amino acid replacements in the N- and C-terminal domains but not in the cyclic core where mutations strongly reduced the vasoconstrictor action of the peptides. These results confirmed the existence of a minimum sequence conserving the activity of the natural peptide. Peptides mutated on the L-cysteine revealed that the nature and the stereochemistry of the disulfide bridge were essential for activity. On the other hand, it was noticed that acetylation of the N-ter amino acid increased activity by 2-fold, whereas amidation of the C-ter residue decreased activity in the same range. At the moment, the importance of several physico-chemical parameters of key amino acids is evaluated via rational mutations and QSAR analysis. Another part of our investigation concerned the molecular modelling of the human receptor for UII[3]. This receptor belongs to the G protein-coupled receptor family and presents similarities to members of the somatostatin/opioid receptor family. Based on sequence homologies with the rhodopsin receptor recently crystallised [4], we built a model for the human UII receptor with the hormone docked inside [5]. This model made it possible to propose a putative docking site. This structure-activity relationship study will provide useful information for the rational design of selective analogues of the UII receptor.

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P D83 - Cyclic peptides with recognition epitope of VCAM-1

M. Malesevic[1], D. Zimmermann[1], U. Strijowski[1], N. Sewald[1]

1. Faculty of Chemistry, University of Bielefeld, D-33501 Bielefeld - Germany

Integrins are α/β-heterodimeric cell-surface proteins that are involved in numerous cellular processes such as migration, growth and differentiation. Several different integrins are involved in disease processes and represent potential targets for drug discovery [1]. $\alpha_4\beta_1$ integrin (very late antigen-4) is expressed on human lymphocytes, monocytes, eosinophiles, basophiles and mast cells. It binds two main ligands: Vascular cell adhesion molecule-1 (VCAM-1) and the CS-1 form of fibronectin. Inhibitors of VLA-4 binding to fibronectin or VCAM-1 are likely to be useful in the treatment of a variety of inflammatory diseases. It has been shown that these cell-adhesion proteins contain minimal sequences, for example Arg-Gly-Asp (RGD), or Leu-Asp-Val (LDV) that bind to integrins at specific sites [2]. The proposed VCAM-1 integrin binding epitope TQIDSPLN in a surface-exposed loop connects two b-strands (the CD-loop) [3].
The targeted application of non-native building blocks (β-amino acids and D-amino acids) in the design of cyclic peptides allows the control of the peptide backbone conformation [4].
Cyclic peptides as conformationally restricted analogues of binding epitope in domain-1 of VCAM-1 are synthetised by the following strategy:
Synthesis of linear peptide precursors on 2-Cl-trityl resins by Fmoc tactics, cyclization under high dilution conditions and finally deprotection. A series of peptides has been synthetised and investigated with respect to conformation and biological activity.

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P D84 - Novel RGD-containing platelet GpIIb/IIIa receptor antagonists: design and synthesis

M. L. Mondeshki[1], L. T. Vezenkov[1]

1. University of Chemical Technology and Metallurgy, Organic Chemistry Department - Bulgaria

A series of novel platelet surface receptor GpIIb/IIIa antagonists: Ile-Pro-β-Ala-Arg-Gly-Asp, Ile-Pro-Ala-Arg-Gly-Asp, Pro- β-Ala-Arg-Gly-Asp and Pro-Ala-Arg-Gly-Asp, containing the Arg-Gly-Asp (RGD) sequence, was designed and synthesized by conventional methods in solution. It was interesting for us to investigate the influence of the N-terminal Pro residue on the antithrombotic activity of the synthesized peptides. Another aspect of the investigation was to establish the enzime stability of the β-Ala containing analogues compared to that of the Ala containing analogues. The synthesis of the protected peptides Boc-Ile-Pro- β-Ala-Arg(NO_2)-Gly-Asp(OBzl)-OBzl, Boc-Ile-Pro-Ala-Arg(NO_2)-Gly-Asp(OBzl)-OBzl, Boc-Pro- β-Ala-Arg(NO_2)-Gly-Asp(OBzl)-OBzl and Boc-Pro-Ala-Arg(NO_2)-Gly-Asp(OBzl)-OBzl was carried out by the stepwise attachment of the Boc-protected aminoacids by DCC/HOBt and HBTU/Et_3N methods. The synthesized products were deprotected by the consequtive treatment with trifluoroacetic acid (TFA) and catalytic reduction in the presence of Pd and purified on Sephadex G-15 by 0.2 N acetic acid.

