

P D29 - The mechanism of signal transduction through membranes by integrins

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Integrins are membrane spanning heterodimeric receptors which couple signal transduction with cell matrix adhesion. Several integrins recognize the tripeptide sequence in RGD-containing peptides and proteins. However, the structural changes, which are involved in the signaling event, are poorly understood. Signal transduction can be initiated by the binding of intracellular proteins to the integrin. Binding leads to a major conformational change. The change is passed on to the extracellular domain through the membrane. The affinity of the extra-cellular domain to certain ligands increases. Thus at least two states exist, a low-affinity and a high affinity state. By using automated docking and molecular modeling starting from the crystal structure of the extra-cellular domains of $\alpha v\beta 3$ [1] as well as by a global search of helix-helix interactions of the transmembrane section of the family of integrins we develop a three state mechanism involving allosteric effects triggered by a ligand induced shift of a Ca^{2+} -ion [2]. We introduce an additional step before dissociation of the head groups, which corresponds to the published crystal structure of the headgroups of $\alpha v\beta 3$ and constitutes a high affinity state. In our model, the inactive state (state one, Fig. 1 *left*) is locked in its position by intra-cellular interaction. Proteins binding at intracellular domains or deletion of the transmembrane and intracellular domains trigger a rotational movement of an integrin subunit, which activates the integrin. This activated or pre-conditioned state (state two, Fig. 1 *middle*) corresponds to the published crystal structure and has a high affinity to extra-cellular ligands. In contrast to the prevailing model the head groups are still associated in this state. Extra-cellular ligand binding leads to further conformational changes, which separate the integrin head groups (Figure 1, *right*). We demonstrate [3] that the family of integrins are capable of adopting a structure similar to the structure of the homodimeric transmembrane protein Glycophorin A. For the $\alpha IIb\beta 3$ integrin this structural motif represents the high affinity state. A second conformation of the transmembrane domain of $\alpha m\beta 3$ is identified as the low-affinity state by known mutational and NMR studies. A transition between these two states was determined by molecular dynamics calculations, which corroborate the existence of a third state.

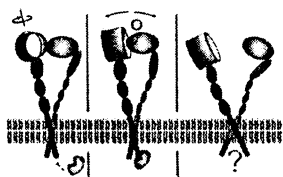


Fig. 1: Three state model of signal transduction in integrins.

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P D31 - Model cyclic peptides for studying the influence of ion cluster in the protein folding and stability

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The interest in protein folding has strongly increased with the development of biotechnology. Two evident reasons are: (a) new polypeptides that are synthesized *in vivo* must be able to fold to a native protein before they can translate their genetic information into biological activity, and (b) thermal and chemical stability are an important properties when proteins are applied in industrial processes.

In protein folding and stability studies, α -lactalbumin (LA small globular protein) has received considerable attention because the transitions between the compact native state, the molten globule (relatively compact intermediate) state, and the more unfolded states can easily be realized. A deep cleft divides the molecules roughly into two lobes. One lobe mainly consists of four α -helices, the other lobe is rather β -sheet-like. In the loop connecting both lobes, all LAs contain 3 critical aspartic acid residues (Asp^{82} , Asp^{87} and Asp^{88}) that are responsible for strong Ca^{2+} binding [1] which considerably stabilizes the native state of the protein. The critical Asp-residues are located in a loop consisting of 10 amino-acids and they participate in one of the negatively charged clusters of the protein. The loop contains two more Asp-residues, the side-chain carboxyl groups of which are not linked with Ca^{2+} but significantly contribute to the local charge.

In the structure of LA, the decapeptide (-KFLDDDLTDD-) forms a considerably twisted loop where the α -C atoms of the first and last amino acid are fixed at about 7.9 Å. Therefore, in a first step we plan to connect the ends of the decapeptide and to look for the most suitable spacer needed for that connection using MM calculations.

We report here the synthesis of the model peptides of the Ca^{2+} binding loop. Their Ca^{2+} binding will be investigated by microcalorimetry (ITC and DSC) and circular dichroism (CD). The results will give information on the impact of the accumulation of negative charges upon the Ca^{2+} binding properties within LA and upon the tendency of the protein to form a partially unfolded state in slightly denaturing conditions.

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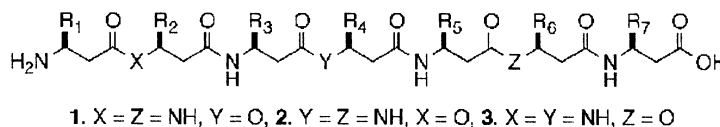
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P D30 - β -Depsipeptides: synthesis and structural studies.

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Oligomers of β -amino acids, β -peptides, are being extensively studied for the past few years [1]. They have been shown to fold into helices or turns, form sheets or stack up in a tubular fashion, depending on the substitution pattern of the peptide backbone. Stable helical structures are formed by peptides containing as few as six residues. The effect of various structural changes, such as use of residues leading to salt-bridge formation [2], introduction of a disulphide clamp [3], replacement of a residue with an α -amino acid, or by varying substituents on the central β -amino acid residue [4], on the helical content of these peptides has been examined. The substitution of a β -amino acid with a hydroxy butyric acid unit lead to disruption of the helical structure of a fully protected β -depsipeptide, as seen from the CD spectra [4]. In order to more closely investigate the effect of hydrogen-bond deletion on unprotected β -depsipeptides, we have synthesized β -depsipeptides 1-3, with the hydroxy butyric acid unit at various positions in the depsipeptide sequence. CD-Spectroscopic and NMR studies of these peptides are in progress. These experiments will give us more insights into the contribution of the hydrogen bonds to the stabilization of the 3_{14} helix in analogous β -peptides.



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P D32 - Circular dichroism and conformation of oostatic peptides: carrier-like role of the C-terminal (Pro)x sequence

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As shown in the previous work [1,2] shortening of the oostatic decapeptide H-Tyr-Asp-Pro-Ala-Pro_n-OH [3] from the C-terminus results in enhancement and acceleration of its effect on egg development inhibition in common flesh fly (*Neobellieria bullata*). We have investigated circular dichroism spectra of the parent and shortened peptide sequences, H-Tyr-Asp-Pro-OH, H-Tyr-Asp-Pro-Ala-Pro_n-OH, n=0-6 and their changes with respect to solvent and pH variation. The study includes also isosteric peptides having methyleneoxy moiety instead of the amide group connecting Pro³ and Ala⁴ residues [4]. The results indicate that the C-terminal oligoproline sequence adopts a quite stable polyproline II like conformation, at least at neutral and acidic conditions and also in 2,2,2-trifluoroethanol solution. The spectra of short N-terminal peptides do not correspond to regular peptidic structures and merely indicate a not very ordered arrangement. These facts support the possibility that the parent decapeptide acts merely like a prohormone from which the active N-terminal peptide is released by gradual cleavage of the C-terminal proline rich part.

