Poster presentations

Topic B

B1 - Analytical and biophysical methodologies
B2 - Peptide structure and analysis
B3 - Molecular design: experimental and computational
B4 - Self assembly and molecular recognition
B5 - Peptide bioavailability

P B1 - Isolation and characterization of low mobility group protein LMG160 from rat liver

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A protein fraction of low mobility group nonhistone chromatin protein designated LMG_{160} was purified from rat liver chromatin using preparative gel electrophoresis. Its mobility on SDS-gel is consistent with a molecular weight of approximately 160 kD. It shows two amino acids of Glycine and Valine as amino terminal acids. Its isoelectrofocusing analysis exhibits two bands focusing between zone of isoelectric points ranging pH 5-5.5. Reduction of disulfide band by DTT in the presence of 9M urea and analysis of the pattern on SDS- gel revealed that reduction of this protein converted almost all of protein to the two faster moving bands with a molecular weight of 84 -70 kDa range. Also enzymatic cleavage of the protein with pepsin, trypsin and chymotrypsin gave 4 and 6 and 5 bands on SDS-gel respectly. The results suggest that this protein fraction have two subfractions that are distinguished under denaturing conditions.

P B2 - Induced axial chirality in the Pro-atropoisomeric Bip α-amino acid for configurational assignment

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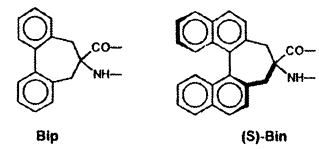
Rosini and coworkers [1] have proposed a novel, general method for the determination of the absolute configuration of diols based on the synthesis and CD properties of conformationally defined derivatives with the pro-atropoisomeric, flexible biphenyl moiety. This approach is potentially suitable even for non-choromophoric substrates. Indeed, non-atropoisomerically stable biaryl compounds, such as the C^{α}-tetrasubstituted Bip α-amino acid, selected peptides of which have been recently synthesized and investigated by our groups [2], are present in solution as racemic mixtures because the free rotation about the C_{ar}-C_{ar} bond precludes the isolation of a single enantiomer. However, stabilization of a preferred enantiomeric conformation (induced axial chirality) can be obtained through appropriate derivatization with a chiral auxiliary.



Bip (S)-Bin

We have synthesized a large series of Boc(Z)/OMe terminally protected di- and tripeptides characterized by a single residue of the pro-chiral Bip and by one or two preceding or following L- or D-Ala residues. A comparison was made with the corresponding peptides containing the rigid, atropoisomeric, chiral (S)-Bin residue [3].

In particular, our CD analysis allowed us to conclude that the pro-chiral Bip residue is an excellent probe for assignment of absolute configuration of α-amino acids (e.g., Ala). In simple dipeptides the chirality transfer is more efficient in the -Bip-Ala- sequence than in the -Ala-Bip- sequence. The negative sign of the band near 250 nm corresponds to a *P* torsion of the biphenyl chromophore and to an (S)-configuration of the chiral α-amino acid, and viceversa. Conversely, the CD spectra of the (S)-Bin peptides are dominated by the extremely intense exciton-splitting of the binaphthyl chromophore near 225 nm and, therefore, are not sensitive to the chirality of the adjacent α-amino acids. We are currently extending the scope of our approach to other chiral α-amino acids (including those with C^{α}-tetrasubstitution), amines and α-hydroxy acids.

References

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P B3 - Chiral high-performance liquid chromatographic enantioseparation of α-substituted proline analogues

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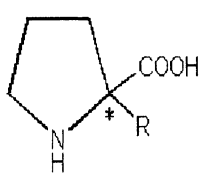
High-performance liquid chromatographic (HPLC) enantioresolution of α-substituted-proline analogues by direct and indirect methods is reported. Direct separations were carried out on quinine-derived chiral anion-exchange chiral stationary phase (CSP). The indirect resolution was achieved by applying pre-column derivatization with chirally pure chiral reagents, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA or Marfey's reagent), 2,3,4,6-tetra-*O*-acetyl--D-glucopyranosyl isothiocyanate (GITC), and (*S*)-*N*-(4-nitrophenoxycarbonyl)phenylalanine methoxyethyl ester ((*S*)-NIFE). The diastereomeric derivatives formed were separated under reversed-phase (RP) conditions. The effects of different parameters on the separation both in direct and in indirect analysis were investigated and chromatographic conditions were optimized to achieve best resolutions. The results allow a straightforward determination of the enantiomeric purity of the compounds.

Structure of the investigated analytes:



R	Compound
methyl	1
n-propyl	2
allyl	3
o-chloro-benzyl	4
o-bromo-benzyl	5
m-chloro-benzyl	6
p-bromo-benzyl	7
p-fluoro-benzyl	8
p-methyl-benzyl	9
1-methyl-naphtyl	10

P B4 - Design and thermodynamic binding studies of peptide ligands for the PDZ protein domain

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A series of linear and cyclic peptides has been designed and synthesized to investigate the binding properties of PDZ protein domains. While in the cellular context PDZ domains engage in protein-protein associations, short peptides with the appropriate sequence have been found to serve as "protein surrogates", and bind to these domains with dissociation constants in the low micromolar range. Using isothermal titration calorimetry (ITC), the thermodynamic parameters of interaction have been studied using peptide ligands that recognize each of the three PDZ domains found within the mammalian neuronal protein, post-synaptic density 95 (PSD95). Initial ITC experiments have focused primarily upon peptide association with the third PDZ domain of PSD95 (PDZ3, a Class I domain), and yielded values for the changes in free energy (DG), enthalpy (DH), and entropy (DS). The first series of linear peptides was designed based on adherence to known consensus sequence information for Class I PDZ domains, and incorporated changes at selected positions to evaluate the affect on binding. The second series consisted of peptides of varying length (4-10 residues) but possessing sequences identical to those of the C-terminal regions of the CRIPT and neuroligan proteins, which are known to bind PDZ3. The consensus and CRIPT-derived peptides were found to bind to PDZ3 with both favorable enthalpic and entropic changes, but association of the neuroligan-derived peptides was driven enthalpically, and marked by unfavorable changes in entropy. Selected substitutions in the consensus peptide sequences demonstrated the relative importance of the first four C-terminal amino acids, and certain conservative residue substitutions were shown to significantly erode affinity. Of all the peptides studied, that exhibiting the highest affinity was the CRIPT-based KNYKQTSV, with a Kd of 0.47 micromolar. Further characterization of PDZ3-peptide interaction was afforded by advanced ITC experiments, either at elevated temperatures to determine the change in heat capacity (DCp), which resulted in small and negative values, or in buffers of differing ionization potentials to calculate linkage to proton exchange with solvent, which was found to be insignificant. Using the binding information gleaned from these structure-activity experiments, a novel cyclic peptide was designed in conjunction with structure-based analysis of PDZ3, in an effort to devise conformationally constrained ligands with enhanced affinity and/or selectivity towards PDZ3. This has lead to a generalized synthesis of sidechain-sidechain bridged cyclic analogues (Fig. 1) that possess the required binding elements for Class I PDZ domain recognition; preliminary studies in which the bridging unit consists of either glycine or beta-alanine have yielded first generation ligands with Kd values ranging from 4.2 to 31 micromolar.