P D85 - Inhibiting viral proteases: challenges and opportunities of induced fit

A. Pessi[1], P. Ingallinella[1], D. Fattori[1], D. Cicero[1], R. Bazzo[1], F. Dal Piaz[2], P. Pucci[2], E. Bianchi[1]

1. IRBM P. Angeletti, Via Pontina Km 30,600, 00040 Pomezia (Rome) - Italy
2. Centro Internazionale Servizi Spettrometria di Massa, Via Cinthia, 80126 Napoli - Italy

Virally-encoded proteases are promising targets for antiviral drugs. For some of these enzymes, like the serine proteases of Cytomegalovirus and Hepatitis C virus, substrate binding and cleavage is better described by an induced-fit rather than a lock-and-key mechanism [1]. We have studied the latter enzyme, NS3/4A, in detail. For S-site binding peptide inhibitors, we found that while different binding modes were possible in the absence of the NS4A cofactor, in its presence a single enzyme structure was achieved [1]. By using as structural probes decapeptide inhibitors spanning the P_6-P_4' subsites, we then found that binding to the prime site produces an additional structural reorganization [2 and manuscript in preparation]. In the absence of NS4A, each inhibitor stabilizes a different tertiary structure of the enzyme. We will discuss new details, obtained by NMR, of one of these inhibited structures. Remarkably, prime side binding induces different enzyme structures *also in the presence* of the cofactor. The picture emerging from our studies is that NS3 exists in solution as an ensemble of many rapidly interconverting structures, with the cofactor and the substrate synergistically selecting those achieving effective catalysis. This flexibility, which is functional to the rapid evolution of the viral quasispecies, presents both an opportunity and a challenge for drug design. As an example of the challenges, we will discuss the development of "serine-trap" type inhibitors. As an example of the opportunities, we will discuss new data on prime site inhibitors developed by combinatorial chemistry, which exploit a binding mode different from the natural substrates.

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P D86 - Design of novel dipeptides with anxiolytic activity on the base of CCK-4

E. Philippova[1], T. Gudasheva[1], V. Briling[1], M. Konstantinopolskiy[1], L. Kolik[1], S. Seredenin[1]

1. Institute of Pharmacology RAMS - Russian Federation

CCK-4 is an endogenous tetrapeptide with panic-like activity in humans. Antagonists of CCK-4 receptors have been shown to produce anxiolytic- like activity in animal models of anxiety. A series of substituted tryptophan-containing dipeptides with CCK-positive and CCK-negative activity were designed on the base of CCK-4 structure using principles of retro-peptide design.

Trp-Met-Asp-Phe-NH_2 (CCK-4)

$Ph(CH_2)_nCO$-Gly-Trp-NH_2 (Dipeptide retro-analog)

We have hypothesized that Trp and Phe hydrophobic side chains play a key role for CCK-4 receptor recognition. In our designed dipeptides the L-Trp moiety was kept, the Phe moiety was replaced by phenalconoyl group. The Gly moiety was taken as a spacer. The reverse direction of peptide chain was used. Normally in the case of reverse-directed peptide chain D-amino acids - containing peptides show agonistic activity, and L-amino acids lead to antagonistic activity. Really, designed substituted dipeptides with D-Trp produces anxiogenesis in animal models of anxiety. L-Trp-containing dipeptides was shown to exert the anxiolytic-like activity. The activity of substituted dipeptides depended on the length of CH_2 chain in phenalcanoyl moiety. The most active dipeptide was $Ph(CH_2)_5CO$-Gly-Trp-NH_2. It demonstrated anxiolytic activity in several anxiogenic models in a dose related manner by both i.p. and oral routes of administration over a dose range 0.006-0.2 mg/kg. Thus, designed dipeptides may be considered as a new group of potential anxiolytics.