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P D33 - Synthesis and solution structure of the two peptides that represent the active -zinc containing- sites of angiotensin converting enzyme (ACE)

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Angiotensin-Converting Enzyme (ACE, EC 3.4.15.1) is a Zn²⁺ containing peptidylhydrolase which plays a crucial role in the regulation of blood pressure due to its ability to remove hydrolytically the C-terminal dipeptide histidylleucine from the decapeptide Angiotensin I producing the potent vasopressor Angiotensin II. ACE belongs to Zincins superfamily and exists in human in two isoforms. The somatic isoform is found on the surface of vascular endothelial cells and the testis isoform in spermatozoa and spermatozoa. ACE enzymes that have been investigated so far have molecular weights in the range of 80kD-160kD depending from the source of enzyme. The large size of these enzymes has been proven to be the main obstacle in its structural elucidation either in solid state or in solution. Consequently, ACE inhibitors are restricted to be designed on the basis of the X-ray crystal structure of Thermolysin or Carboxypeptidase A, which present the characteristic metal-binding motifs HEXXH and EXIXD of various Zn metalloproteases, observed also in ACE. In this work two peptides bearing 36 amino acids, which correspond to the N- (ACE I) and C- (ACE II) metal binding sites, were synthesized and studied in solution by ¹H NMR spectroscopy in order to gain further information concerning the ACE active sites (Figure 1). Complete sequence specific assignment was performed for the apo- and holo- form of the 36 residues fragment of both active sites, in order to probe the structural differences upon the zinc complexation. Preliminary analysis of sequential, short and medium range NOEs revealed that the helical structure is the main structural feature of the peptide skeleton. The two 36-residue peptides without the zinc metal ion (His390...Ala425 for ACE I and His988...Ala1023 for ACE II native sequences) adopt α -helical structure at their two termini and in a fragment which covers the region 19-22 of the primary sequence (Figure 2). The region covered by the residues in positions 13-15 of the sequence is susceptible to form a turn when the peptides are folded upon zinc coordination.

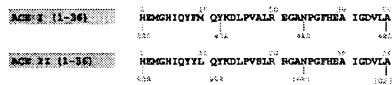


Fig 1 - Amino acid sequence of the synthesized peptides

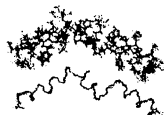


Fig. 2 - Family of 30 structural models of ACE II peptide calculated through NMR data

P D35 - Mapping of the active sites of PSA modulating peptides

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Introduction and objectives: PSA, a member of kallikrein protease family, is widely used marker for prostate cancer in humans. Several prostate specific antigen (PSA) binding peptides have been identified by using phage display libraries. These peptides are PSA specific and they do not bind to any other serine proteases. Cyclic peptide analogs have been shown to promote PSA protease activity. The active sites of the three cyclic modulating peptides with the lengths of 10 to 13 amino acids we studied by using point mutations and located into the three dimensional structure determined by NMR.

Methods: Cyclic peptides were synthesized with Fmoc synthesis strategy. Peptides were cyclized via cystein side chains with the Iodination method and Acetaminomethyl (Acm) as a side chain protection group. NMR analyses were made with a Bruker Avance 500 NMR spectrometer. In 1D experiments the temperature coefficients (dd/dT) of the amide protons were calculated by analysing the chemical shifts at five temperatures (300-320 K). The 2D experiments were recorded either at 305 K or at 310 K. The amino acids were assigned and peptide structures were determined with help of TOCSY and NOESY spectra. Structure calculations were performed by DYANA software.

According to the alanine replacement studies, the two aromatic amino acid side chains are essential for the biological activity of all three peptides. The amino terminal Phe(3) or Tyr(4) and the carboxy terminal Tyr(7) or Trp(11) were found essential respectively. In the three PSA modulating peptides the aromatic rings turn near each others and are suggested to form the interaction site with PSA.

P D34 - Synthetic peptides as molecular recognition probes: recognition of a hydrophilic patch on the surface of p53 tetramerization domain

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The increasing interest in protein-protein interactions has stimulated the study of protein surface molecular recognition. Protein-protein interactions are usually mediated through large areas which have complementary shape and charge. The recognition of these surfaces could therefore allow the modulation of a protein function. This kind of recognition is however poorly developed in comparison to inhibitors that bind to the active site of enzymes. The extensive solvation of charged and polar groups on the protein's exterior is the greatest barrier to achieve this recognition. Synthetic peptides, thanks to their large chemical diversity at the side-chain level, provide a good opportunity for the design of protein-specific ligands, including those addressed to highly hydrophilic surface-patches. In the present work, we have designed and synthesised a peptide ligand which is able to recognise a highly hydrophilic patch located on the surface of the tetramerization domain of the tumor suppressor protein p53. This hydrophilic motif is formed by four acidic residues arranged into an α -helix in an *i,i+3* distribution. We have expressed and purified this domain labeled with ¹⁵N and ¹³C in *E. Coli*. The design of the peptide has been assisted by molecular modeling, and the interaction has been studied by the combined use of fluorescence spectroscopy, nuclear magnetic resonance and surface plasmon resonance (SPR). We suggest that this recognition event could be used to interfere with the interaction of p53 with other proteins or DNA, and/or to modulate the stability of the domain, and therefore the stability of the whole protein.

P D36 - Conformational studies of leptin fragments by circular dichroism

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The adipocyte-derived protein hormone leptin inhibits food intake and increased energy expenditure through brain receptors in the hypothalamus. This protein contains 146 amino acids and shows high identity among the mammals. The leptin receptor belongs to the class I cytokine receptor family that are found in many hypothalamic regions including arcuate, paraventricular and ventromedial nuclei. In a previous study, we observed that after intravenous administration of 1 mg/kg of six leptin fragments {Ac-Lep₂₂₋₂₆-NH₂ (I), Ac-Lep₂₇₋₅₀-NH₂ (II), Ac-Lep₅₁₋₅₇-NH₂ (III), Ac-Lep₇₁₋₉₄-NH₂ (IV), Ac-[Se⁹⁶]-Lep₉₅₋₁₁₉-NH₂ (V) and Ac-Lep₁₂₀₋₁₄₃-NH₂ (VI)}, only I and V were able to induce Fos-like immunoreactivity (Fos-ir) in the lateral arcuate, ventromedial and dorsomedial nuclei of the hypothalamus, although not with the same intensity than the leptin [1]. Fragments I, III, IV and VI exhibit an α -helical structure, while fragments II and V shape the loops in the native leptin molecule [2,3]. In this work, we decide to study and compare the secondary structures of the six-isolated leptin fragments by circular dichroism. The peptides were synthesized by solid-phase using the *t*-Boc strategy on the MBHA resin. After HF treatment, they were purified by RP-HPLC, characterized by LC/MS, AAA, and CZE and tested in their ability to induce Fos-like immunoreactivity in adult male rats. CD spectra, as a function of the pH (4-10) and the TFE concentration, were recorded on a Jasco instrument (J180) using 15 mM PBC as buffer. From these studies we found that most of the fragments presented random coil structure in the pH range tested. The only exception was the fragment IV, which presented a typical α -helix profile. As expected, a significant content of helical structure was observed in all the compounds, with concomitant reduction in random coil structures, when the TFE content was increased. The decrease in the biological activity might be correlated to an unexpected lower helical content of the fragments under physiological conditions. Even though the peptide design needs some refinement, these data show that synthetic peptides may be an essential tool for the development of leptin-related compounds having potential application in human or veterinary medicine.

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P D37 - Structure analysis of cyclo[Ala-β-Ala-Pro-β-Ala] by NMR spectroscopy and *ab initio* computing

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Cyclo[Ala-β-Ala-Pro-β-Ala] was synthesised for modelling the inverse γ-turn. In order to identify the possible conformers in solution we combined two three dimensional structure analysis methods: NMR spectroscopy and *ab initio* molecular computation.

The energy and structure of several conformers of the cyclic peptide were computed by *ab initio* methods. Calculations were carried out at three different levels of theory: first AM1, second RHF/3-21G, third B3LYP/6-31+G(d).

Two-dimensional NMR spectra (TOCSY and NOESY) were recorded in TFE-d₂. Following chemical shift assignment, distance restraints were collected and several conformers computed using XPLOR 3.851. However, all NMR restraints seem to result from possible conformational averages in solution.