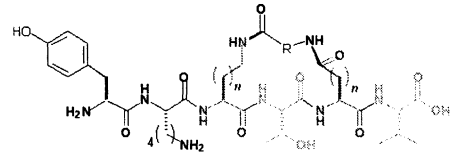

Fig. 1 - General Form for PDZ-Binding Cyclic Peptides

P B5 - Acridine-based fluorophores in peptide studies

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Acridine derivatives belong to a group of very interesting and potentially useful compounds for methods applying fluorescence resonance energy transfer (FRET) between two chromphores. High quantum yield of fluorescence and high values of decay times, high molar absorption coefficients and shift of the absorption and emission spectra towards longer wavelengths allow to apply acridine chromophores as effective energy donors or acceptors. Additionally, the acridinyl probes are resistant to fotobleaching, so they can be effectively used in some investigations requiring prolonged irradiation (e.g. scanning solid-phase libraries of labelled compounds). In order to prove possibility of further applications of acridine derivatives in peptide investigations we synthesised some analogues of 9-acridone: compounds bearing carboxyl group directly on aromatic ring or shifted by methylene groups, and non-coded amino acid having acridone fragment in a side chain (compound obtained by modification of (p-nitro)phenylalanine). The acridine-type molecules of our interests (acridone derivatives) were obtained, starting from simple and cheap compounds, in a multi-step reaction involving modified Ullmann–Jourdan condensation followed by cyclodehydratation step [1]. Such obtained compounds contain carboxyl and/or amino group, so they could be directly incorporated into peptide chain and serve as a donor or acceptor of energy.
In our communication we present some synthetic aspects and extended photophysical studies of acridine derivatives, including the influence of substituent position in the acridine skeleton analysis, and their application in peptide investigations (enzymatic and conformational studies), as well.

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P B6 - New consensus hydrophobicity scales extended to non-proteinogenic aminoacids

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Hydrophobicity is a fundamental attribute of amino acid residues that determines protein folding, interactions between protein subunits, receptors and ligands, and protein segments or peptides and biological membranes. Numerous attempts have been made at establishing hydrophobicity index scales, but these are characterised by a bewildering variation in the absolute values of the indices for each amino acid, in the relative placing, and in the range of values covered in each scale. The most used of these scales reflect the popularity of the methods they are associated with rather than the accuracy of the indices they contain. As our work with synthetic membrane-interacting antimicrobial peptides required an accurate determination of their overall hydrophobicity and hydrophobic moment, we have set out to obtain a new consensus scale based on the widest possible set of literature base scales, that would also include indices for non-proteinogenic residues. Two novel consensus scales have been determined; The first was derived from 162 published scales determined by theoretical, statistical or experimental methods, while the second used 33 scales determined with strictly experimentally methods. The refinement of these two sets of base scales, using two alternative standardisation and two alternative filtering methods resulted in a 'general' (GCS) and an 'experimental' (XCS) consensus scale that vary in the positioning of only a limited set of amino acids, in particular Trp and Cys. These consensus scales perform favourably with respect to a set of commonly used published scales in a number of tests. In particular, both scales showed a very good correlation with experimental descriptors for hydrophobicity such as the octanol/water partition coefficients for N-acetylated and C-amidated amino acids (respectively $R2 = 0.90$ and 0.98) and the reversed-phase HPLC retention times for Fmoc N-capped and C-amidated aminoacids ($R2 = 0.96$ for both scales). This suggested simple means of extrapolating indices for non-proteinogenic amino acids; i.e. a rapid computational method based on extrapolation from calculated Po/w values, and a chromatographic method based on extrapolation from Fmoc-amino acid reversed-phase retention times. Both methods have been evaluated for a range of non-proteinogenic residues with aliphatic (Aib, Abu, Nva, Nle), polar (HoSer) and charged (Orn, Dab, Dap) side-chains.
The GCS scale has been used to quantify the hydrophobicity and amphipathicity of a self-consistent set of linear membranolytic antimicrobial peptides in which these properties, degree of helical structuring and charge were systematically and independently varied. These four parameters were correlated to the antimicrobial potency of the peptides against E. coli and S.aureus, in terms of log2(MIC) indicating a fundamental difference in the manner with which they inactivate these bacteria, which may in part be due to different membrane properties.

P B7 - Stability of modified linear and cyclic epitope peptides in human serum and toward lysosomal enzymes

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The development of synthetic, peptide-based immunogens which could undergo processing under required circumstances, but are stable before reaching the targeted site would be important for more efficient immunisation [1]. In order to investigate the effect of chemical modification (e.g. cyclization, D-amino acid substitution) on enzymatic stability epitopes from two proteins were chosen. Epitope ^{268}LAPEDPEDSALLEDPVGTVA^{287} of the glycoprotein D (gD) from herpes simplex virus (HSV) is capable to induce virus neutralising antibody response [2]. Epitope ^{15}TPTPTGTQTPT^{25} from the tandem repeat unit of mucin 2 glycoprotein (MUC2) is recognised by protein specific antibodies even in tumour tissue [3]. Two types of strategy were applied. Cyclic peptide variants with thioether, disulfide and amide linkage were prepared from the 268-287 sequence of HSV gD-1 [4], while certain amino acids were replaced by the D-analogue in the flanking regions of the epitope peptide in the case of the MUC2 [5].
The stability of these peptides was studied in human serum (concentration: 10% and 50%) and in the presence of various lysosomal enzymes (e.g. dipeptidyl peptidase, aminopeptidase). Peptides were incubated for 96h at 37°C and samples were taken at various time points. Digested samples and untreated control compounds were analysed by RP-HPLC and the peaks detected were identified by mass spectrometry. From time vs AUC curves the kinetics of degradation were determined. We found that the bond introduced for the cyclisation in HSV gD-1 peptide has some influence on enzymatic degradation. Also our results show that the number of D-amino acids in MUC2 peptide increase the stability of the peptide.

