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Structural Studies

FT-IR STUDIES ON THE CONFORMATIONAL CHANGES OF β-AMYLOID[1-42] EFFECTED BY ITS FRAGMENT ANALOGUES

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One of the important factors in the development of Alzheimer's disease is the aggregation of β-amyloid (Aβ) peptide adopting antiparallel β-sheet conformation. The connection of the aggregation of Aβ with Alzheimer's disease has not been cleared up yet. It is assumed that inhibiting or retarding of Aβ aggregation could be a possible method to prevent the progress of the disease. Previously, it was found that Aβ[16-20] is capable of inhibiting the aggregation of Aβ[1-42] by intermolecular forces between the whole length of Aβ and this pentapeptide[1]. The aim of our work was to investigate how Aβ[16-20] and different fragments and fragment analogues of Aβ[1-42] influnce on the conformational transitions of Aβ[1-42].
The investigated 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 analitical HPLC. In measurements, Aβ[1-42] and the short peptides were used in a molar ratio of 1:3. For standardisation purposes, peptides were disaggregated before each experiment in dimethyl-sulfoxide. After dilution with phosphate buffered heavy water saline (pH=7.0), conformational changes were followed by time dependent Fourier transformed infrared spectroscopy (FT-IR) and two dimensional FT-IR correlation analysis (2D FT-IR) techniques in the amid I region. The measurements were carried out from 5 min to one week. It was observed that in the present of the investigated short peptides, transition from α-helix and random coil to β-sheet occurred, but in a lower rate then by β-amyloid[1-42] itself, and the adopted β-sheet structure was less aggregated than without the investigated peptides.

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SELF ASSEMBLY OF PEPTIDES RICH IN PROLINE RELATED WITH γ-ZEIN

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γ-Zein is a major constituent of maize endosperm and is found lining the membrane of endoplasmic reticle (ER) derived protein bodies (1, 2). The N-terminal domain of this protein contains a highly repetitive sequence $(VHLPPP)_8$, which is necessary for sorting γ-zein into the ER, and this sequence suggests the formation of a long amphipathic helix that may be related to the peripheral location of γ-zein into the protein body. Circular dichroism studies of synthetic peptides with the formula $(VHLPPP)_n$ (where n=2-8) have shown that they adopt a polyproline II conformation in aqueous solution that increases with the length of the oligomer and the concentration (3). Samples of peptides $(VHLPPP)_n$ (where n=3, 5 and 8) dried on highly oriented pyrolitic graphite were studied by atomic force microscopy. They self assembled forming monolayers with organized domains of nanofibrils (5-7 nm wide). We proposed a model to interpret the images, and in order to understand the molecular interactions that drive to the self assembly we carried out molecular dynamics simulations.
The biological importance of these results comes from the fact that, taking into account the alternating hydrophobic character of V and L *vs.* H side chains, it can be easily anticipated that the monolayers formed by $(VHLPPP)_8$ must be amphipathic. This suggests a possible mechanism for the γ-zein targeting and protein body formation. AFM experiments using the γ-zein are in progress in order to validate the model here proposed.

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STUDIES ON THE BINDING OF β-SHEET BREAKER (BSB) PEPTIDES.

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Alzheimer's disease starts after an overproduction of β-amyloid peptides in the brain. These peptides contain 40-42 amino acids and have a high tendency to form parallel or antiparallel β-pleated sheet structure. Small changes of pH or ionic media trigger conformation change and association of β-amyloids into supramolecular structures: diffusible aggregates containing 50-100 peptide units or highly aggregated fibrils. The aggregates are neurotoxic and resistent againts proteolytic enzimes and can form plaques. Short peptide fragments can bind specifically to β-amyloids and hinder association and aggregation, these compounds call β-sheet breaker (BSB) peptides. They are putative drugs of the Alzheimer's disease.
The structure of the binding of Soto- (LPFFD), Tjernberg-peptides (KLVFF) and a series of new BSB peptides synthesized in our laboratory were studied theoretically. The structures of the peptides were calculated by molecular dynamics methods in the presence of implicit and explicit water model with AMBER and a modified GROMOS87 force field. In the calculations of the structures, simulated annealing and isotherm molecular dynamics methods were applied. The association of the BSB peptides to the βA(1-42) and its fragments were performed by AUTODOCK. Our results support that the binding of hydrophobic side chains are important in the association of the BSB peptides to βA(1-42).