By comparing the *ab initio* and the most likely NMR structures the most probable solution conformers could be identified.

P D38 - Location of copper(II) within the prion PrP(106-126) fragment and influence of the metal ion on the conformational change of this peptide.

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The prion protein (PrP) is the infectious agent responsible for the spongiform encephalopathies such as Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. These diseases appear to be caused by the post-translational and conformational transition of the normal cellular prion protein isoform (PrP^C), into the abnormal pathogenic isoform (PrP^{Sc}). [1] The highly conserved region encompassing the residues 106-126 of human PrP^C seems to have a key role in this conformational conversion. *In vitro* studies on the synthetic peptide homologous with this sequence (PrP 106-126: KTNMKHMAGAAAGAVVGGGLG) indicate it adopts different secondary structures in different environments, is highly fibrillogenic and toxic to neuronal cell cultures. [2] Since the PrP(106-126) exhibits some of the pathogenic and physicochemical properties of PrP^{Sc}, it has been used as a model for the study of PrP^{Sc} neurotoxicity. Studies with recombinant or native prion protein and peptides related to its sequence have suggested that PrP^C is a copper binding protein. Recombinant protein binds between four and five copper atoms. Four of these copper ions were specifically retained by the octapeptides (PHGGGWGQ)₄ from amino acids 60-91 within the flexible N-terminal half of PrP^C. [3] The data for the structured C-terminal domain PrP(121-231), containing three histidine residues, revealed one further possible copper binding site. [4] Interestingly, recent evidence indicates that PrP(106-126) can interact with copper(II) ions and this interaction modulates either PrP(106-126)'s fibrillogenicity and neurotoxicity. [5] We have been studying the synthesis and the interactions of metal ions with peptides corresponding to either structurally and functionally relevant regions of the prion protein. [6] In this communication we report a spectroscopic study on the copper(II) complexes formed with PrP(106-114: KTNMKHMAG), corresponding to the N-terminal polar head of PrP(106-126) sequence, and the whole PrP(106-126) itself. The study has been carried out by visible absorption (UV-vis), circular dichroism (CD), nuclear magnetic resonance (NMR) and electron spin resonance (ESR) spectroscopic methods. Moreover, electrospray ionisation mass spectrometry (ESI-MS) has been used to directly determine the stoichiometry of the copper(II) complexes. The results presented here indicate that both peptides interact specifically with copper(II) and that metal binding strongly affects their conformation. Moreover, the comparison of the obtained data allowed us the unambiguous determination of PrP(106-126)'s metal binding site.

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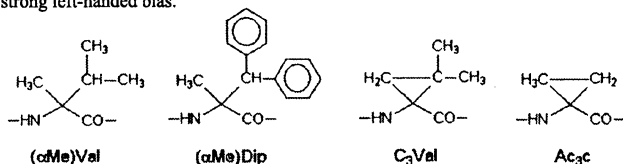
P D39 - Conformational properties of c₃Val, a C^α-tetrasubstituted, C^β-branched, C^α₁ ↔ C^α₂ cyclized, chiral α-amino acid

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The relevance of stable secondary structure elements in peptides and proteins has inspired considerable effort toward the design of rigidified artificial molecules. In particular, significant progress has been made in engineering and synthesizing turn and helix inducers and mimetics. Indeed, turn and helical motifs are fundamental constituents of many biologically active peptides and are often critical for their biological activity. Among the many approaches to the synthesis of rigidified peptides, a widely used strategy has been represented by the exploitation of structurally restricted, C^α-tetrasubstituted α-amino acids able to drastically reduce the conformational freedom of the peptide in which they are inserted. Among the C^α-tetrasubstituted, chiral α-amino acids with a single β-branched side chain the *aliphatic*, C^β-trisubstituted (αMe)Val is an extremely efficient β-turn and 3₁₀-helix former, while the *aromatic*, C^β-trisubstituted (αMe)Dip may induce either a folded or a fully-extended (C₅) conformation.

To expand the investigation of the preferred conformation of this sub-class of C^α-tetrasubstituted α-amino acids, we embarked on a program directed toward the first structural characterization of peptides based on c₃Val, characterized by a C^β-tetrasubstituted, *aliphatic* side chain. c₃Val can also be viewed as a C^β,β'-dimethyl derivative of the C^α₁ ↔ C^α₂ cyclized residue Ac₃C, known to form distorted β-turn and 3₁₀-helical conformations. Optically pure (R)c₃Val was produced by a combination of chemical and chiral chromatographic steps. *Via* step-by-step solution methods, using the EDC/HOAt coupling procedure, we synthesized the -c₃Val-(Gly)- and -c₃Val-(Aib)-c₃Val-(Aib)- peptide sequences. For comparison, the related (αMe)Val-based tripeptide sequence was also prepared. The conformational preferences of these peptides were assessed by FT-IR absorption, ¹H NMR, and X-ray diffraction techniques. Our experimental results strongly support the view that: (i) the c₃Val residue is a good β-turn and 3₁₀-helix former, although apparently less efficient than (αMe)Val; and (ii) the relationship between α-carbon chirality and screw sense of the folded structure formed is the same as that of (αMe)Val, *i.e.* the (R) residue has a strong left-handed bias.

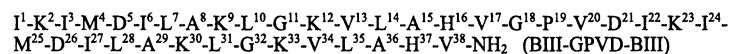


P D40 - Conformational studies of a bombolitin III-derived peptide mimicking the four-helix bundle structural motif

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Bombolitins are five structurally-related heptadecapeptides originally isolated from the venom of a bumblebee [1]. The biological activity of these peptides is related to their ability to form amphipathic helices in the presence of phospholipid aggregates or membrane-mimetic systems. In aqueous solution and in the absence of detergent micelles or phospholipids, bombolitins at sufficiently high concentration form oligomeric aggregates with consequent conformational transition from a random coil to the α-helical structure [2]. The aggregation propensity is sequence-dependent and is higher for bombolitin II and III. In previous work we have studied a bombolitin III analog containing the photo-reactive residue benzoyl-phenylalanine in position 3. By irradiation of Bpa³-bombolitin III aggregates, covalently bound dimers were obtained with the α-helices oriented in an antiparallel fashion [3]. These studies also suggested the possibility that two dimers further aggregate forming a four-helix bundle motif. On the basis of these results we designed and studied the following peptide formed by two bombolitin III sequences linked head-to-tail by the tetrapeptide bridge -Gly-Pro-Val-Asp-



It has been shown by Baltzer and coworkers [4] that the tetrapeptide GPVD connecting helical peptide sequences favors the formation of the helix-loop-helix structural motif. Thus dimerization of peptide sequences in a loop-helix-loop structure should lead to the formation of the four-helix-bundle motif. The conformational properties of the peptide were studied by CD, NMR and molecular dynamics calculations. The results indicate the presence of a loop-helix-loop conformation at 10⁻³M concentrations. At higher concentration NOESY connectivities were detected which are compatible with the presence of a four-helix bundle formed by dimerization of peptide molecules in the helix-loop-helix structure.