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P B8 - High sensitivity phosphoprotein analysis using a combination of variable flow chromatography and precursor ion discovery on a Q-Tof mass spectrometer

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Mass spectrometry has firmly established itself as the primary technique for identifying proteins. Currently, the mass spectrometry technique providing the highest degree of specificity and sensitivity is electrospray LC-MS/MS. However in the case of post translationally modified peptides only a very limited sub set of the peptides present are required to be fragmented and often these low intensity peaks can be missed. A solution to this problem is a method that allows specific post translationally modified peptides to be identified during the course of an LC-MS experiment. In the case of phosphotyrosine, a low mass immonium ion at m/z 216 can be detected. This characteristic ion is used to direct the mass spectrometer to fragment potential phosphopeptide precursor ions which are present at that time point in the low energy data. In this case several precursor ions may require MS/MS interrogation at one decision making time-point and implementation of a chromatographic technique known as variable flow chromatography allows greater time to interrogate these peaks. This approach will be discussed with examples of where this methodology has been used for the targeted analysis of phosphorylated peptides.

P B9 - Three dimensional structure of the cathelicidin motif of the Protegrin-3 precursor

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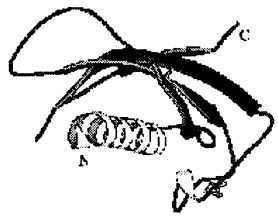
In mammalians, numerous precursors of antibacterial peptides with unrelated sequences share a similar prosequence (96-101 residues, 2 disulphide bridges) referred to as the cathelicidin motif[1]. The 3D structure of this widespread motif has not yet been reported and in order to determine it, the cathelicidin motif of protegrin-3 was overexpressed in *E. coli* using a Tag-His/thrombin site/ProS construct. The His-tagged protein was purified on nickel column and after the thrombin cleavage, ProS was characterised by using CD, mass spectrometry and NMR. NMR spectra indicated that ProS adopts a well-defined structure[2]. Surprisingly, at neutral pH, the ^{1}H-^{15}N HSQC spectra clearly revealed the presence of two and for some residues four sets of cross-peaks, resulting from several conformers in slow exchange on the ^{1}H chemical shift time scale. Although, this conformational exchange is pH mediated, the involvement of the cis/trans isomerization of one or two proline amide bonds (the sequence contains 10 prolines) cannot be ruled out in this conformational exchange. The full assignment is in progress by using a uniformly ^{15}N- and ^{13}C-labeled sample. Besides this solution structure study, well diffracting crystals of ProS were obtained and X-ray data were collected at 2.2 Å resolution[3]. Since the molecular replacement and the heavy metal derivatives failed, the phasing information was obtained by the MAD technique (Multi wavelength Anomalous Dispersion). The structure consists of a global cystatin fold with a N-terminal helix and a four-stranded ß-sheet. On the basis of the ProS and of the protegrin structures a model for the protegrin precursor was built. In this presentation, the NMR study and the X-ray structure of the cathelicidin motif as well as the original method used to solve the phase will be presented. Then, the roles of the widespread cathelicidin motif in the maturation of antibacterial peptides will be discussed.



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P B10 - Exploration of the Ramachandran surface of beta–peptides computed at *ab initio* and DFT levels of theory

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Unnatural oligomers, such as β-peptides are subject of increasing interest. We are about to provide basic information with others[1,2], for understanding the conformational properties of β -peptides.
Minima and transition state structures of β-Ala, β-Abu were carried out using models of For-β-Ala-NH_2, Ac-β-Ala-NHMe and For-L-β-Abu-NH_2, at different levels (RHF/3-21G, B3LYP/6-311G++(d,p)//RHF/3-21G) of theory. Furthermore, the 4D Ramachandran-surface E=E(φ,μ,ψ) (torsion angles φ, μ, ψ and the corresponding energy) of the models listed above was determined at RHF/3-21G level of *ab initio* theory.
For determining the Ramachandran surfaces we calculated the conformational energies of 7500 different conformations in the case of For-β-Ala-NH_2 and Ac-β-Ala-NHMe, and 15000 conformations in the case of For-L-β-Abu-NH_2.
We found 7 minima of For-β-Ala-NH_2 and 18 corresponding transition state structures, 10 minima and 21 corresponding transition state structures in the case of Ac-β-Ala-NHMe and 15 minima with 35 corresponding transition state structures in the case of For-β-Abu-NH_2 .
We believe, that using the 4D Ramachandran-surfaces and the various transition state structures could present a complete dataset of the conformational properties of the molecules mentioned above.

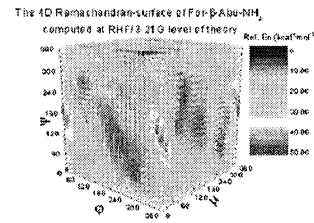

Fig. 1 - 4D ramachandran-surface of For-β-Abu-NH_2

References
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P B11 - Characterization of the 3_{10}-helix by HRMAS NMR spectroscopy

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High-resolution magic angle spinning (HRMAS) NMR spectroscopy is a very promising technique in the field of solid phase organic chemistry for the characterization of resin-bound compounds, including small molecules and peptides [1]. HRMAS NMR has been successfully applied to the conformational characterization of bioactive peptides covalently attached to different resins [2]. It has been found that the 141-159 peptide sequence from Foot-and-Mouth Disease Virus covalently linked to the POEPOP (polyoxyethylene-polyoxypropylene) resin, swollen in organic solvent, is able to fold into a regular helical structure, which is very close to the secondary structure adopted by the free peptide in solution. Using HRMAS NMR, model poly(Ala)$_n$ sequences have also been studied to determine their tendency to self-aggregate when bound to a polystyrene-type resin [3]. On the contrary, the same peptides are able to fold in an α-helix when their loading on the solid support is decreased. In this Communication we present a HRMAS conformational analysis of a series of Aib-rich peptides (Aib: α-aminoisobutyric acid) linked to the POEPOP resin. A detailed information on the preferred conformation adopted by these compounds was obtained from 1D and 2D NMR experiments. In DMSO unambiguous assignments for all NH proton resonances were achieved by NOESY, DIPSI and RFDR experiments. The determination of the temperature coefficients of the NH proton resonances in DMSO confirmed the tendency of these peptides to fold in a 3_{10}-helix. It has been already demonstrated that Aib-rich peptides tend to adopt helical structures of remarkable stability [4]. In this connection Aib-rich peptides can be exploited as useful templates in molecular recognition studies even if covalently linked to polymeric matrices. The present HRMAS NMR characterization of the structure adopted by solid-supported model peptides containing Aib residues can be viewed as the first step along this direction.