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A LINEAR PRION PEPTIDE FOLDS INTO A WATER–STABLE MONOMERIC β–SHEET

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In order to understand the molecular driving forces of prion conversion, conformational properties of a synthetic linear peptide derived from the globular core of sheep prion protein have been studied by circular dichroism and nuclear magnetic resonance spectroscopies. This peptide encompasses the sheep prion region 145–169, corresponding to the human 142–166 region. Under physiological conditions, the peptide folds into a highly stable monomeric β–sheet, whereas, in the benign protein the peptide is engaged in largely helical and coiled secondary structures. These findings suggest that corresponding region in the whole prion polypeptide chain can serve as a transconformational site during the prion conversion. Based on this consideration, a structural background for the mechanism of prion conversion is proposed.

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THE NMR THREE-DIMENSIONAL STRUCTURE OF THE PEPTAIBOL ANTIBIOTIC, LONGIBRACHIN LGA I

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Longibrachin LGA I is a 20-residue antibiotic peptide produced by the fungus *Trichoderma longibrachiatum* and belonging to the peptaibol class. It modifies the permeability of membrane bilayers, forming voltage-gated ion channels according to the barrel-stave model which involves transbilayer aggregates of helical monomers[1]. LGA I differs from the well-known alamethicin F50 (Alm F50) by a single Pro2→Ala substitution. This naturally occurring sequence modification was used to probe the role of the residue at position 2 in the channel stability, as a significative decrease in the channel lifetimes was observed for LGA I, as compared to Alm F50.
The three-dimensional structure of LGA I was thus studied, based upon 2D-NMR spectroscopy and molecular modeling. The resonance assignments and conformational parameters (NOEs, $^3J_{NHC\alpha H}$ coupling constants and thermal coefficients of amide protons) were obtained for LGA I in methanol solution. The 3D structure was calculated, using the program X-PLOR, starting from 155 NOE-derived interproton distance constraints and 11 Φ dihedral angle restraints. A total of 123 converged structures, consistent with the NMR data, were generated by restrained molecular dynamics and energy minimization calculations. LGA I forms a 33 Å long amphipathic helix, mainly α-type, apportioned into two segments (1-9 and 14-20) linked by a bend. The bend angle is about 150°. The structure is very close to that of Alm F50, the residue at position 2 occupying the same location in the hydrophilic sector of the helix for both peptides. Shortening of the LGA I channel lifetimes should thus be due to lesser anchoring of the N-terminal part of the helices at the *trans*-bilayer interface, when position 2 is occupied by alanine which is considered as more hydrophobic than proline[2].

LGA I Ac U[1]**A**-U-A-U-A-Q-U-V-U-G-L-U-P-V-U-U-Q-Q-Fol[20]

Alm F50 Ac U[1]**P**-U-A-U-A-Q-U-V-U-G-L-U-P-V-U-U-Q-Q-Fol[20]

(U (Aib): α-aminoisobutyric acid; Fol (Pheol): phenylalaninol)

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DESIGNING HETERODIMERIC TWO-STRANDED α-HELICAL COILED-COILS: THE EFFECT OF CHAIN LENGTH ON PROTEIN FOLDING, STABILITY, AND SPECIFICITY.

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We are studying the two-stranded α-helical coiled-coil as a model protein to investigate the relative contributions of electrostatics and hydrophobicity in heterodimer formation. In addition to providing fundamental answers to protein folding and stability, de novo designed coiled-coil heterodimers have many biomedical applications. Coiled-coils are a natural dimerization domain formed when two or more amphipathic α-helices wrap around each other in a left-handed supercoil. Their sequences are characterized by a heptad repeat (abcdefg) where positions "a" and "d" are occupied by hydrophobic residues which form a continuous hydrophobic core. We have designed heterodimeric coiled-coils whose specificity is controlled by electrostatic interactions in positions "e" and "g". The "E-coils" have two glutamic acid residues per heptad and the "K-coils" have two lysine residues per heptad in these positions. This destabilizes the homodimers through electrostatic repulsions and stabilizes the heterodimer through electrostatic attractions. In order to design coiled-coils with varied affinities we have synthesized coiled-coils of 3, 4, and 5 heptads by solid-phase techniques. Their folding, stability and oligomerization were characterized by circular dichroism spectroscopy and analytical ultracentrifugation. Heterodimer formation was observed during redox equilibrium experiments. We found that chain length played a significant role in the rate of heterodimer formation and affinity in the early stage of protein folding prior to secondary structure formation. In addition, electrostatic attractions appear to play a significant role. The kinetics of association and disassociation were also investigated by BIAcore, and found to be significantly influenced by chain length.