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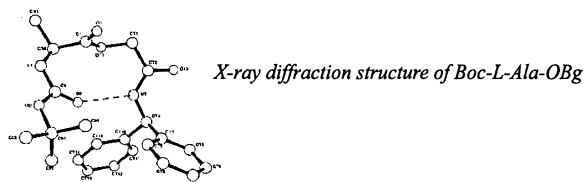
P D41 - Conformational bias induced by the OBg C-protecting group

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The N-benzhydryl-glycolamide ester (OBg ester) is a useful C-protecting group in peptide synthesis which is stable under the conditions used for removal of the Z, Boc, and Fmoc protecting groups, and can be selectively cleaved upon a mild alkaline treatment [1] or by tetrabutylammonium fluoride in organic solvents [2]. Owing to the presence of the bulky benzhydryl group, OBg esters of N^α-protected amino acids are often crystalline compounds of relatively high melting point. The occurrence of a type I γ-turn at the C-terminus in the crystal structure of the peptide Boc-D-Pro-L-Pro-L-Pro-D-Pro-OBg, where the N-H group of the benzhydrylamino moiety is intramolecularly hydrogen bonded to the carbonyl oxygen of the L-Pro(3) residue [3], prompted us to investigate whether the conformational motif shown by the C-terminal OBg group in the above mentioned structure could be of more general significance. The minimal chain length of model compounds suitable for testing such an hypothesis is represented by N^α-protected aminoacyl-OBg esters.

The preferred conformation of the OBg esters of selected N^α-protected amino acid derivatives, which can be viewed as N^α-acylated dipeptide derivatives, in structure-supporting solvents was investigated by FT-IR absorption and ¹H NMR techniques. The results indicated significant populations of intramolecularly H-bonded conformers. In addition, the X-ray diffraction structures of Z-Aib-OBg, Boc-Ala-OBg, and Piv-Pro-OBg provided definitive evidence for the occurrence of β-turn conformation in each of the above compounds. These results indicate that the OBg group is able to promote a folded conformation in the preceding amino acid, a unique property among C-protecting groups.



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P D43 - The crystal structure of a D-Lysine-based chiral PNA-DNA duplex.

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Peptide nucleic acids (PNAs) are nucleic acid analogues in which the sugar-phosphate backbone has been replaced by a pseudo-peptide skeleton, made up of N-(2-aminoethyl)glycine units [1]. Nucleobases are linked to this skeleton through a carboxymethyl moiety, which allows for a two atom spacer between the backbone and the bases. PNAs recognize DNA and RNA sequences with high specificity and selectively by forming Watson-Crick base pairs leading to RNA-PNA and DNA-PNA hybrids more stable than the corresponding regular nucleic acid complexes. The high thermal stability and the high resistance to proteases and nucleases make PNAs ideal candidates as anti-sense or anti-gene therapeutic agents [2], and they are currently used as very powerful tools in molecular biology and in diagnostics. However, the development of PNA based therapeutics is somewhat prevented by the poor solubility of PNAs in physiological conditions and by their limited uptake into cells. Furthermore, PNAs can bind to complementary DNA strands in both parallel and antiparallel orientations. Thus, several variants of the basic PNA structure have been proposed in order to improve the binding specificity, solubility and uptake into cells [3]. In this context, recently Sforza *et al.* [4] reported the synthesis of a chiral PNA decamer (GTAGAD_D-Lys₈TPD-Lys₂CD_D-Lys₂ACT) containing three adjacent chiral monomers ("chiral box") based on D-lysine, in the middle of the PNA sequence. Binding studies demonstrated that this 10-mer PNA hybridizes with the complementary DNA sequence only in the antiparallel mode; furthermore, the difference between the melting temperatures of its full-matched and single-mismatched hybrids with DNA strands was greatly enhanced compared to those observed for other achiral or chiral PNA. Thus, it possesses many of the properties required for the detection of point mutation in diagnostics, and for the development of gene therapeutics. In a general project, aimed at understanding at the molecular level the role of chirality in DNA recognition, and in order to investigate the structure-activity relationships of chiral PNAs, we have undertaken a crystallographic study on this PNA decamer, hybridized with its complementary antiparallel DNA strand (5'-AGTGATCTAC-3'). Here we report the crystal structure at 1.6 Å resolution of this duplex, solved by a single wavelength anomalous diffraction experiment on a brominated derivative.

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P D42 - Ac-ΔXaa-NMe₂: conservative conformation

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α,β-Dehydroamino acids belong to specialized amino acids, which are modifiers of a peptide chain. On the other hand, numerous modifications of peptides and peptide mimetics are based on the tertiary amide bond. This bond can be modeled by N',N'-dimethylamide group. Within the Ac-ΔAla-NMe₂, Ac-(Z)-ΔPhe-NMe₂, and Ac-(Z)-ΔAbu-NMe₂ molecules, we combined C^α=C^β double bond together with the N',N'-dimethylamide group, and investigated conformations of these compounds by X-ray diffraction *in vacuo* calculations.

X-ray data analysis revealed that investigated compounds keep specific conformation of the torsion angles φ, ψ = ~ -45°, ~ 130°. That occurs neither for the known Ac-ΔAla-NHMe and Ac-(Z)-ΔPhe-NHMe nor saturated Ac-L/DL-Xaa-NMe₂ analogues, where conformations in the crystal vary depending on the amino acid moieties, and no conformational preferences are observed. The conformation adopted by the series of studied Ac-ΔXaa-NMe₂ is located in the high-energy region of the Ramachandran diagram, which is not so readily accessible for saturated peptides. Besides, the Ac-ΔXaa-NMe₂ conformers show high degree of non-planarity of their tertiary amide bond.

The conformations in the crystal of the studied compounds correspond to their minima calculated on the basis of HF/3-21G conformational maps and re-optimized with the DFT/B3LYP/6-31G** method. It was found that the conservative conformation is the lowest in energy for Ac-(Z)-ΔPhe-NMe₂ but not for Ac-ΔAla-NMe₂ and Ac-(Z)-ΔAbu-NMe₂. The conformations in the crystal of the studied compounds correspond to their minima calculated on the basis of HF/3-21G conformational maps and re-optimized with the DFT/B3LYP/6-31G** method. It was found that the conservative conformation is the lowest in energy for Ac-(Z)-ΔPhe-NMe₂ but not for Ac-ΔAla-NMe₂ and Ac-(Z)-ΔAbu-NMe₂. In the case of the Ac-(Z)-ΔPhe-NMe₂ molecule the angle χ² does not exceed value [30°]. Similarly to the conformation in the crystal, all calculated conformers reveal the non-planarity of the tertiary amide bonds.

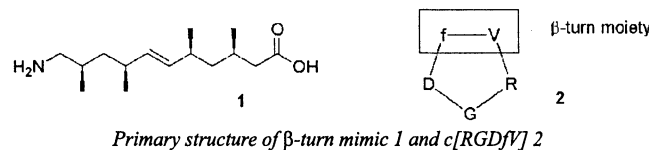
In summary, obtained results indicate that both the conservative conformation with the torsion angles φ, ψ = ~ -45°, ~ 130° and the non-planarity of the tertiary amide bond are the intrinsic features of the studied compounds.

P D44 - Evaluation of structural features of a designed β-turn mimic by incorporation into a cyclic RGD peptide

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Conformation design similar to that used by nature in polyketide natural products led to compound 1. Based on avoidance of allylic strain and syn-pentane interactions in the 2,4-dimethylpentane units this linear molecule folds to a βII-turn mimic format as shown by IR-spectroscopy and NMR coupling constants.[1]



The cyclic pentapeptide c[RGDfV] which is a selective antagonist for α_vβ₃ integrins and contains a βII-turn [2] was chosen in order to evaluate further structural features of the turn mimic 1. The β-turn mimic 1 was incorporated by SPSS into the cyclic RGD peptide instead of the D-Phe-Val-moiety.

The three-dimensional structure in water was determined by 2D-NMR spectroscopy, metric matrix distance geometry (DG) calculations and molecular dynamics (MD) simulations in order to evaluate to which extent the β-turn mimic 1 adopts a βII-turn motif in this environment.