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P B12 - Alamethicin sequences reconsidered

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Among the first peptaibol sequences that could be elucidated were alamethicins (ALM) I and II, isolated from the mold *Trichoderma viride* NRRL 3199. The two major sequences recorded in [1] were those commonly reported in the literature. It was realized, however, that actually two microheterogeneous groups named ALM F-30 (acidic peptides, the major components corresponding to ALM I and II according to [1]; here named F-30/I and F-30/II) and ALM F-50 (neutral peptides), were produced in a complex fermentation medium. Each group represents a microheterogeneous mixture of peptaibols. The ratio of the two groups was to a certain extent dependent on fermentation conditions. ALM were isolated from the culture broth using XAD adsorber resin, and peptide groups F-30 and F-50 separated by silica gel column chromatography. The groups were fractionated by preparative HPLC. Individual peptides were separated by analytical HPLC and subjected to on-line sequencing using electrospray ionisation mass spectrometry. Chiral gas chromatography revealed the L-configuration of components and the presence of D-isovaline (D-Iva) in minor sequences. Positions of isomeric amino acids were assigned by GC-MS after methanolysis as demonstrated previously with peptaibols trichovirins [2]. Here we report on sequences of ALM F-50 (see below) and minor sequences of ALM F-30 (Ac, acetyl; U, α-aminoisobutyric acid; J, isovaline; Fol, phenylalaninol).

Ac-U-P-U-A-U-A-Q-U-V-U-G-L-U-P-V-U-U-Q-Q-Fol	F-50/I
Ac-U-P-U-A-U-U-Q-U-V-U-G-L-U-P-V-U-U-Q-Q-Fol	F-50/II
Ac-U-P-U-A-U-A-Q-U-V-U-G-L-U-P-V-U-J -Q-Q-Fol	F-50/III
Ac-U-P-U-A-U-A-Q-U-V-U-G-L-U-P-V-U-U-E-Q-Fol	F-30/I
Ac-U-P-U-A-U-U-Q-U-V-U-G-L-U-P-V-U-U-E-Q-Fol	F-30/II

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P B13 - Sequences of peptaibol antibiotic trichoaureocins from *Trichoderma aureoviride*

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Peptaibols are fungal polypeptides containing a high proportion of Aib (α-aminoisobutyric acid) and a C-terminal bound 2-amino alcohol. The mold Trichoderma aureoviride (strain IMI 91968 from Commonwealth Mycological Institute, Kew, UK) was cultured in complex medium. Fermentation was conducted in nineteen 2-L shake flasks, each containing 400 ml medium, for 7 d at 27 °C. Mycelia were obtained by filtration and treated with MeOH and MeOH/chloroform. Extracts were evaporated to dryness and subjected to Sephadex LH-20 (eluent MeOH) and silica gel chromatography (eluent chloroform/ MeOH/AcOH/water 65:25:3:4). Amounts of 0.9 g crude peptaibol mixture named trichoaureocins (TAC) were obtained, uniform on TLC. Peptides could be separated by analytical and semipreparative HPLC (Nucleosil 100 C-18; 250 x 8 mm ID; 3 µm). The latter provided six fractions each of which was subjected to sequencing using on-line HPLC on a special fluorocarbon stationary phase (Fluofix 1 EW 425) and ESI-MS/MS as described for peptaibols trichovirins [1], antiamoebins [2], and stilboflavins [3]. The TAC fraction 1 was uniform using Nucleosil stationary phase but was resolved in peptides 1a-1e on the fluorocarbon phase. TAC sequences are presented in the Figure.

No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1a	Ac	A	A	U	A	U	A	Q	U	V	U	G	L	U	P	V	U	V	Q	Q	Fol
1b	Ac	U	A	U	A	A	A	Q	U	V	U	G	L	U	P	V	U	U	Q	Q	Fol
1c	Ac	U	A	U	A	U	A	Q	U	V	U	G	L	U	P	V	U	A	Q	Q	Fol
1d	Ac	U	A	U	A	U	A	Q	U	V	A	G	L	U	P	V	U	U	Q	Q	Fol
1e	Ac	U	A	U	A	U	A	Q	A	V	A	G	L	U	P	V	U	U	Q	Q	Fol
3	Ac	U	A	U	A	U	A	Q	U	V	U	G	L	U	P	V	U	U	Q	Q	Fol
4	Ac	U	A	U	A	U	A	Q	U	X	U	G	L	U	P	X	U	X	Q	Q	Fol
5	Ac	U	A	U	A	U	U	Q	U	V	U	G	L	U	P	V	U	U	Q	Q	Fol
6	Ac	U	A	U	A	U	U	Q	U	X	U	G	L	U	P	X	U	X	Q	Q	Fol

Figure. Sequences of trichoaureocins; Ac = acetyl; protein amino acids are abbreviated according to one-letter-nomenclature; U = Aib, X = valin (V) or isovaline (Iva); Fol = phenylalaninol; Fol and chiral amino acids are of the L-configuration with the exception of D-Iva.

The 20-residue peptaibols represent a natural peptide library and cause hemolysis of sheep erythrocytes and exert antibiotic activity against Bacillus subtilis and Staphylococcus aureus.

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P B14 - Molecular Mmodeling of structurally related µ-opioidmimetics: parameters defining µ-and δ-receptor selectivity

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Molecular modeling and ^{1}H NMR analysis of structurally related µ-opioid mimetics revealed a paradigm for m-opioid receptor binding and δ- and µ-opioid receptor selectivity. The structures of the µ-agonist, H-2,6-dimethyltyrosine(Dmt)-Pro-Phe-NH-6-quinoline, (GPI IC_{50} = 0.6 nM; MVD IC_{50} >10,000), the µ/δ-agonists H-Dmt-Pro-Phe-NH-5-quinoline (GPI IC_{50} = 0.3 nM; MVD IC_{50} = 0.6), and H-Dmt-Pro-Phe-NH-1-napthalene (GPI IC_{50} = 0.5 nM; MVD IC_{50} = 5.5) were evaluated using ^{1}H NMR and extensive conformational searching. Final structures were docked into δ-opioid receptor sites through interactions between Gln105 and Dmt, W274 and Phe and Y129 with the C-terminal amide. The amide of H-Dmt-Pro-Phe-NH-6-quinoline was shielded by quinoline prohibiting H-bonding between the C-terminal amide and Y129. NOE cross peaks also indicated that orientations of the C-terminal amide and the ring system of H-Dmt-Pro-Phe-NH-6-quinoline were different than those of the µ/δ-agonists, H-Dmt-Pro-Phe-NH-5-quinoline and H-Dmt-Pro-Phe-NH-1-napthalene. The results suggested that a direct interaction between the C-terminal amide and the δ-opioid receptor is required for δ-agonism but not µ-agonism. Based on this hypothesis ligands without the C-terminal amide were evaluated to determine minimal µ-receptor binding requirements. A pyrazinone derivative, H-Dmt[K,K-pyrazinone]Dmt, (GPI IC_{50} = 1.9 nM; MVD IC_{50} = 41.5) was evaluated for potential electronic interaction between pyrazinone and Trp295. Structurally related diaminoalkanes H-Dmt-$(CH_2)_4$-Tyr, and H-Dmt-$(CH_2)_4$-Dmt were evaluated to explore the effect of hydrophobicity on the µ-receptor binding site.