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CONFORMATION STUDIES ON SYNTHETIC GALANINS (GALs) AND THEIR FRAGMENTS

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GALs are nowadays among the best-studied neuropeptides with neurotransmitter and neuromodulator roles. All known GALs have 29/30-amino acid residues with amide or free carboxyl terminals. We have synthesized human, pig, rat, bovine and chicken GALs and their N- and C-terminal larger or smaller fragments by means of solid-phase synthesis, using both Boc and Fmoc strategies, and with 1% Merrifield and MBHA resins. The purified peptides were controlled and identified by RP-HPLC, HPCE and FAB- or ESI-MS, and some of them by Maldy-MS. The conformations of all the GALs and their derivatives were investigated by CD, FTIR and NMR techniques. *The CD spectra* of the different GALs and their derivatives reveal ordered structures in trifluoroethanol. In the case of human GAL, the spectrum reflects a helical structure: prediction and the shorter sequences of GAL suggest 3_{10}-helix. The spectra of shorter sequences of GALs point to conformer mixtures. Both the *CD* and *FTIR spectra* in water and D_2O, respectively, indicate a predominantly flexible unordered structure. The infrared spectra are dominated by two bands, centered at about 1672 and 1650 cm^{-1}, which may be related to the free (non-solvated) and the solvated amide group /1/. Unfortunately, interpretation of *FTIR spectra* in trifluoroethanol is quite difficult in consequence of contribution of the side-chain functionality's. For more conformational information *^1H NMR* were carried out by DOF-COSY, TOCSI and ROESY methods with a Brucker 400-MHz wide-beam spectrometer. Our results are in accord with earlier findings /2/ as regard the secondary and tertiary structures, especially of human GAL.

These studies are supported by Hungarian Research Fund OTKA (T 030526 and T 019306).

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CD MONITORING OF CATION BINDING BY HISTIDINE-CONTAINING PEPTIDES

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In an attempt to understand the role of cations on the bioactive conformation of peptides and polypeptides, we have investigated the interactions of different metal ions with "model" peptides. Metal binding[1] can stabilize or destablize pre-existing secondary stuctures in peptides. In most cases, cations tend to satisfy their own chemical and geometrical requirements upon complexation and might induce specific conformations, after, for example, binding to the imidazole side-chain of histidine[2]. The toxicity of certain metal ions is often due to replacement of vital metals due to their stronger affinity to specific sites or cause by inducing unfavourable conformational changes of the peptide skeleton[3]. Most calcium channels contain antagonist binding sites for naturally occurring toxins, small organic and inorganic compounds. Divalent and trivalent metal ions, in particular cadmium, nickel and zinc, are well-known blockers.[4] We report here systematic studies the effect of Ca^{2+}, Mg^{2+} Zn^{2+}, Ni^{2+}, Cd^{2+}, VO^{2+} ions on synthetic histidine-rich oligopeptides and histatins (H3 and H5). These peptides were synthesised by either the Boc- or Fmoc strategy on solid support. Circular dichroism was used to monitor changes of the backbone conformation in terms of the structure of the peptides (absence and presence of side-chain functionalities). In water cation binding does not have a significant effect upon backbone conformation, but by increasing the ratio of cation, we have detected some side-chain ligation to the metal ions. In trifluoroethanol, cation-specific changes were also observed upon increasing the amounts of cations.

This study was supported by the Hungarian Research Fund OTKA (T025829, T022913, F026632) and the National Sciences and Engineering Research Council of Canada

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LOCAL STRUCTURES INDUCED BY THE UREA FRAGMENT IN PEPTIDE CHAINS

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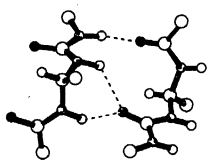
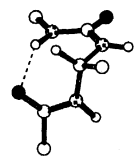
In the solid state, the *N,N'*-disubstituted ureas are shown by x-ray diffraction to be rigid fragments with two *trans* amide bonds, so that the N-H bonds are oriented in a direction opposed to that of the carbonyl. Ureas are thus often involved in multiple intermolecular hydrogen bonds giving rise to polymeric aggregates, as in the case of crystallized urea-containing peptides.
We have synthesized model peptides such as Boc-Xaaψ[NH-CO-NH]NHR **1** (Xaa = Leu, Phe, Ser(Bzl); R = Me, iPr), Piv-Pro-Leuψ[NH-CO-NH]NHMe **2** and Piv-Proψ[NH-CO-NH]Leu-NHMe **3** by an original method using *O*-succinimidyl carbamates as the activated intermediates. Their conformational properties have been investigated in solution by IR and NMR spectroscopy, and in the solid state by X-ray diffraction.