We will present a comparison of cyclo[RGDfV] 2 and cyclo[RGD1] with relevance to the structure of both the turn mimic and the RGD moiety and to biological effects.

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P D45 - Synthesis and characterization of the transmembrane peptide region of the sodium pump γ subunit: a link between its oligomeric state and renal hypomagnesaemia

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The Na,K-ATPase, or sodium pump, is a ubiquitously-expressed membrane-bound enzyme that controls the transmembrane gradients of sodium and potassium ions in animal cells. The enzyme comprises two subunits, α and β , and in the kidney, is also associated with a small single-spanning membrane protein, the γ subunit [1]. Recent evidence suggests that the γ subunit may form homo-oligomers [2]. In order to characterize the quaternary structure of the γ subunit, we used the Lys-tagged peptide approach previously described in our laboratory [3] to synthesize a peptide, γ TM, that contains its transmembrane (TM) region. Initial structural characterization of this 32-amino acid peptide (KKKTVRKGGGLIFAGLAFVVGLLILSKRFKKK) using circular dichroism spectroscopy indicates that it is a random coil in aqueous buffer but spontaneously adopts an α -helical conformation in the presence of detergent micelles (sodium dodecyl sulfate (SDS), and perfluorooctanoate (PFO)). Polyacrylamide gel electrophoresis (PAGE) and fluorescence resonance energy transfer (FRET) experiments demonstrated that γ TM forms oligomers in PFO, a detergent that tolerates relatively weak associations between membrane proteins, but not in the more disruptive SDS. In addition, oligomerization of γ TM is abrogated upon mutation of a highly conserved Gly residue (G41, using the numbering for the full-length γ_a isoform) to either Arg or Leu. Significantly, the G41R mutation had previously been shown to be associated with a form of dominant renal hypomagnesaemia [4].

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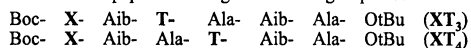
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P D46 - Structural features of linear peptides in solution carrying new fluorescent analogues of tryptophan

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Foldamers represent a new class of molecules characterized by a few conformations in solution. In peptide chemistry foldamers are formed by specific substitutions on the C α atom, leading to restricted torsional rotations. These compounds have been shown to be appropriate models for structure/activity relationship in molecular recognition processes and for the study of intermediates in protein folding. We present here the most relevant structural features of two series of hexapeptides having the following sequences:



where X stands for the fluorophore (A=Aal or N=NTrp) and T for Toac, as shown in Fig. 1. IR absorption spectra in the amide stretching region reveal a predominant presence of intramolecularly H-bonded NH groups in all compounds examined, indicating the occurrence of helical conformations in solution. CD spectra are definitely less diagnostic because of the extensive absorption of the aromatic fluorophores in the peptide spectral region. Steady state and time resolved fluorescence measurements show a strong quenching of both Aal and NTrp emissions by Toac. In addition, these experiments indicate that AT₁ forms a ground-state complex involving the Aal-Toac donor-acceptor pair. The data of molecular mechanics calculations fully match the results obtained by time resolved fluorescence experiments only when the backbone of all peptides, but AT₁, is in the α -helical conformation. By contrast, the Aal-Toac ground-state complex can be theoretically obtained only when the backbone chain populates a 3_{10} -helical conformation. Within experimental errors, fluorescence measurements on the diastereomeric L- and D-NTrp-based peptides do not exhibit significant differences. Therefore, despite the difference in energy between the diastereomers, the donor-acceptor pairs should attain similar distance and orientation, leading to comparable quenching efficiencies. Interestingly, the chiral NTrp moiety, located at the N-terminus of the backbone, deeply affects the screw sense of the secondary structure, despite the presence of L-Ala residues in the sequence. Three main conclusions can be drawn from the foregoing results. (i) All compounds investigated populate only a few ordered conformations in solution. (ii) The screw sense of the helix is controlled by the NTrp probe. (iii) Azulenyl and azatryptophanyl derivatives are good photophysical alternatives to the naturally-occurring tryptophan residue.

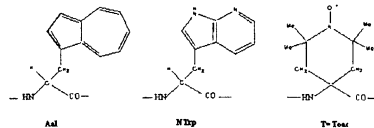


Fig. 1 - Schematic representation of the chromophores.

P D47 - Binding modes of histidine in copper(II) complexes of oligopeptides - a circular dichroism study

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The imidazole group in the histidine side chain is one of the most effective metal ion binding site in metalloproteins. In most cases it is supposed to bind in a monodentate way to the metal ions, however, some examples are already known of bidentate or chelate type coordination. Our earlier studies on the copper(II) complexes of various hexapeptides revealed that histidine offers a broad variety of metal ion binding sites. [1] Circular dichroism (CD) measurements in the copper(II) d-d transition region proved to be a unique and simple tool for determination of the coordinating donor groups of the ligand molecules in aqueous solution. Equilibrium CD measurements in the systems containing copper(II) and glycine-L-alanine tripeptides allowed us to assign the CD spectra to two types of the species with the compositions of CuLH₁ and CuLH₂ (where L denotes the ligand molecule deprotonated at the carboxylate and amino groups and the negative index of the proton is devoted to the deprotonation of the ligand molecule at the peptide group(s) as a consequence of the coordination to the metal ion) [2]. The sign and the intensity of the spectra are well related to the chirality and distance of the chiral centre(s) in the ligand from the metal ion chromophore and the stability of the metal complexes. As a continuation of this work the same type of experiments have been performed with tripeptides containing histidine in various sequences. Among these the X-X-His type peptides (where X may be Gly or Ala) are models of human serum albumin binding site. These form well defined copper(II) complexes involving the amino, deprotonated amide and imidazole nitrogens into the metal ion binding. At the same time the His-X-X tripeptides behave in a different manner forming stable bis complexes with histamine type coordination unless the pH is increased to form the MLH₂ species. For this complex we supposed a new structure instead of the triglycine-like coordination, proposed in the literature, with the N-terminal imidazole nitrogen involved in the coordination sphere. The ligands with histidine in the second position form diverse species, with a tetrameric one among these, featuring bridging bidentate imidazole groups.

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P D48 - Profiling protein expression changes in monocytic U937 cells exposed to PMA using two-dimensional LC-MS/MS on a hybrid quadrupole orthogonal acceleration time-of flight (Q-ToF) mass spectrometer.

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The monocytic cell line U937 undergo cellular differentiation to macrophage upon exposure to phorbol myristate acetate, PMA [1]. This differentiation process is playing an important role in inflammation conditions, autoimmune diseases, and in the formation of atherosclerotic plaques [2]. In this investigation, we have used a proteomics approach to monitor the global protein expression changes between the monocytes and derived macrophages in both the total cell lysates and in enriched fractions of plasma membrane. The analysis of hydrophobic membrane components was facilitated using a non-gel separation system that provided identification of high molecular weight proteins such as integral membrane proteins, ion channel receptor and transporters. This was made possible through recent advances in both HPLC and mass spectrometry instrumentation which enabled the analysis of complex protein mixtures typically not separated on a two dimensional gel. These experiments involved separation of the complex digest mixture by microcapillary liquid chromatography with on-line mass spectral detection using automated acquisition modes whereby conventional MS and MS/MS spectra are collected in a data dependant manner. Protein identification is then achieved via database searching of the MS/MS spectra, providing qualitative information on the protein distribution. Hundreds of MS/MS spectra can be acquired in a fully automated fashion, resulting in the identification of significant numbers of proteins, including low copy number proteins, from a single LC-MS/MS experiment [3]. If a complex protein mixture is to be investigated then a fractionation step prior to reverse-phase separation of the tryptic peptides becomes advantageous [3]. We have, therefore, utilised a 2D LC-MS/MS approach using a capillary LC system (CapLC) operating at nanoliter per min flow rates coupled to a Q-ToF mass spectrometer. By placing a strong cation exchange (SCX) cartridge followed by a C18 trap cartridge it is possible to pre-fractionate the peptides prior to separation on a C18 column. After loading the sample, discrete fractions are sequentially eluted from the cation exchange cartridge using a salt step gradient; the eluted peptides are then retained on the trapping C18 cartridge whilst they are desalted. Finally the peptides are eluted from the C18 pre-column, at 200nL/min, onto a 75 μ m ID x 10cm Waters Symmetry analytical column for separation and elution into the mass spectrometer. This analytical approach will be compared and contrasted to results obtained using 1D gel separation followed by reverse phase LC-MS/MS of *in-gel* protein digest of excised bands.