P D87 - Modern peptide synthesis of intelligent gadolinium-delivery systems enables molecular imaging methods in tumor cells

R. Pipkorn[1], W. Waldeck[1], S. Heckl[1], J. Debus[1], J. Jenne[1], R. Rastert[1], G. van Kaick[1], K. Braun[1]

1. Deutsches Krebsforschungszentrum Heidelberg - Germany

At present, inefficient delivery and rapid excretion of the contrast agents are the major obstacles in Molecular Imaging. Despite the efforts in development of drug delivery systems, the lack of cell specifity was hampered distinguish tumor and normal cells. Amplification or overexpression of oncogenes eg. the myc family was determined in the DU 145 and AT1 prostate carcinoma cell lines. In this study, a transmembrane-transport-system of human origin was coupled to the Gadolinium-complex to enable passage into the intracellular space and to differentiate between tumor and non-tumor cells.
DU 145 and AT1 prostate carcinoma cells as well as non-tumor cells (lymphocytes) were incubated with a Gadolinium-complex which was, in turn, linked to a oncogene expressing cell specific peptide-module (100 pM). In order to improve the efficiency of cytoplasmic transport, a transmembrane-transport-system (human origin) was cleavably covalently bonded to the Gd-complex-peptide-module. A Gd-complex without transporter (Magnevist®) was used as a control. Influx and efflux were measured every 10 minutes with MRI (1,5 T; Siemens Magnetom Vision Plus; circular polarised head-coil). Additional the cellular localization of the Gd-complex was performed using confocal laser-scanning-microscopy.
After just ten minutes, Gd-complex-peptide-modules could be detected in cancer cells and non-tumor cells. Gd-complexes without transporter did not enter the intracellular space. The Gd-complex-peptide-module was transported rapidly out of non-tumor cells but remained in tumor cells due to target-specificity. Apoptosis was not detectable.
A rapid and specific uptake of gadolinium into the cytoplasm of the DU-145 and AT1 prostate cancer cells and non-tumor cells is possible. The complex-peptide-module persists only in the specified oncogene expressing tumor cells and thus allows a demarcation of tumor versus non-tumor cells. Intra-operative MRI in neuro-surgical procedures does not allow a clear delineation of malignant versus healthy tissue due to the out-flow of the interstitial contrast agent Magnevist® from the opened interstitium. This Gd-complex-peptide-module could be an elegant solution.

P D88 - Preferred conformation and membrane activity of the LP237-F lipopeptaibols

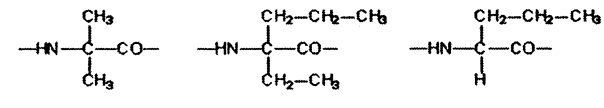
M. Rainaldi[1], A. Moretto[1], C. Peggion[1], F. Formaggio[1], S. Mammi[1], E. Peggion[1], J. A. Gálvez[2], M. D. Díaz-de-Villegas[2], C. Cativiela[2], C. Toniolo[1]

1. Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova - Italy
2. Department of Organic Chemistry, Institute of Materials Science of Aragon, University of Saragoza-CSIC, 50009 - Spain

The lipopeptaibol antibiotics of the LP237-F family are cytotoxic and antibacterial metabolites isolated from the cultures of the fungus *Tolypocladium geodes*. The three 10-peptide members sequenced so far (F5, F7, and F8) differ by the amino acids in positions 3 (Tyr or Phe) and 8 [Etn, C^{α}-ethyl norvaline, or Aib]. All have an N-terminal fatty acid and a C-terminal 1,2-aminoalcohol. The sequences synthesized and investigated in this work are as follows:

1				5				10			
Oc-	Aib-	Pro-	Phe-	Aib-	Gln-	Gln-	Aib-	Aib-	Gln-	Ala-	Lol	F7
Oc-	Aib-	Pro-	Phe-	Aib-	Gln-	Gln-	Aib-	Etn-	Gln-	Ala-	Lol	F8
Oc-	Aib-	Pro-	Phe-	Aib-	Gln-	Gln-	Aib-	Nva-	Gln-	Ala-	Lol	Nva analogue

(where Oc is *n*-octanoyl, Nva is norvaline and Lol is leucinol).
Here we describe their synthesis, conformational analysis (by FT-IR absorption, CD, and bidimensional NMR), and membrane permeability measurements. In particular, we examined the effect induced by hydrophobicity (higher in Etn and Nva than in Aib) and C^{α}-substitution (higher in Aib and Etn than in Nva) in position 8.