all-*trans* urea in the solid state



cis-trans urea in solution

Spectroscopic experiments on **1** in solution indicate that the urea unit is intramolecularly hydrogen bonded to the preceding carbonyl and closes an 8-membered cycle. It results that the NH-CO bond is capable of a rapid *cis/trans* equilibrium, since only the *cis* conformer is compatible with the aforementioned interaction. Spectroscopic experiments are in progress on the three urea-peptide series.

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CONFORMATIONAL ANALYSIS OF TWO ANGIOTENSIN II ANTAGONISTS USING NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND MOLECULAR MODELING

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Angiotensin II (AII) is an octapeptide product of the renin enzymatic cascade and is responsible for the regulation of blood pressure and vascular volume. We recently reported the conformation of AII adopted in a phospholipid micelle environment as determined by NMR spectroscopy and molecular modeling (1). AII was found to assume a well-defined hairpin turn structure with its C- and N-termini approaching to within 7.6 Å of each other. A hydrophobic cluster formed by the side chains of Tyr^4, His^6 and Phe^8 was found on one side of the molecule. We have performed NMR and conformational analysis of the AII antagonists [Sar^1]-AII_{1-7} and [Sar^1,Val^5,Ala^8]-AII in a SDS micelle environment. For these two peptides a large number of intermolecular and intramolecular NOEs were observed. A combination of molecular dynamics, simulated annealing and energy minimization was used to determine the conformational preferences for these two molecules. For both peptides a well-defined β-turn structure between residues 2-5 was determined. The C-terminal segment of [Sar^1,Val^5,Ala^8]-AII diverged into two sets of conformations extending in different directions following the His^6 residue, whereas in [Sar^1]-AII_{1-7} the C-terminus was more flexible and less well defined, but also diverged in two directions following the Ile^5 residue. The β-turn structure in the N-terminal segment of the two antagonists and one set of conformations identified in their C-terminal regions showed close structural similarity with the lipid-bound conformation of AII. The existence of a common β-turn structure in the N-terminal segment of these two peptides as well as in AII appears to be a common feature of all angiotensin analogs and has been observed in several other experimentally derived structures of AII. It is possible that the antagonist behavior of the two peptides studied may be due to the increased structural flexibility and multiple conformations found in their C-terminal regions.

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$\beta^{2,2}$-, $\beta^{3,3}$-, AND $\beta^{2,2,3}$- PEPTIDES: SYNTHESIS AND UNEXPECTED CIRCULAR DICHROISM FOR SOME OF THEM

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In 1996 we have discovered that β-peptides, i.e. oligomers of β-amino acids, containing as few as six residues form suprisingly stable helices. Since then, three types of β-peptide helices and two turn motifs, an antiparallel and a parallel sheet structure, a hairpin motif, and tubular arrangements of cyclic β-peptides have been identified by circular dichroism (CD), 2D-NMR spectroscopy and X-ray crystal structure analysis.
We report now on the total synthesis and CD measurements of new β-peptides, which consist of up to six chiral residues, $\beta^{2,2}$-, $\beta^{3,3}$-disubstitued and $\beta^{2,2,3}$-trisubstituted. The new β-peptides should not be able to form any of the secondary structures known to date (models are showing that geminally disubstitued β-amino acids are helix- and pleated- sheet breaking residues in β-peptides). Inspired by research in our group dealing with the diastereoselective alkylation of β-heterosubstitueted carbonyl compounds via dianionic species, we developed a convenient method for the synthesis of chiral 2,2- and 3,3-disubstituded derivatives of 3-aminopropanic acid, starting from (*S*)-(-)-malic acid. The $\beta^{2,2,3}$ amino acids with side-chains of alanine, valine, and leucine were prepared from the corresponding α-amino acids by *Arndt-Eistert* homologation, followed by double α-methylations. By CD investigations of these peptides in MeOH solution, we obtained some interesting results. As expected, the CD pattern of the $\beta^{3,3}$-peptide shows a new type of pattern with a relatively strong *Cotton* effect (ellipticity $\theta = 6.5 \times 10^4$). But to our surprise, the CD spectrum of a $\beta^{2,2,3}$-peptide shows a pattern similar to that observed for a 3_{14}-helix. Ongoing NMR experiments will hopefully lead to elucidation of the structures.