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P D49 - Urotensin-II: spectroscopic and molecular dynamic investigation in several environmental conditionsE. Zampelli⁽¹⁾, A. Carotenuto⁽¹⁾, P. Rovero⁽¹⁾, P. Grieco⁽²⁾, P. Campiglia⁽²⁾, E. Novellino⁽²⁾

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Human Urotensin II (hU-II) is a disulfide bridged undecapeptide recently identified as the ligand of an orphan G protein-coupled receptor. hU-II (H-Glu-Thr-Pro-Asp-cyclo[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) has been described as the most potent vasoconstrictor compound identified to date. We have recently analyzed the solution structure of hU-II in DMSO solution. In this medium the cyclic residues of hU-II are well defined, although no secondary structural element could be observed. Interestingly, the side chains of the aromatic residues of the core region Phe⁶, Trp⁷, and Tyr⁹ form a hydrophobic cluster, probably implicated in receptor binding. We have now investigated in details hU-II structural features in a variety of environmental conditions through CD, NMR, and Molecular Dynamic techniques. We employed a DMSO/water mixture as a medium compatible with transport fluids, a HFA/water mixtures that can probe the intrinsic tendency of the peptide to assume helical conformation, and a SDS micellar medium as membrane mimic environment.

In this work we report the structural properties of hU-II and their possible biological implications.

P D50 - A comparison of the own and Root-Bernstein's hypotheses on the peptide complementarityR. Zbozień-Pacamaj⁽¹⁾, P. Stefanowicz⁽¹⁾, I.Z. Siemion⁽¹⁾

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Recently we have proposed a new hypothesis on amino acid complementarity based on the periodicity of the genetic code [1,2]. According to this proposition the complementary amino acids are coded by „equivalent” codons, in which the two first bases are complementary and the third one is the same, eg. Gly and Pro, which should form a complementary pair, are coded by GGG and CCG codons, respectively. Our scheme of amino acids pairing is very similar to that proposed by Root-Bernstein [3,4] (on the basis of very different arguments), and differs from it in two main points only (according to our scheme Phe is complementary to Asn and Leu to Lys while in the Root-Bernstein's case Phe is complementary to Lys and Leu to Asn).

We tested our hypothesis on the series of peptides related to the 90-99 fragment of TGF-β2 (transforming growth factor) examining their tendency to form dimers with respective complementary peptides. The results obtained indicate that peptides interact selectively with “antipeptides” [2].

Studies on interactions between cyclolinopeptide A and its “antipeptides” allowed to compare experimentally the hypothesis of Root-Bernstein and the theory of codon equivalency.

We examined the following peptides:

cyclolinopeptide A

I) c-(Leu-Ile-Ile-Leu-Val-Pro-Pro-Phe-Phe),
linear precursor sequence Leu-Ile-Ile-Leu-Val-Pro-Pro-Phe-Phe

“antipeptides” according to our hypothesis

II) Lys-Tyr-Tyr-Lys-Gln-Gly-Gly-Asn-Asn,

III) c-(Lys-Tyr-Tyr-Lys-Gln-Gly-Gly-Asn-Asn),

“antipeptides” according to Root-Bernstein's hypothesis

IV) Asn-Tyr-Tyr-Asn-Gln-Gly-Gly-Lys-Lys

V) c-(Asn-Tyr-Tyr-Asn-Gln-Gly-Gly-Lys-Lys)

Our hypothesis seems to be more suitable for the investigation of peptide-“antipeptide” complexation process.

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P D51 - Role of the 1-16 N-terminal region in the *trans* conformation involved in Alzheimer's disease.S. Zirah⁽¹⁾, S. Kozin⁽²⁾, A. Blond⁽¹⁾, M. Cheminant⁽¹⁾, P. Debey⁽²⁾, S. Rebuffat⁽¹⁾

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The amyloid peptide Aβ is the major component of senile plaques, a characteristic brain lesion of Alzheimer's disease. Aβ assembly into fibrillar structures is assumed to be initiated by a conformational transition of the peptide from an α-helix or random to a β-sheet secondary structure. Involvement of Zn²⁺ ions in this *trans* conformation has been strongly suggested. The 1-16 Aβ N-terminal region : D¹AEFR⁵HDSGY¹⁰EVHHQ¹⁵K (Aβ(1-16)) corresponds to the amino acid sequence between the cleavage sites of β- and α-secretases, the former releasing Aβ, while the latter only releases nonamyloidogenic peptides. The 1-16 N-terminal sequence contains the three histidines hypothesized to be implicated in Zn²⁺ binding, together with the three mutations (R⁵G, Y¹⁰F and H¹³R) that distinguish the human Aβ from the rat protein. As rat does not develop Alzheimer's disease and Aβ_{rat} is not able to form amyloid deposits, we investigated the role of the 1-16 Aβ region in the *trans* conformation, and focused particularly on the conformational behavior of this fragment upon addition of Zn²⁺ ions.

A conformational study of Aβ(1-16)_{human} has been undertaken by circular dichroism and NMR spectroscopy. Depending on the medium, Aβ(1-16)_{human} adopted either a random coil or an α-helical structure including a turn centered at D7-S8. The conformational transition toward β-sheet occurring in the presence of Zn²⁺ ions that has been previously observed by CD [1] was here examined by NMR. The zinc-bound form, which exhibits strong specific induced shifts affecting histidine residues was further characterized. The whole results are compared to those obtained with Aβ(1-16)_{rat}, allowing to discuss the influence of the rat mutations on the conformation and Zn²⁺ binding properties.

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P D52 - Effects of alanine analogs of Mast Cell Degranulating (MCD) peptide on mast cell activity and bindingA. Buku⁽¹⁾, M. Mendlowitz⁽²⁾, J. A. Price⁽³⁾

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MCD peptide is known to have a concentration-dependent histaminic/antihistaminic effect on mast cells and to inhibit IgE binding to its mast cell receptor. MCD peptide is a highly basic 22 residue bicyclic peptide characterized by four cysteine residues at positions 3,15 and 5,19: IKCNCKRHHVIKPHICRKCIGKN-NH₂. To understand the role of the basic residues of the MCD peptide, we synthesized analogs in which lysine residues at positions 2, 11, and 21 were replaced by alanine. The peptides were synthesized by solid phase and characterized by standard analytical methods. Histamine-releasing activity was tested in rat mast cells. The alanine analogs had a five- to ten-fold decrease in activity compared to the parent MCD peptide. The histamine-releasing activity of the analogs was as follows: MCD>Ala²¹>Ala¹¹>Ala². Binding studies by flow cytometry showed that the fluorescence labeled alanine analogs bound to basophilic leukemia (RBL) mast cell receptors in a manner similar to MCD peptide.