P B15 - Structural studies of amyloid beta-peptide-(25-35)

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The major components of neuritic plaques found in Alzheimer disease (AD) are peptides known as amyloid β-peptides (A-β-peptides), which range in length between 39 and 42 residues. The A-β-(1-42) is the most prone to aggregation and is produced in larger quantities in familial forms of AD.
A-β-(25-35), sequence GSNKGAIIGLM, is a synthetic derivative of amyloid β-peptide, that is highly toxic and forms fibrillar aggregates typical of β-amyloid. It has been proposed that it represents the biologically active region of A-β-peptide. Since it retains both the physical and biological properties of A-β-peptides it can be used as a suitable model of full-length peptides, for testing inhibitors of aggregation and toxicity. Like the A-β-(1-42), A-β-(25-35) undergoes a conformational transition from a soluble, alpha-helical form to aggregated fibrillary β-sheet structures which are neurotoxic [1]. A detailed investigation of the influence of different environmental conditions on the conformational state of A-β-(25-35) peptide could help to clarify the mechanism of aggregation and its implications in the formation of fibrillar aggregates. We have recently studied the solution structure of A-β-(1-42) in a hexafluoroisopropanol (HFIP)/water mixture as determined by homonuclear 2D NMR [2]. Here we report the conformational characterization of A-β-(25-35) in mixtures of water and organic solvents by CD and NMR spectroscopy. Spectra were recorded in water/DMSO, water/SDS, water/HIFP. The poor quality of NMR spectra in DMSO/water hampered the building of a 3D model in this solvent. 3D structures of A-β-(25-35) were calculated on the basis of NMR data recorded in water/SDS and in water/HIFP. HFIP has been chosen in view of its solvent power and of the ability to stabilize helical structures. The comparison of the A-β-(25-35) NMR structures with the different NMR models of the full A-β-(1-42) suggests that the 11-mer derivative A-β-(25-35) and the full length A-β-peptide share the same conformational peculiarities which are intriguingly dependent on the medium environment.

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P B16 - Conformational analysis of peptides derived from feline immunodeficiency virus tm-glycoprotein.

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Feline immunodeficiency virus (FIV) provides a valuable model system by which criteria for antilentiviral vaccines and drugs development can be tested. A 20-mer synthetic peptide spanning amino acids ^{767}L-G^{786} of the membrane-proximal ectodomain of FIV transmembrane glycoprotein potently inhibited growth of a tissue culture-adapted strain of FIV in fibroblastoid CrFK cells. Furthermore, the 20-mer ^{767}L-G^{786} blocked replication of cell-free and cell-associated primary isolates in lymphoid cultures very effectively, with IC_{50} ranging between 0.03 and 0.63 µg/ml. By testing deleted or substituted peptides, peptide ^{770}W-I^{777} was identified as the minimal sequence needed for full antiviral activity and conservation of the three tryptophan residues was found to be essential. Here we report a conformational analysis of the 20-mer ^{767}L-G^{786} and its 8-mer derivative ^{770}W-I^{777} by means of CD and NMR spectroscopies. A comparative structural analysis is performed aimed to understand the factors determining the stability of 8-mer peptide, as well as the role played by the remaining residues of the 20-mer peptide in conditioning the conformational properties of the ^{770}W-I^{777} peptide.

P B17 - TOAC-labeled bradykinin analogues: conformational studies in model membranes and correlation with the biological activity

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Bradykinin (BK) analogues labeled with the TOAC (2,2,6,6-tetramethylpiperidine-N-oxide-4-amino-4-carboxylic acid) spin probe have been synthesized and biological assays indicated that, whereas the $TOAC^3$-BK is devoid of activity, $TOAC^0$-BK still retains about 70% of the activity of BK in isolated rat uterus (Nakaie et al., 1998, 2002). Initial EPR and CD studies in solution varying the pH or the TFE amount have indicated that both derivatives are characterized by extended structures. However, the active $TOAC^0$-BK presents a higher degree of conformational flexibility and resembling more closely the molecular dynamics of the native hormone, BK. In TFE, the two analogues acquire a more folded conformation but again, a clear distinction is observed between the analogues. A higher movement restriction is detected in the inactive $TOAC^3$-BK compound. To further extend this combined EPR-CD approach for membrane-mimetic systems, the present report investigated comparatively the structural features of these BK analogues in the anionic SDS and in the zwitterionic HPS surfactants. CD spectra indicated stronger interaction of both peptides with SDS micelle but in contrast to the extended structures observed for BK and the active $TOAC^0$-BK analogue, the mid-chain labeled $TOAC^3$-BK displayed a more folded conformation trending to acquire an α-helical-type conformation as the amount of surfactant increased. EPR spectra corroborated these findings as prominent incorporation of both peptides occurred in SDS than in HPS. Noteworthy, stronger interaction was observed with the inactive $TOAC^3$-BK analogue revealed by the higher values of the measured rotational correlation time values ($\tau \sim 50 \times 10^{-10}$ s) if compared with $TOAC^0$-BK ($\tau \sim 14 \times 10^{-10}$ s). The stronger interaction of peptides in SDS was also revealed by interpreting the variation of the isotropic hyperfine splitting parameter (a_0) values of the EPR spectra. This parameter is dependent on the polarity of the medium and a very pronounced decrease in this values was measured for analogues in SDS, thus indicating their incorporation in a more apolar environment. The EPR parameter which considers the two existing τ values, τ_C / τ_B, may also help unravel the nature of the molecular movement of peptide segments. The higher τ_C / τ_B ratio calculated for $TOAC^3$-BK than for $TOAC^0$-BK (1.4 against 1.2) suggested a higher asymmetry of movement for the former peptide, thus in accordance with its more folded structure. All together the findings confirmed the structural similarity between BK and its active $TOAC^0$-BK analogue, regardless of the media. Secondly, a clear demonstration of a direct structure-biological activity relationship was verified for these paramagnetic BK analogues. [(1)*Peptides 1996* (1998) 673; (2) *Peptides* (2002) 23, 65.]