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CONFORMATIONAL SEARCHING OF THE MAMMALIAN LHRH AND ITS FRAGMENTS BY MEANS OF ^{17}O KAI ^1H-NMR SPECTROSCOPY.

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Luteinizing hormone-releasing hormone (LHRH) stimulates the release of Luteinizing hormone (LH) and follicle stimulating hormone (FSH) from anterior pituitary. Up to date, at least ten different types of LHRH have been intendified from various species. The amino acid sequence of the human form was varified as: pyroglu-His-Trp-Ser-Tyr-Gly-Leu Arg-Pro-Gly-NH_2. This hypothalamic neuropeptide not only acts as a fertility but also as an antifertility drug. Furthemore, there have been good results in the treatment of some forms of cancer. Several conformational studies have been reported for mammalian LHRH with some contradicted results concerning the existence of the β-turn structure in the Try^5-Arg^8 fragment. On the other hand it is worth noting that whilst the first conformational studies (theoretical calculations and NMR) of mammalian LHRH were carried out immediately after its discovery, later the research activities were exclusively oriented towards the synthesis of as many as possible analogues. Very vecently the interest on the conformational studies was renewed. In this work we present the synthesis and the conformational analysis by means of ^{17}O and ^1H-NMR spectroscopy of a number of selectively ^{17}O enriched LHRH analogues and its fragments. Three residues (Tyr^5, Pro^9 and Gly^{10}) ^{17}O enriched in the carbonyl oxygen were investigated for their participation in intramolecular hydrogen bonding interactions. ^1H and ^{17}O-NMR conformational studies were performed in DMSO-d_6 solution. The ^{17}O chemical shift and line-width values as well as the ^1H-NMR data (chemical shifts, coupling constants, NOE effects e.t.c.) will be discussed in conformational terms.
(Grants from GGSRT).

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CONFORMATIONAL STUDY OF A TRYPTOPHANE CONTAINING TAT(1-9) ANALOGUE AS POTENT INHIBITOR OF DIPEPTIDYL PEPTIDASE IV

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N-terminal HIV-1 Tat peptides represent natural inhibitors of the cell surface ectopeptidase dipeptidyl peptidase IV (DP IV/CD26) mediating the immunosuppressive effect of the HIV-1 Tat protein via inhibition of DP IV, at least in part. Structure-activity studies based on systematic replacement of certain amino acids of the parent peptide Tat(1-9) (MDPVDPNIE) resulted in a series of nonapeptides displaying different inhibitory potencies on DP IV. Conformational studies based on NMR spectroscopy in conjunction with restrained molecular dynamics simulations could provide relevant information. The results of these studies revealed that the hydrophobic and bulky side chains of isoleucine in I^5-Tat(1-9) and of leucine in L^6-Tat(1-9) prevent optimal interaction with DP IV leading to the inactivity of these peptides. Interestingly, replacement of Asp in the second position led to analogues with considerably enhanced inhibition. The key compound turned out to be the Trp^2 analogue which was most potent in the DP IV inhibition assay. In this work we will present results of conformational analysis in water solution of W^2-Tat(1-9). This peptide was shown to exist in two equally populated conformations, one characterized by a *cis* amide bond between Trp^2 and Pro^3. Analogous to Tat(1-9), the second conformer of W^2-Tat(1-9) showed *all-trans* peptide bonds. The backbone atom superposition of the structural ensembles of both *cis* and *trans* W^2-Tat(1-9) and the parent peptide Tat(1-9) indicated remarkable similarities between them. The backbone along the residues Pro^3 to Pro^6 adopts in both cases a fairly rigid conformation. Obviously, hydrophobic interactions between the side chains of certain amino acids of the compounds are in great part responsible for the stabilization of the conformations in aqueous solution of Tat(1-9) as well as of W^2-Tat(1-9). The considerable enhancement of the inhibition capacity of the W^2-Tat(1-9) analogue can only be due to the presence of a tryptophane in the second position promoting proper interaction with DP IV or adopting a favourable local conformation of the entire peptide upon binding to DP IV.