D3 - Peptide interactions with membranes and in signal transduction

P D53 - Studies on a synthetic analog of the TM V segment of the mt1 melatonin receptor

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Melatonin (N-acetyl-5-methoxytryptamine, MLT) is a hormone produced mainly by the pineal gland in most vertebrate species including humans. The physiological role of MLT is related to both chronobiology and modulation of the body hormonal milieu. MLT appears to exert these effects through specific high affinity receptors. MLT has also been shown to have powerful antioxidant properties acting as an efficient free radicals scavenger.

Our attention is focused on characterization of MLT interaction with its high affinity membrane-bound mt₁ receptor, which belongs to the G protein-coupled receptors (GPCR) superfamily. A number of structure-affinity relationships were identified and recently many authors proposed molecular models of the putative MLT binding site [1 - 4] in which residues belonging to the fifth transmembrane segment (TM V) of the receptor are involved. As there are strong suggestions that individual TM segments first fold independently and subsequently arrange into their optimal packed conformation in the membrane [5], studies of peptide sequences corresponding to TM segments can provide important structural information about the TM region of the receptor.

In this work we examined the interaction of the mt₁ TM V segment with phospholipid membranes by means of CD, NMR, IR and fluorescence spectroscopy studies. In these studies we utilized the synthetic peptide analog illustrated in Fig. 1.

KKKKTIAVVVFHFIVPNleIIVIFTYLKKKK

Fig. 1 - Sequence of the synthetic peptide analog of the mt₁ TM V segment.

This peptide was constructed by adding eight Lys residues, four at N-terminus and four at C-terminus respectively, to a peptide analog corresponding to the receptor TM sequence 191-211, in which Met²⁰³ was replaced by Nle and Cys²⁰⁹ by Thr. Addition of Lys clusters greatly improves the peptide solubility and prevents its aggregation [6].

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P D54 - Modelling of protein P2 from *Haemophilus influenzae*: the role of surface exposed loops on MEK1/MEK2/MAPK cascade activation

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Bacterial lipopolysaccharide (LPS) and associated outer membrane proteins (OMPs) are potent initiators of fever, coagulation disorders, multiple organ failure and shock in humans and experimental animals. Among the OMPs, porins are known to form specific or non specific diffusion channels, allowing small, polar molecules to diffuse across membranes. Hence, porins play a fundamental role in the communication between bacteria and the environment. Being a major component of the gram-negative bacterial outer membrane, porins also play an important role in bacterial pathogenesis and are involved in adherence and invasion.

To identify the role of surface exposed porin loops in the induction of signalling pathways we have synthesised and tested a set of synthetic peptides.

The porin P2 of *Haemophilus influenzae* is one of the best characterised porins in terms of its functional characteristics. Sequence analyses of P2 genes indicate that the transmembrane regions are relatively conserved among strains while considerable heterogeneity exists in the loop regions of the molecule. The activation of the inflammatory and immunological response is initiated by interactions with the bacterial cell or one of its components and involves the phosphorylation of the main signal transduction networks. The activation takes place as a consequence of eukaryotic cell interactions with molecules localised on the surface of the bacterial cell such as LPS or OMPs. In this work, we verified the signal activity of *H. influenzae* P2 porin and investigated in details which domains of the protein are involved in the signalling pathways. The results obtained, showed that P2 is an activator of tyrosine phosphorylation. We were able to test peptides corresponding to the amino acid sequences of variable loop regions facing the cell exterior and thus more probably involved in the initial interaction with the host cell. The results obtained showed that peptides corresponding to the sequences of surface exposed loops are able to activate the MEK1-MEK2/MAPK cascade.

P D55 - Analysis of functional domains in glycoprotein H of Herpes Simplex Virus type 1

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Herpesviruses are ubiquitous pathogens of all vertebrates. Herpes Simplex Virus Type 1 (HSV-1) is one of at least eight herpesviruses that persistently infect man and is the best studied. Like all herpesviruses, HSV encodes a large number of glycoproteins and other integral membrane proteins, at least ten of which are incorporated into the lipid bilayer (envelope) of the virus. Three of these proteins, designated glycoprotein B (gB), gD and the gH/gL heterodimer, are absolutely required for virion infectivity and for virus-induced cell-cell fusion. HSV entry into the host cell is achieved by fusion of the viral envelope with the plasma membrane of the host cell. The aim of this proposal is to investigate further the function of the gH/gL complex in membrane fusion and virus transmission, since this molecule is required for membrane fusion by all herpesviruses. The first step in our strategy was to identify specific functional domains in HSV-1 gH. We constructed a panel of 33 different mutants with insertion scattered fairly evenly and randomly throughout the gH gene. Before undertaking experiments to assess structural and functional properties of mutant proteins, the ability of each mutated gene to express a full-length polypeptide has been examined by in vitro-coupled transcription and translation in rabbit reticulocyte lysate, and SDS polyacrylamide gel electrophoresis. An eukaryotic in vitro system such as expression in Cos-7 cells has then been used to characterize the mutants in terms of their ability to express at the cell surface, to support virus-cell and cell-cell fusion and to form an antigenically authentic gH:gL heterodimer. Three mutants were severely if not completely compromised in both virus entry and syncytium formation assays. These mutant proteins all contained insertion in a hydrophobic segment of 108 amino acids in length which precedes the predicted transmembrane domain of the gH molecule. This region is therefore involved in mediating membrane fusion during cell-to-cell spread and virus penetration. These results represented a starting point to design a set of peptides to investigate with deeper details the mechanisms of membrane fusion, signal transmission pathways and infection due to HSV-1. Overlapping peptides from gH, representing consecutive segments of the peptide chain in the region where most of the non-fusion mutants are located, have been produced by solid phase peptide synthesis (SPPS) with standard Fmoc strategies. These overlapping peptides can be used to locate individual residues and peptide sequences that are essential and non-essential for anti-herpes activity.

In order to study the activity of the synthesized peptides it is important to determine whether their activity can be uncoupled from the confounding effect of cellular toxicity. We have used a colorimetric assay for the quantification of cell death and cell lysis, based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. All of the peptides tested resulted non-toxic.

We are now testing the biological properties of our peptides in terms of plaque reduction assay and virus-yield reduction assay. The same peptides are being tested for identifying their role in the induction of signalling pathways.

P D56 - Membrane interactions of the internal fusion peptide of Ebola glycoprotein

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The fusion peptide of the Ebola virus envelope glycoprotein (Ebola GP) [1,2] is an internal sequence including residues 524-539 within the N-terminal region of the GP2 transmembrane subunit [3]. In this work we perform experimental and theoretical analyses of the interaction with membranes of EBOwt (GAAIGLAWIPYFGPAE) and EBOmut (GAAIGLAWIPYFGRAAE), representing the Ebola fusion regions in the wild-type and a defective mutant glycoprotein GP2 [4], respectively. The sequences are predicted to partition into membranes and adopt a characteristic topology, according to hydrophobicity distribution analyses based on the Wimley-White scales [5,6]. Determination of the structural requirements by circular dichroism and infrared spectroscopy confirms that both peptides adopt similar structures in aqueous solution and when associated with membranes. However, only the wild-type sequence induces the type of membrane perturbations that compromise overall bilayer architecture.