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P B18 - Structure-activity relationship of lactam bridge gomesin analogues

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Gomesin is an antimicrobial peptide (Pyr-CRRLCYKQRCVTYCRGR-NH_2) isolated from hemocytes of spider *Acanthoscurria gomesiana*. It contains two disulfide bridges ($Cys^{2\text{-}15}$ and $Cys^{6\text{-}11}$) and is effective against several gram-positive and negative bacteria strains and fungi, although exhibits some hemolytic activity [1]. Recently, we showed the importance of the disulfide bridges in the biological activity of gomesin at physiological salt concentration [2]. In the present work we explored the hypothesis that the disulfide bridge could be replaced by lactam. Our ultimate goal is to obtain active analogues that are more selective and/or resistant to serum proteases degradation. Thus, a series of nine lactam-bridged gomesins were synthesized, allowing the exploration of the ring size and bridgehead chirality. The peptides were synthesized by solid phase methodology on MBHAR using the t-Boc strategy. The peptidyl-resins had their lactam bridges formed in 20%DMSO/NMP using BOP as coupling reagent. After lactamization and HF treatment, the disulfide bridge was achieved by air oxidation. The analogues were purified by RP-HPLC and characterized by CZE, AAA, RP-HPLC/MS and circular dichroism. The antimicrobial activity was monitored by a liquid growth inhibition assay against *M. luteus*, *E. coli*, and *C. albicans* [1]. In low salt concentration, [$Ser^{2,15}$,Asp^6,Orn^{11}]-; [$Ser^{2,15}$,Orn^6,Asp^{11}]-; [$Ser^{2,15}$,D-Asp^6, Orn^{11}]-; [Asp^2, $Ser^{6,11}$, Orn^{15}]- and [D-Asp^2, $Ser^{6,11}$, Orn^{15}]-Gm showed to be 4 to 32-fold less active than the gomesin in the three organisms strains. On the other hand, [Asp^2, Orn^{15}]-; [Asp^6, Orn^{11}]-; [Glu^2, Lys^{15}]- and [Glu^6, Lys^{11}]-Gm on *M. luteus* and *C. albicans* were just 2–fold less potent than gomesin, although equipotent to it on *E. coli*, with the exception of [Glu^6, Lys^{11}]-Gm that was 4-fold less potent. When the assays were performed under physiological salt concentration, [$Ser^{2,15}$,Asp^6,Orn^{11}]-; [$Ser^{2,15}$,Orn^6, Asp^{11}]-;[$Ser^{2,15}$, D-Asp^6, Orn^{11}]-;[Asp^2, $Ser^{6,11}$, Orn^{15}]- and [D-Asp^2, $Ser^{6,11}$, Orn^{15}]-Gm showed to be 8 to 16-fold less active than the gomesin on *M. luteus* and were inactive on *E. coli* and *C. albicans*. [Asp^2,Orn^{15}]-Gm and [Asp^6,Orn^{11}]-Gm were just 2-fold less potent than gomesin on *M. luteus* and *E. coli* and 5-fold on *C. albicans*. [Glu^6,Lys^{11}]-Gm showed to be 2- and 8-fold less active than gomesin on *M. luteus* and *E. coli* respectively, and inactive (until 20 μM) on *C. albicans*. Interestingly, [Glu^2,Lys^{15}]-Gm presented almost the same activity than gomesin. From these results we can concluded that the disulfide bond can be replaced by lactam-bridge, specially using Glu and Lys as bridgehead components, in the position 2/15 of the gomesin molecule. No obvious correlation can be found between biological activity and secondary structural features observed in CD.

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P B19 - Structural analysis of the interaction of diepoxybutane with haemoglobin by LC/ES/MS. Evaluation of alkylated peptides as biomarkers of the occupational exposure to 1,3-butadiene

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1,3-butadiene (BD) is a compound used in the rubber plastic manifacture and also an environmental pollutant. The metabolic pathway of BD leads to the formation of highly reactive species such as 1,2,3,4-diepoxybutane (DEB). Although the exact mechanisms of BD-induced mutagenesis and carcinogenesis are unknown, chemical reactions of epoxy-metabolites with DNA result in covalently bound adducts, causing genetic mutations and possibly initiating the carcinogenic response. BD-epoxy metabolites react also with a variety of nucleophilic sites on plasmatic proteins, and on haemoglobin (Hb) to form Hb adducts. Protein adduct formation is a valuable surrogate for DNA adduct formation since, particularly in animal models, many chemical carcinogens bind to both DNA and proteins in blood with similar dose-response kinetics. Hb is the protein of choice for such measurements because it is readily accessible, and has known rates of turnover. The purpose of the present study was the identification of suitable biomarkers for monitoring human exposure to BD. This required, as preliminary step, the structural characterisation of the adducts formed by *in vitro* interaction of Hb with DEB, which was obtained by liquid chromatography/electrospray ionisation-mass spectrometry (LC/ESI/MS) analysis of tryptic peptides of hemoglobin chains. The reactive sites of human hemoglobin were identified by tandem mass spectrometry. LC/ESI/MS analysis of peptides coming from tryptic hydrolysis of haemoglobin incubated with DEB showed that two different adducts are formed: hydroxyepoxy-butyl (HEB)-derivative and trihydroxy-butyl (THB)-derivative. For both types of adducts LC/ESI/MS analysis demonstrated that major reactivity belongs to the amino group of N-terminal valine of alpha and beta globins and to peptide containing cysteine 93 of beta globin. The modification in N-terminal valine tryptic peptide could be utilized as a specific marker for DEB in humans exposed to DEB or 1,3-butadiene. A procedure was set up based on this characterisation, allowing Hb modification to be assessed by monitoring alkylated peptides using SIR mass spectrometry with the use of a octa-deuterated trihydroxy-butyl peptide standard, in order to investigate the feasibility of using N-terminal THB-peptides as biomarker of human exposure to BD. The use of peptides as biomarkers compared to that of the intact proteins was advantageous for high accuracy and high resolution quantitative measurement by mass spectrometry. By this methodology, it was also possible to compare advantages and disadvantages of presently available strategies for the measuring of Hb adducts. The results obtained can lead to the optimisation of molecular dosimetry of BD adducts, and the analytical procedure described herein colud be applied to the biological monitoring of exposure on the workplace.

P B20 - Cross interaction of beta-sheet protein fragments of prions and beta-amyloid

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In the last decade the mechanism of neurodegenerative diseases development has been described on the molecular level. It is postulated that Alzheimer (AD), Creutzfeldt-Jacob (CJ) and similar diseases have a common genesis, which is a base for a common name "conformational diseases". In these diseases, the main postulated factor responsible for the nerve cells degeneration is formation of the protein aggregates. The main proteins that form aggregates in various diseases are different, in AD the aggregates are formed from beta-amyloids, in CJ aggregates of prions are responsible for degenerative diseases. Nevertheless, the transformation of the protein to beta-sheet is a common, necessary factor igniting the aggregation. Mechanism and kinetics studies of peptide and protein amyloidgenesis are important and necessary to understand the basis of the illness and to plan further therapy. The mechanism of transformation of helical to beta-sheet conformation is not well established. It is postulated that the conformation changes are induced by "seed" factor(s), like already existing beta-sheet peptide fragments. It is possible that exists cross-interaction between beta-formed proteins. To check such possibilities we have performed kinetic studies of interactions of beta-amyloid fragments responsible for beta-sheet formation with respective prion fragments. To analyze possible homo- or hetero-aggregates, the combination of mass spectrometry and fluorescent HPLC analysis has been applied.