(This work was supported by the Deutsche Forschungsgemeinschaft, SFB 387.)

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INTRAMOLECULAR SIGNAL TRANSDUCTION IN THE BRADYKININ B2 RECEPTOR

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PolyHis-tagged human bradykinin B_2 receptor (pHis-BKR) has been expressed and functionally characterized in our lab [Biol. Chem., Vol. 381, April 2000, in press]. The advantage of the tagged receptor consists in its easy purification and detection. Thus, the pHis-BKR can be easily purified using Ni-NTA columns and is suitable for both, immunoprecipitation and immunoblotting with anti-polyHis antibodies. The pHis-BKR displayed slightly modified pharmacological properties compared with the wild type receptor (WT-BKR). Thus, pHis-BKR seems to be an useful tool for studying the expression rate of mutants and their signal transduction in COS-7 cells.

To investigate the intramolecular signal transduction in the BKR certain mutants were obtained and expressed. The contribution of cytosolic loops to G-protein activation was studied with as well as single and cassette mutants. Simultaneously substitution of second and third loop by a sequence from the anionic transporter from erythrocytes prevents the signal transduction, demonstrating the importance of the second and third cytosolic loop. To examine the role of the sequence DRY in the second intracellular loop (highly conserved among GPCRs) we replaced D176 and R177 both by Ala. The role of cysteine residues C304 and C348 was checked by their replacement by serine. D315 and D407 were replaced by alanine to proof the hypothesis about the ion-tunnel-like interface for signal transduction within GPCRs. For all receptor mutants binding of different agonists and antagonists was correlated with intracellular signal transduction (inositol phosphate level, arachidonic acid release and MAP-kinase activation).

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CONFORMATIONAL ANALYSIS OF N-GLYCOPEPTIDES BUILT UP OF α-HELICAL PEPTIDES AND SUGAR RESIDUES CORRESPONDING TO N-GLYCANS' CORE-REGION.

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The chemical synthesis of structurally uniform N-glycopeptides is of great interest to understand the properties of natural N-glycoproteins and the conformational effect of the sugar residues on the peptide backbone in particular. An α-helical peptide sequence Ac-AEAAAKENAXKEAAAKA-NH_2 (X = A, S) was made accessible to N-glycosylation by incorporating β-allyl-protected aspartic acid and adjacent 2-hydroxy-4-methoxybenzyl(Hmb)-protected alanine within a solid phase synthesis protocol using Fmoc/t-Bu chemistry. After selective allyl-deprotection, the peptides were N-glycosylated with oligosaccharides of four different sizes corresponding to the core common to all N-linked oligo-saccharides. The glycosylation yield was 70% for the monosaccharide and 30% for the pentasaccharide. Conformational analysis of the unmodified and N-glycosylated peptides included CD- and NMR-spectroscopy to detect trans-formations from α-helical to β-turn structures. The CD-spectra of the N-glycopeptides showed only little deviation from the unmodified peptides. Only the NAS peptide glycosylated with the pentasaccharide showed an increase in random coil population according to CD. Corresponding to the results of CD-measurements the ^{1}H 1D NMR-spectra and ^{1}H 2D-NOESY-spectra at 600 MHz in phosphate buffer/pH 5.5 revealed a high helicity for the majority of the examined glycopeptides as determined by the chemical shift index (CSI), ^3JH_N-H_α coupling constants and intensities of sequential NOEs. Nevertheless, no characteristic i, i+3 NOEs were found except for the unglycosylated peptides. The glycopeptides with one or five sugar residues, respectively, showed a notable decrease of helicity in the N-terminal region; remarkably the glycopeptides containing the pentasaccharide core had two NMR signals for the N-terminal and some internal amino acid residues. Additionally capillary electrophoresis measurements in phosphate buffer/pH 8 showed that the glycopeptide containing the sequence NAS and the pentasaccharide core showed two peaks whose ratio was temperature dependent. This work reveals, that the global structure of model N-glycopeptides is sensitive to the number of N-linked sugar oligomers in combination with the kind of amino acid residues close to the glycosylation site.

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NMR Study on the Interaction Between MHC Class I Protein and Its Antigen Peptide

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Major histocompatibility complex (MHC) class I protein consists of heavy chain and light chain (B2m). MHC class I protein binds to the foreign peptide inside the cell and displays it on the cell surface. The class I complexis recognized by T-cell receptor (TCR) of cytotoxic T-cell, leading to lysis of the infected cell.