Aib Etn Nva

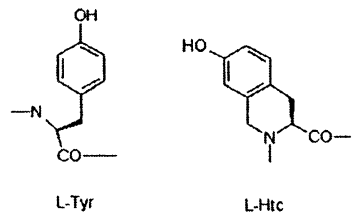
Preparation of the novel, enantiomerically pure, C^{α}-tetrasubstituted α-amino acid Etn was performed by chemical methods. Total synthesis of lipopeptaibols was achieved by condensation of the 1-4 and 5-10 segments, the latter with three Glu(OMe)-Gln replacements, followed by triple methyl ester aminolysis with NH_3. The three 10-peptides, characterized by a high percentage (40%) of C^{α}-tetrasubstituted α-amino acids, are markedly α-helical in $CDCl_3$, MeOH and DMSO solutions, particularly in their central and C-terminal regions. The α-helix topology is responsible for their marked amphiphilic character and the related remarkable membrane permeability properties. In position 8 the combination of high hydrophobicity and C^{α}-tetrasubstitution, as in the Etn-containing LP237-F8 antibiotic, has a positive effect on membrane interaction.

P D89 - Htc residue as useful tool in the synthesis of selective tyrosine kinase substrates

P. Ruzza[1], A. Calderan[1], A. Donella-Deana[2], A. Osler[1], B. Biondi[1], A. M. Brunati[2], L. A. Pinna[2], G. Borin[1]

1. Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova - Italy
2. Department of Biological Chemistry, University of Padova - Italy

Phosphorylation of proteins on tyrosyl residues plays a key role in the regulation of cell proliferation and differentiation, cell cycle control, as well as in the transmission of various signals from the extracellular compartment to the cytoplasm and into the nucleus. To study protein tyrosine kinase activities and substrate specificity in cell extracts, we synthesized different peptides reproducing natural phosphorylation sites, including the conserved autophosphorylation site of the Src-PTK family, and the HS1 phosphorylation site. We found that the replacement of the phosphorylatable tyrosine with its Htc constrained residue strongly inhibits the phosphorylation of peptide-substrates by Lyn and c-Fgr, two enzymes of Src-family. On the contrary, Syk can phosphorylate Htc-analog, albeit with a lower efficiency with respect to the native sequence. Kinetic data determined for Syk indicate the Htc-analog as a potential specific substrate for this enzyme [1, 2]. In order to develop useful peptides for the screening of the phosphorylation properties of PTKs, we elongated Htc containing peptides with a cationic tail. The presence of this poly-arginine sequence does not affect in any way the phosphorylation properties of these peptides, whereas it allows the quantitative binding of the screening peptide to phosphocellulose filters. Although a number of different techniques are available to separate the phosphorylated peptide from other ^{32}P-labeled material present in the assay mixtures, the most efficient method is to make use of the ability of peptides containing several basic aminoacids to bind via ionic interactions to phosphocellulose paper. The preliminary phosphorylation data and the evaluation of these peptides as diagnostics of specific protein tyrosine kinase as been reported.



Stucture of the phosphorylatable residues.

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P D90 - Design of a novel fluorogenic peptide substrate for aggrecanase activity

S. Singh[1], M. W. Pennington[1], J. Westling[2], S. Botsko[1], J. Sandy[2,3]

1. Bachem Bioscience Inc. - U.S.A
2. Shriners hospital for crippled children - U.S.A.
3. University of South Florida - U.S.A.