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P D57 - Possible explanation of the role of the water medium in the mechanisms of action of peptides in low and super-low doses

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In our previous reports we proposed a possible mechanism of distant signal transmission from peptides to receptors (target cells) in the water medium without ligand-receptor complex formation [1, 2]. This mechanism can be parallel to the existing classical contact one. Among the most important and still unsolved problems in the theory of signal substances is the mechanism of their action in low and super-low doses. To study this phenomenon, we regarded the interaction of peptide structures and conformations, their impact upon the dynamic characteristics of water and dose/biologic effect dependencies in a wide range of concentrations. Biologically active peptides KE, EK, KW, AEDG, AEDP, KEDP and KEDA synthesised at the Institute were used as the objects. Their effect upon the properties of water (formation of solitons with parameters changed due to interaction with the molecules of the dissolved substance) was studied by temperature (5-45°C) dependencies of infra red spectra of the solutions in the near (5180 cm⁻¹) and far (200 cm⁻¹) areas. *In-vitro* biotesting included the determination of the proliferative activity of thymocytes and study of the effects on the activity of neutral sphingomyelinase in the membranes of murine thymocytes, mRNA IL-2 synthesis in murine spleen lymphocytes, retinal explant growth, spinal marrow ganglia and thymuses of newborn rats. In the majority of cases, we registered a bimodal curve in the dose/biologic effect dependencies and its second maximum – in low and super-low concentrations (10⁻¹⁰-10⁻¹⁵ M). We presume that it is in low and super-low doses of an active substance where the formation, distance transmission and detection of a signal from ligand molecules to a target cell take place with the active role of the water medium according to the soliton mechanism that we described earlier.

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P D59 - Inhibition of cellular signal transduction with cell-permeable peptides: correlation of inhibitory activity with intracellular proteolytic stability

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Cell-permeable peptides are a versatile tool for a non-disruptive delivery of peptides, proteins, and oligonucleotides into living cells *in vitro* and in whole organisms. The Penetratin peptide was derived from the third helix of the DNA-binding domain of the *Drosophila* Antennapedia Homeodomain protein. This peptide is 16 amino acids in length and rich in basic amino acid residues. Interference with intracellular molecular interactions and reactions can readily be achieved by conjugating the cell-permeable peptide to inhibitors of these processes. Earlier work by our group has addressed the import capacity of fluorescently labelled analogues of this peptide as well as compared the import efficiencies of different cell-permeable peptides and peptide-cargo constructs. It was shown that import is strongly dependent on the peptide cargo. Fluorescent labelling of these molecules is therefore required to normalize biological activities with respect to import efficiencies. In addition, however, these inhibitors are expected to undergo intracellular proteolytic breakdown. For this reason, the concentration of imported peptides derived from the intracellular fluorescence will deviate from the concentration of biologically active compounds. To address the interdependence of intracellular proteolytic breakdown and biological activity a series of cell-permeable peptide inhibitor constructs were generated, in which the linker amino acids connecting the cell-permeable peptide moiety with the inhibitor peptide were varied or replaced by non-proteinogenic amino acids. In addition peptides with free versus amidated C-termini were compared. As inhibitor a peptide interfering with the ZAP-70 kinase active site was selected [1]. This tyrosine kinase plays a key role in T cell receptor-dependent T-lymphocyte activation. In experiments employing cell-permeable peptide constructs for addressing the MHC-class I loading pathway we had realized that pulse-chase experiments of peptides labelled either at the N-terminus of the peptide or within the peptide sequence provided a robust and easily accessible read-out of intracellular stability towards proteolysis. Using read-outs for T-lymphocyte activation the information on intracellular proteolytic processing was correlated with the inhibitory biological activity of the respective construct. Our approach, taking into account the import efficiencies as well as the intracellular break-down, enables a quantitatively controlled interference with intracellular signalling pathways. Furthermore this combination of read-outs will considerably accelerate the optimisation of peptidic and non-peptidic signalling inhibitors with respect to their intracellular stability in applications such as target validation.

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P D58 - Synthesis of SXWS peptides and their chemotactic activity on a ciliated protozoan *Tetrahymena pyriformis*

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In our previous study [1] we found that tetrapeptide SEWS exhibits a prominent chemotactic effect at 10⁻¹² M on a ciliated protozoan, *Tetrahymena pyriformis*. In this presentation the solid phase synthesis and chemical characterisation of 18 other members of SXWS sub-library as well as the comparison of their chemotactic properties with those of SEWS will be shown.

For solid phase synthesis the Fmoc^tbutyl technique was used, with an *in situ* active ester (HOBt/DIC) coupling. In position X all common L-amino acids were applied except Cys. Peptides were purified by RP-HPLC and their structure was confirmed by ES-MS.

Chemotactic activity of peptides was measured by capillary method [2]. The 19 tetrapeptides could be divided into three main groups. Majority of them were more or less chemoattractant. Within this group, SEWS and STWS possessed strong activity. Peptides containing A and M in position X were attractant with a low concentration optimum, while SIWS, SNWS, SPWS and SSWS showed their maximal activity at higher concentration. SHWS and SQWS had two maxima, suggesting the existence of more than one chemotactic receptors. The second group consisted of 4 peptides having no chemotactic activity: SDWS, SGWS, SLWS and SRWS. Peptides with F, K, V, W and Y in their position X possessed chemorepellent activity.

No correlation have been found so far between the chemotactic character and the side chain moiety of the amino acid residue in position X. Based on calculations we assume that the solvent exposed area of the different amino acids [3] plays a role in the chemotactic response.

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P D60 - Contrasting, species-dependent functional activity of mammalian cholecystokinin (CCK) peptides and drosulfakinin-1 at the CCK-A, CCK-B and *Drosophila* sulfakinin receptor type 1 (DSK-R1)

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The insect sulfakinin family of neuropeptides show function and sequence homology with the hormonally active C-terminal portion of the mammalian peptides cholecystokinin (CCK) and gastrin. Recently, we cloned the first *Drosophila melanogaster* drosulfakinin receptor, DSK-R1, which shares ~40% identity with both mammalian CCK receptor subtypes, CCK-A and CCK-B. We showed that DSK-R1 was functionally activated by [Leu⁷]-DSK-1S, FDDY[SO₃H]GHLRF-NH₂, a sulfated, Met⁷ → Leu⁷ substituted analog of *Drosophila* drosulfakinin-1.

The importance of the sulfate moiety was underscored by about 3,000-fold lower potency of [Leu⁷]-DSK-1, an unsulfated counterpart of [Leu⁷]-DSK-1S. In this study, we utilized a calcium mobilization assay to evaluate functional activity of [Leu⁷]-DSK-1S, [Leu⁷]-DSK-1, CCK4 (MGWMDF-NH₂), sulfated CCK8 (DY[SO₃H]MGWMDF-NH₂), and gastrin-II (pEGPWLEEEEEEAY[SO₃H]MDF-NH₂) at the DSK-R1, CCK-A and CCK-B receptors expressed in mammalian cells. The three vertebrate hormones released intracellular Ca²⁺ in CHO-K1 cells expressing CCK-A or CCK-B with potency orders in subnanomolar to nanomolar ranges, comparable to those reported in literature. These peptides were, however, completely inactive in DSK-R1-CHO-10001A or DSK-R1-SHEP cells up to 10⁻⁵ M. Both [Leu⁷]-DSK-1 and [Leu⁷]-DSK-1S were completely inactive at CCK-A up to 10⁻⁵ M. and [Leu⁷]-DSK-1S showed ca. four orders of magnitude lower potency than CCK4 or CCK8 at CCK-B. Our finding at a molecular level parallel *in vivo* reports that CCK, which is inhibitory on intestinal smooth muscle of vertebrates, is unable to alter spontaneous contractions of insect hindgut. These contrasting, species-dependent functional activity of the mammalian CCK-like peptides and fly drosulfakinin might be consistent with the evolutionary distance between their hosts.