In this paper, we established the large scale expression system of MHC class I protein in E. coli. The folded protein was identified by sandwich type ELISA method.

We measured 1H NMR spectra of antigen peptide and MHC class I protein using Bruker DMX 750 instrument. The large line broadenings of the peptide signal were observed indicating the existence of the interaction between the peptide and protein. We could also observe the transfer NOE (TR-NOE) by measurement of 2D NOESY spectra, due to the complexation between the peptide and protein. The detailed results of these analysis of the interaction will be presented.

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Structural Studies

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SOLUTION CONFORMATIONS OF OSTEOGENIC FRAGMENTS OF PARATHYROID HORMONE: COMPARATIVE NMR STUDIES OF THE 1-31 PEPTIDE AND CYCLIC ANALOGUES

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The 1-31 peptide fragment of the human parathyroid hormone (hPTH) and a side-chain lactam derivative, [Leu^{27}]cyclo(Glu^{22}-Lys^{26})-hPTH-(1-31)NH_2, represent a new generation of recently-discovered anabolic/osteogenic agents. We have determined the solution structures of these osteogenic peptides at neutral pH and close to physiological conditions. The linear peptide assumes a relatively well-defined helix-turn-helix conformation. The N-terminal helix spans residues 3-11 and the C-terminal helix is defined by residues 16-30. Both helices contain well-structured capping motifs, including specific hydrophobic interactions. There are also a number of long-range NOE contacts which constrain the relative orientations of the N- and C-terminal helices. Side-chain cyclization in the C-terminal helical region was found to increase only the C-terminal helical population, leaving intact the global shape of the peptide. The conformational properties of these osteogenic peptides will be compared with those for longer hPTH fragments in order to determine whether conformational alterations may render the shorter hPTH-(1-31)NH_2 fragments as selective osteogenic agents. This work was greatly facilitated by the very-high-field NMR at 800 MHz, which is funded by the National Research Council of Canada, the parent institution of both the Biotechnology Research Institute and the Institute for Biological Sciences.

STRUCTURE-ACTIVITY RELATIONSHIP OF VIP/PACAP; N-TERMINAL STRUCTURE DEPENDENT RECEPTOR-BINDING SPECIFICITY AND BIOLOGICAL ACTIVITY ON PC-12 CELLS.

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VIP and PACAPs, naturally occurring neuro-polypeptides, are categorized into the glucagon-secretin family due to their structural similarities and biological activities. Both of VIP and PACAPs also share the same receptors, named PACAP/VIP receptors, which were classified into mainly three subtypes, "PAC 1", "VPAC 1" and "VPAC 2". VIP and PACAPs had been also observed significant differences among themselves at the binding assay with respect to "PAC 1". Therefore, we examined their structures in the phase of liquid on the basis of NMR measurements, log P values and CD spectrum of their derivatives in order to confirm the different factors between themselves. In addition, we investigated the effect of their N-terminal modified derivatives on rat pheochromocytoma cells (PC-12 cells). In the neurite outgrowth examination, N-terminal modified VIP (PACAP-like) still kept the effect at even low concentration (10^{-10} M). On the contrary, N-terminal modified PACAP 27 (VIP-like) reduced their activity as compared with native PACAP 27. These phenomena were also confirmed in the inhibitory effect of cell growth at relatively high concentration (10^{-7} M). The results clearly suggested that the amino acid residues at position 4 and 5 in VIP/PACAP had an important physicochemical role in their activities and even specification.

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CONFORMATIONAL AND BIOLOGICAL STUDY OF THE TYROSINE PHOSPHORYLATION SITES OF THE NICOTINIC ACETYLCHOLINE RECEPTOR (ACHR) β-SUBUNIT.

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Phosphorylation of tyrosine affects several functions of the AChR receptor. It has been also shown that mAbs against these phosphorylation sites efficiently inhibit the opening of the ion channel, suggesting that these sites are capable of controlling the channel. Binding experiments of mAbs to the AChR, in the presence of the phosphorylated and unphosphorylated peptides, revealed significant inhibition ranged from 80 to100% (peptide concentrations from 0.005 to 0.1μg). With the aim to investigate the structural and functional role of the β Tyr 355 phosphorylation site, four peptides corresponding to the phosphorylated and unphosphorylated Torpedo (ANDEY(PO_3H_2)FIRKPAG) and Human (GTDEY(PO_3H_2)FIRKPPSG) sequence β 353-364 have been examined in their free forms in DMSO, and bound to the mAb 148 in water by NMR spectroscopy and restrained MD calculations.