Aggrecan is the major osmotically active component of cartilage matrix. It confers on the tissue a capacity to resist compression under load. Increased levels of aggrecan fragments in synovial fluids indicate the onset of osteoarthritis and inflammatory joint diseases. It is widely accepted that the loss of aggrecan is promoted by the action of one or more of the aggrecanases, ADAMTS1, -4 and -5. Aggrecanases cleave human aggrecan at five sites, the most destructive cleavage being at the Glu^{373}-Ala^{374} bond [1]. This cleavage site, within the interglobular domain of the proteoglycan core protein has been shown to be the major cause of aggrecan destruction in human joint diseases [2]. The aim of this study was to design an internally-quenched fluorogenic substrate for assaying this cleavage activity. Thus, a peptide with the following sequence was developed: NH_2-Leu-Pro-Arg-Dpa-Ile-Thr-Glu-Gly-Glu*-Ala-Arg-Gly-Lys(MCA)-NH_2. It was expected that upon enzymatic cleavage of the Glu*-Ala bond, fluorescence quenching would be alleviated causing a continuous increase in fluorescence, which can then be measured fluorometrically.
The peptide substrate was synthesized using Fmoc chemistry on an amide resin. The side chain of C-term Lys was protected with ivDde, which allowed its selective derivatization with MCA after deprotection with 2% hydrazine/DMF.
The peptide substrate was assayed for its enzyme specificity. Thus, 20 μM substrate was incubated with 13 nM of rADAMTS-4 for 3.5 hours at 37 °C in 20 mM Tris, pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$. The cleavage of the substrate was followed by both HPLC and MS analyses. A plot of peak area of the cleaved peptide, NH_2-Ala-Arg-Gly-Lys(MCA)-NH_2 versus time clearly showed a steady increase in peak area with time. MS analysis of the cleaved sample also confirmed the expected cleavage. Molecular ion peaks $(M+H)^+$ corresponding to two cleavage fragments: NH_2-Leu-Pro-Arg-Dpa-Ile-Thr-Glu-Gly-Glu-CO_2H and NH_2-Ala-Arg-Gly-Lys(MCA)-NH_2 were readily identified. Fluorometric determination of the kinetics of substrate cleavage by rADAMTS4 is in progress.

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P D91 - Influence of polymer polypeptides and IL-6R peptides on chemotaxis of the macrophage-like cell line J774

R. Szabó[1], E. Illyés[1], G. Mező[1], N. Schiess[2], L. Kôhidai[2], F. Hudecz[1]

1. Research Group of Peptide Chemistry, Hungarian Academy of Sciences Budapest, 112, P.O. Box 32, H-1518 - Hungary
2. Department of Genetics, Cell- and Immunobiology, Semmelweis University, H-1445, Budapest - Hungary

Chemotaxis is one of the most important biological functions of the living cells. Migration of monocytes and macrophages plays an important role in the early steps of the immune response. Chemotactic activity is often induced by oligopeptides [1]. We found before, that IL-6R peptides with the sequence of SXWS are chemoattractant to the unicellular *Tetrahymena pyriformis* GL [2]. Polylysine based branched chain polymeric polypeptides (e.g. poly[Lys(dl-Ala_m-X_i)], (XAK) and poly[Lys(X_i)], (X_iK)) are used as macromolecular carrier of drugs and epitopes. Charge and polarity of the amino-acid components of their side chains influence the biological properties like cytotoxicity [3]. In the present studies the chemotactic effect of (W)SXWS peptides and ten polymers containing different side chains were investigated on the monocyte-macrophage tumour cell-line J774. Cytotoxicity of the polymers was measured by MTT-assay. Chemotactic ability of compounds was analyzed in 96 well NeuroProbe® chamber for 3 hours using a wide concentration range. The dependence of chemoattractant/repellent features of (W)SXWS peptides on the nature of amino acid X was documented [4]. Except the polycationic poly(Lys) and H_iK, the polymers were not toxic for the J774 cells after 1 hour. After 24 and 48 hours the toxicity of P_iK, SAK and Suc-EAK were slightly increased (20-25%). In the case of polymeric polypeptides a weak chemoattractant effect of polycationic polylysine, poly[Lys(dl-$Ala_{4,0}$-$Ser_{0,9}$)] (SAK), and poly[Lys(DL-$Ala_{3,1}$-$Thr_{1,0}$)] (TAK), containing hydroxyl-group in their side chain, and polyanionic poly[Lys(DL-$Ala_{4,0}$-Suc-$Glu_{1,0}$), Suc-EAK) were observed.

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P D92 - Evaluation of the GPIIb/IIIa regions participating in the platelets aggregation

V. Tsikaris[1], M. Sakarellos-Daitsiotis[1], N. Biris[1], M. Abatzis[1], E. Tenente[1], P. Stathopoulos[1], A. Tambaki[1], J. Mitsios[1], D. Tsoukatos[1], A. Tselepis[1], M. Elisaf[2], K. Soteriadou[3], D. Sideris[2], C. Sakarellos[1]

1. University of Ioannina, Department of Chemistry - Greece
2. University of Ioannina, Medical School - Greece
3. Hellenic Pasteur Institute - Greece