P B21 - NMR chemical shielding analysis of N-formyl-serinamide, a model for polar side chain containing peptides

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The existence of structure - chemical shift relationships for peptides could greatly aid in the NMR chemical shift assignment and structure refinement processes that occur during peptide/protein structure determination. Previously certain structure-chemical shift correlations were determined for the glycine, alanine, valine and phenylalanine dipeptides. To determine whether such correlations can also exist for polar side chain containing amino acid residues the serine dipeptide, For-L-Ser-NH_2, was studied. Using the GIAO-RHF/TZ2P and GIAO-RHF/6-31+G* levels of theory the NMR chemical shifts of all hydrogen ($^1H^N$, $^1H^\alpha$, $^1H^{\beta1}$, $^1H^{\beta2}$), carbon ($^{13}C^\alpha$, $^{13}C^\beta$, $^{13}C'$) and nitrogen (^{15}N) atoms has been calculated for all 44 stable conformations of serine previously determined by our group. An attempt was then made to linearly correlate each type of chemical shift with the ω_o, ϕ, ψ, ω_1, χ^1 and χ^2 structural parameters. At both levels of theory a visible linear correlation can be seen between $^1H^\alpha/\phi$, $^{13}C^\alpha/\phi$ and $^{13}C^\alpha/\psi$, however even the highest R^2 value is only 0.695 ($^1H^\alpha/\phi$; GIAO-RHF/TZ2P level of theory). These results indicate that for serine the molecule's structure has a strong influence on its chemical shifts, nevertheless there are other significant factors that must be considered as well during the chemical shift assignment and structure refinement processes.

P B22 - Conformational analysis of natural marine cyclopeptides with antitumour properties

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According to the WHO estimations in 2002 there will be 20 millions cancer patients worldwide, 70% of which in developing countries. From a financial point of view the market of anti-tumor products reached 14.8 billion dollar with a 12.5% annual growth rate. It is therefore not surprising that the search for new anti-tumor agents has become particularly active, and in this context natural products of marine origin are particularly interesting. Our laboratory has been working for more than 10 years, in collaboration with PharmaMar, in the synthesis and structural elucidation of anti-tumor ciclodepsipeptides of marine origin [1-3] . As a consequence of this the compounds aplidine and kahalalide F are in an advanced stage in the clinical and preclinical studies respectively. The study of the conformation of this kind of compounds is particularly difficult because these molecules tend to combine a high spectral complexity with a high flexibility. NMR, however, is a very powerful technique in this kind of studies. The possibility of using very high field spectrometers and multidimensional techniques allows removal of most spectral overlap problems. As for flexibility, in our opinion, this issue can only be tackled by studying these molecules in a variety of experimental conditions and through the use of molecular dynamics calculations as a means of analyzing the data. In this communication we will present our results on three molecules: aplidine, trunkamide and kahalalide F. We will discuss the case of aplidine which, in solution, presents the coexistance of several rotamers with a low interconversion rate which allows the two conformers to be resolved using HPLC. We will also present data, especially for trunkamide and kahalalide F, which show the great impact that changes of configuration can have on the conformational behavior of the molecule. Finally, we will discuss the impact of NMR in the determination of the configuration of stereogenic centers of unknown stereochemistry.

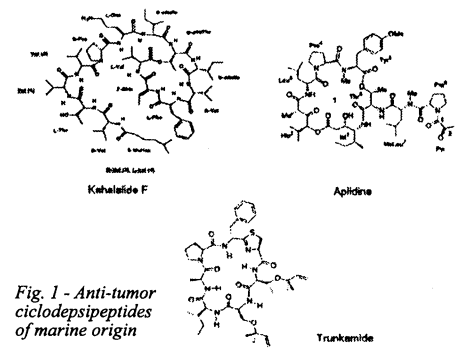

Fig. 1 - Anti-tumor ciclodepsipeptides of marine origin

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P B23 - Conformational studies of ampullosporin A and synthetic analogues by circular dichroism in comparison to the crystal structure of the native peptide

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In a previous work we have described the synthesis and biological activities of analogues of the peptaibol ampullosporin A:
Ac-Trp[1]-Ala-Aib-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Gln-Leu-Aib-Gln-Leu[15]ol which was isolated from the fungus *Sepedonium ampullosporum* in 1997 [1,2]. Besides displaying moderate narrow-spectrum antibacterial and antifungal activity, ampullosporin A was found to enhance pigmenation on *Phoma destructiva* and to induce hypothermia in mice [2]. Further investigations using behavioural tests have confirmed the assumption of a neuroleptic activity of ampullosporin A [3].
For structure-activity-relationship studies we synthesized a series of related structures containing modifications in the N-terminal Trp, the C-terminal Leuol as well as in the different Aib-positions of the sequence. Whereas compounds with C-terminal Leu or Leu-NH_2 or N-terminal D-Trp or Tic showed similar activities as ampullosporin A we were able to generate peptides which were active in the pigmentation assay but showed no hypothemric response in mice. In order to find out explanations for the differences in the biological activities investigated the conformational and membrane-modifying properties of these peptides.
In this paper we now report the conformational study of ampullosporin A analogues using circular dichroism. The CD-spectra of the analogues and the crystal structure of natural occurring ampullosporin A, determined in 2001 [4], will be discussed considering the biological results.

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P B24 - Structural and biosensor analyses of a biotinylated peptide probe for APC tumor suppressor protein

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Many colon tumours arise from mutations in the gene coding for the production of the adenomatous polyposis coli (APC) tumour suppressor protein. This large molecular weight (>300kDa) protein contains a coiled coil amino terminal domain that is responsible for homodimerization. Previous work by others [1] has led to the design of a specific 54-residue peptide (anti-APCp1) that dimerizes preferentially with this domain. We have undertaken the chemical synthesis of a modified form of this peptide (anti-APCp2) that bears a biotin moiety at its amino terminus for use in subsequent micro-affinity purification and analysis of differentially regulated protein complexes. The peptide was subjected to comprehensive chemical characterization to confirm its purity. Secondary structural analysis by CD and FTIR spectroscopy indicated that the peptide could assume a wide range of potential conformations, depending upon the precise microenvironment. Significantly, a stable α-helical structure was generated when the solvent conditions supported intramolecular salt-bridge formation along the helix barrel. The biotinylated anti-APCp2 was immobilised onto a streptavidin sensor surface, in a specific orientation leaving all amino acids available to form a coiled structure. A high molecular mass fraction (greater than 600 kDa) was detected in the eluant from a size-exclusion column following injection of colonic carcinoma cell line (LIM1215) lysates that reacted specifically with the immobilised anti-APCp2 on the biosensor surface. A high molecular mass protein (Mr >250kDa on SDS-PAGE) could be specifically immunoprecipitated from this peak using either the anti-APCp2 peptide or an anti-APC polyclonal antibody. A multidimensional micropreparative chromatographic /biosensor/ proteomic protocol for the purification of APC alone and APC complexed with different biopolymers in various cell lines, and stages of tumor development is currently being developed using this strategy