The GPIIb/IIIa receptor is the most abundant glycoprotein on platelet plasma – membranes consisting, like other integrins, of a non – covalently associated heterodimeric complex of two subunits, the GPIIb or αIIb (α-subunit) and the GPIIIa or β3 (β-subunit) [1]. This glycoprotein undergoes substantial conformational changes on activation to gain high affinity to fibrinogen, an event essential to platelet aggregation and thrombus formation [2]. Small RGD containing peptides, hydrids and non – peptide mimetics have been applied as potent and highly specific inhibitors of the GPIIb/IIIa – fibrinogen interaction as it is mediated by the RGD cell – adhesion region of fibrinogen. However, binding of the RGD – like inhibitors to GPIIb/IIIa maintains the receptor in the activated stage, which enhances the platelet activation event, despite the initial RGD-inhibitory effect on platelet aggregation. Mapping of the two subunits by soluble 20-peptides covering their entire extracellular regions revealed the occurrence of potent inhibitors of the platelet aggregation. Their anti-aggregatory activity is ranged from 80 to 20% for GPIIb and from 60 to 25% for GPIIIa at 500μM peptide concentration. In particular, the sequences ETGGVFLCRWRAEGGQCPSL (49-68 GPIIb) and YMESRADRKLAEVGRVYLFL (313-332GPIIb),as well as the sequences YPSLGLMTEKLSQKNINLIF (289-308GPIIIa),SSNVLQLIVDAYGKIRSKVE(337-356GPIIIa) and FKDSLIVQVTFDCDCACQAQ(421-440GPIIIa) display the best anti-aggregatory effect. The defined binding sites, some of them being in agreement with the literature [3], are found on the hydrophilic regions of both subunits suggesting their localization on the exposed and highly accessible areas of the receptor. The great advantage of this study is that in all assays the receptor was utilized in the conformationally active form.

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P D93 - Peptide analogues of the GPIIb 313-332 region of the GPIIb/IIIa receptor: design, synthesis and antithrombotic activity

V. Tsikaris[1], M. Abatzis[1], N. Biris[1], A. Tambaki[1], J. Mitsios[1], M. Sakarellos-Daitsiotis[1], D. Tsoukatos[1], A. Tselepis[1], M. Elisaf[2], K. Soteriadou[3], D. Sideris[2], C. Sakarellos[1]

1. University of Ioannina, Department of Chemistry - Greece
2. University of Ioannina, Medical School - Greece
3. Hellenic Pasteur Institute - Greece

Platelet activation, aggregation and thrombus formation are all directly involved in acute coronary syndromes such as Acute Myocardial Infraction and Unstable Angina. The GPIIb/IIIa receptor, which is the major fibrinogen receptor and belongs to the integrin family, plays an important role in this process. In previous studies, we realized the mapping of the extracellular domain of both subunits of the GPIIb/IIIa receptor, which resulted in the determination of the possible fibrinogen binding sites. The GPIIb 313-332 region YMESRADRKLAEVGRVYLFL has been chosen for further studies, as it has shown an increased inhibitory effect on platelet aggregation. It has to be noted that this sequence is reported for the first time as a binding site of the receptor, while there are reports for the vicinal 294-314 and 300-312 sequences [1,2] as binding sites of GPIIb/IIIa. In order to determine the minimum length of the site, which is required for the maintenance of the inhibitory effect, seven overlapping by 6 amino acids, peptides were synthesized. The three octapeptides, GPIIb 313-320, GPIIb 315-322 and GPIIb 317-324, which cover the GPIIb 313-324 region, showed a gradual reduction of the inhibitory effect (from 70% to 15%) on platelet aggregation. Amino acid replacement in these three octapeptides led to the synthesis of new analogues that are possibly characterized by a different inhibition mechanism. The structural study of the above analogues has been performed by ^{1}H-NMR spectroscopy. The conformation-biological activity relationship will be discussed thoroughly.

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P D94 - Cyclic RGD peptides influence the interaction between the integrin αvβ1 and fibronectin

D. Zimmermann[1], A. Müller[2], M. Malesevic[1], K. Stembera[1], N. Sewald[1]

1. Faculty of Chemistry, University of Bielefeld, D-33501 Bielefeld - Germany
2. Evotec OAI, D-22525 Hamburg - Germany

Integrins are cell surface receptors that bind to different components of the extracellular matrix. They are heterodimers that consist of noncovalently associated α and β chains. The integrin αvβ1 is a fibronectin receptor expressed on lymphocytes and leucocytes. In contrast to other fibronectin receptors it is specialised in the interaction with fibronectin and does not bind to any other ligand [1].
This integrin plays an role in extravasation and migration of activated lymphocytes during immune response and is involved in inflammatory diseases [2].
Fibronectin, its natural ligand, belongs to the group of the extracellular matrix proteins. It is composed of three different domains, each being responsible for specific binding to components of the extracellular matrix.
The sequence -Arg-Gly-Asp- (RGD) in the integrin binding domain is essential for the interaction between fibronectin and the integrin αvβ1.
A series of cyclic peptides containing the RGD sequence together with amino acids that stabilize the overall secondary structure (D-amino acids, β-amino acids) has been synthesized and tested.
Especially cyclic peptides with defined secondary structure like c-(-Arg-Gly-Asp-D-Phe-Val-β-Ala-), c-(-Arg-Gly-Asp-D-Phe-Val-Gly-), c-(-Arg-Gly-Asp-D-Phe-β-Leu-Gly-) and c-(-Arg-Gly-Asp-D-Phe-β-Leu-Ala-) have been investigated.
The influence of this peptides was analyzed by several biological assays like cell adhesion assays or surface plasmon resonace (SPR).

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P D95 - Virtual high throughput screening using LigandFit as an accurate and very fast tool for docking, scoring, and ranking

M. Lim-Wilby[1], J. Jiang[1], M. Waldman[1], C. M. Venkatachalam[1]

1. Accelrys Inc., 9685 Scranton Road, San Diego, CA 92121 - U.S.A

The imperative for virtual high throughput screening arises from the availability of multiple targets, millions of compounds in screening libraries, and limited resources for even the best-endowed pharmaceutical enterprises. LigandFit, a docking and scoring application, has been developed to address this need. A suite of algorithms is provided to (1) aid the user in the detection and definition of binding sites, (2) provide various docking modes with user-defined options, and (3) rank ligands using LigScore2 and published scoring functions. We will present considerations that affect accuracy in both docking & scoring, including the effects of disproportionately large binding sites, extremely flexible ligands, metal ions, and the presence of flexible protein side chains. Recent advances have allowed reasonably large (~50k) ligand libraries to be screened in a matter of hours, such that the bottleneck in virtual screening is no longer docking, but the preparation and analysis of the datasets.

P D96 - The bioactivity of sugar - amino acid / peptide interaction products

K. F. El-Massry[1], A. H. El-Ghorab[1], A. Farouk[1]

1. Flavour & Aromatic Chem. Dep., National Research Center, El-Tahrir st., Dokki, Cairo - Egypt

During Maillard reaction (thermal treatment of foods) reducing sugars react with amino acids or peptides, as a result, a concentrates with different chemical classes of volatiles are formed which affect the sensory evaluation, physical, chemical properties and the nutritional value of the product and influence customer acceptability. Maillard reaction products are not only responsible for browning and flavour formation, but there are also products formed which have antioxidative activity. Although the antioxidative activity of Maillard reaction mixtures is well established, hardly anything is known about the molecular and physiological mechanisms of theses effects. Many aroma concentrates were prepared using different amino acids and peptide with different functional groups and different sugars. The sensory properties were evaluated according to the ISO rules. The degree of browning, the phenolic content as well as the antioxidative activity were also assayed for the different concentrates. It was found that, there is an obvious structure - activity relationship between the reactants and the formed concentrates. Also, this study showed for the first time that Maillard reaction products can have beneficial influence on LDL oxidation in vivo. Furthermore it was possible to relate the activity to the amino acid or peptide structure and even to distinct Maillard reaction products. Theses results suggest that certain Maillard products have beneficial physiological activity. Biological studies were done to learn more about their fate in vivo and about their potential activity. The study showed that glutathione model system have a hepatoprotective activity by hindering the elevation of hepatic TBARS content as well as the ALT level in the liver and sera of the CCl4-intoxicated rats. Therefore, there is a possibility that the hepatoprotective properties of glutathione model system might be attributed to the antioxidative activities in CCl4-intoxicated rats. Also it was found that glutathione model system has no effect on the different biochemical parameters of rats which given this product for 30 days.