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P B25 - Structure analyses of HSV epitope peptides with NMR spectroscopy and molecular dynamic computation

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Herpes simplex virus (HSV) with its two closely related serotypes, is one of the most common infectious agents in humans. Sequences 1-23 in glycoprotein D (gD) were found as an epitope region responsible for virus neutralizing antibody production [1]. The peptide 9-21 (LKMADPNRFRGKDL) was determined as a core epitope in this sequence. Two cyclic versions of the peptide 9-21 (SG-2: H-LKc[HcyADPNRFK]GKDL-NH_2 and SG-3: H-LKc[CADPNRFK]GKDL-NH_2) were synthesised. In the ELISA tests the SG-2 showed binding activity, but the SG-3 was not recognised by antibodies [2]. The question was that, what cause the differences between the behaviour of two peptides.
The three-dimensional structures of peptides were mapped by NMR spectroscopy and molecular dynamic computation. After the comparative analysis of counted dominant conformers it was found that the SG-2 has two main conformers and the SG-3 has a more rigid structure. Although the difference is only one methylene group between the two peptides this raise the deviation in their structures and conformational stability.

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P B26 - FT-IR studies on the effect of β-sheet breaker peptides on the conformation and aggregational properties of β-amyloid[1-42] and PrP[106-126]

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Alzheimer's disease (AD) and Creutzfeldt-Jakob disease (CJD) are progressive neurodegenerative disorders, characterized by accumulation of fibrillar deposits followed by neuronal cell death. In AD, the major component of the plaques is β-amyloid (Aβ), a 39-42 residue long peptide. In CJD, the deposits consist primarily of the conformationally altered prion protein (PrP). In both cases, the neurotoxic effects are due to the aggregation of these peptides, adopting a highly insoluble and protease resistent β-sheet conformation. It is assumed that inhibiting or retarding of the aggregation process by β-sheet breaker peptides (BSB) can prevent fibril formation and the neurotoxic effects of these peptides. The aim of our work was to investigate the effect of different BSB peptides on the secondary structure and aggregational properties of Aβ[1-42] and PrP[106-126].
The peptides were synthesized on MBHA resin by solid phase methodology using Boc-chemistry. The compounds were purified by preparative RP-HPLC and characterized by ES-MS and analytical HPLC. Because of the high TFA content (absorbance at 1673 cm^{-1}) of the purified peptides, the compounds were reeluted on a C18 column using AcOH in the solvents. In measurements, Aβ[1-42] or PrP[106-126] and the BSB peptides were used in a molar ratio of 1:3. Peptides were dissolved in dimethyl-sulfoxide or trifluoro-ethanol and diluted with phosphate buffered heavy water saline (pH=7.4). The effect of the BSB peptides on the conformational transitions of Aβ[1-42] and PrP[106-126] were followed by time dependent Fourier transform infrared spectroscopy (FT-IR) and two dimensional FT-IR correlation analysis (2D FT-IR) techniques in the amide I region.

P B27 - Biological activity, NMR and conformational analysis of peptide analogs of amino-terminal sequence of RANTES

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RANTES (regulation upon activation normal T-cell expressed and secreted) is a member of the CC-chemokine subfamily that acts as a chemoattractant for several kinds of leukocytes. Its biological activity results from binding to CCR5 receptors. It was shown that RANTES might play an important role in inhibiting of HIV replication of certain T-tropic strains in primary PBMC and analogs of amino-terminal sequence with anti-HIV activity were developed [1].
A number of both linear and cyclic peptides corresponding to the amino-terminal RANTES sequence 3-10, which is known to be a crucial region for chemotaxis, have been synthesized for ongoing conformational and biological studies. NMR investigation of Ac-(Tyr1-Gly2-Ser3-Asp4-Thr5-Thr6-Pro7-Ala8)- NH_2 demonstrated that this peptide adopted no structure in solution. However amide proton of residue 5 might take part in hydrogen bonding in at least a part of low-energy conformers. Chemoattractant activity of synthesized analogs was investigated by modified method of Nelson. Only one cyclic analog cyclo[10 Ala-RANTES 3-10] showed significant level of chemoattractant activity. Insertion into peptide chain beta-alanine or gamma aminobutyryl residues abolished biological activity. Position 3 seemed to be insignificant for manifestation of biological activity. Conformational analysis of Ac-(10 Ala-RANTES 3-10)NH_2 resulted in a set of low-energy structures, characterized by bend around residues 3-5 and extended conformation of residues on both ends of molecule. One conformer had a compact structure in which of C- and N-terminuses located in close proximity. Taking into account that cyclo[10 Ala-RANTES 3-10] possessed biological activity of the whole RANTES, and N-terminal part of RANTES is very flexible, we suppose that amino terminal sequence of these chemokine may adopt quasi cyclic structure during receptor-binding process.

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P B28 - Structural effect of metal replacement in a Cys2His2 zinc finger domain

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In the last years it has been demonstrated that zinc ions are structural components of a large number of proteins. The binding of zinc stabilizes the folded conformations of domains not able to fold by themselves, so that they may facilitate interactions between the proteins and other macromolecules such as DNA. Zinc has a key structural role in transcription factors that contain zinc finger domains, the largest group of eukaryotic DNA binding proteins know to date [1]. NMR studies of single and double zinc fingers without DNA verified that the domains could fold as isolated units with each finger consisting of a ββα fold clustered around a compact hydrophobic core. At the center of the fold was a single zinc ion chelated in a tetrahedral geometry by two residues of cysteine and two residues of histidine.
Since a number of metal ions, including xenobiotic ions, are believed to have etiological roles in carcinogenesis and other disease processes, recent studies have examined the effects of metals other than zinc on the structure and function of hormone receptors [2,3]. Analogously, elucidating mechanism of metal ion-zinc finger protein interactions could provide molecular details of the structure and function of these proteins, as well as insights into potential disease processes.
In the present study, the effect of Cd^{2+}/Zn^{2+} substitution in the solution conformation of a 37 amino acids zinc finger domain from SUPERMAN protein [4] will be examined in order to gain understanding of the metal ion structural role in zinc finger proteins.

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