Due to the presence of the KPP sequence, the free unphosphorylated peptide experiences an equilibrium between one trans and one cis isomer in 80/20 ratio. A β-folded structure is observed for the YFIR sequence. In the presence of mAb148, the cis-trans ratio increases up to 50/50. The two isomers are equally recognized and bind stronger to mAb148 than the phosphorylated one. The TR-NOESY data suggest that both antibody-bound isomers adopt an extended structure. On the contrary, the phosphorylated peptide adopts a compact folded structure in the presence of mAb 148 with the formation of two adjacent type I β-turns involving respectively the DEYF and the YFIR sequences. Only the all-trans conformation of the peptide is observed. These conformational findings correlate well with the inhibition experiments. The in depth investigation of the AChR structure will facilitate the understanding of the structural basis of the ion channel function.

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Conformational variability of the synthetic peptide homologous to residues 129-141 of the mouse prion protein

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4 Laboratorium voor Eitwitbiochemie en Eitwitengineering, Universiteit Gent, K.L. Ledeganckstraat, B-9000 Gent, Belgium.

The effect of solution conditions on the conformation of the peptide corresponding to residues 129-141 of the mouse prion protein has been examined by experimental and theoretical tools including circular dichroism, secondary structure predictions, and Molecular Dynamics simulations.

The conformational properties of the peptide observed by CD confirm the prediction results: the peptide is chiefly random coil in water. We show however that the peptide samples hairpin conformations in one of several ~1-ns Molecular Dynamics simulations in water. Interestingly the analysis of the CD spectra obtained in this study suggests the presence of β-structure, which given the length of the peptide can only consist in β-hairpin.

The peptide can also be induced to form a modest percentage of helical structure in the presence of organic cosolvents such as trifluoroethanol, or detergents such as sodium dodecyl sulfate and lysophosphatidylcholine. This result is different from that obtained for a homologous hamster fragment, which differs from the mouse sequence by the single substitution of Ile 139 to Met. Interestingly, this substitution is crucial for the barrier in the transmission of the prion disease between hamsters and mice.

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CONFORMATIONAL PREFERNCE OF AZAAMINO ACIDS: For-AzGly-NH_2

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Azaamino acids are formed by the replacement of a α-carbon of amino acids with a nitrogen atom. Since the N-N bond (ϕ) of these peptidomimetics is between amide and urea in the peptide structure and the N-C(O) bond (ψ) has partial double bond character, the azaamino acid has been thought to have unique conformational characteristics. To identify conformational preference of azaamino acids in peptides, we have carried out *ab initio* studies of For-AzGly-NH_2 which are the simplest model that bear the structural characteristics of the amino acids, have provided information regarding the accessible conformation of the azaglycine in long peptides.

To obtain preferred conformations, we use IRC methods (HF/3-21G*) and the resulting conformers were fully optimized using 6-31G*, 6-31G**,..... Then hydrogen bonding and lone pair interactions are precisely analyzed and thus we can explain stability of each conformer and preference of torsion angles.

We believe that these results will be highly useful for the design of constrained peptidomimetics in drug discovery and nanotechnology (peptide engineering).

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OVEREXPRESSION AND STRUCTURAL STUDY OF THE PRO-SEQUENCE OF PROTEGRIN-3, A CATHELICIDIN MOTIF

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Protegrins are a family of five short antibacterial peptides (16-18 residues) characterised by their high content in cysteine and arginine residues and which structure consists of a ß-sheet stabilised by two disulphide bridges. Protegrins are initially produced as an inactive precursor protein of 149 residues which consists of a peptide signal (1-28), a pro-sequence (ProS) of 101 residues (29-130) and of the protegrin sequence (131-148). The active protegrin is released by cleavage of the $V^{130}R^{131}$ amide bond by an elastase-like enzyme. Interestingly, very similar pro-sequences are encountered for more than 15 antibacterial peptides of unrelated structures and such a pro-sequence is referred to as cathelicidin motif.

The three-dimensional structure of this wide spread motif which contains 4 conserved cysteine residues engaged in 2 disulphides bonds is not yet known. However, despite a low sequence similarity (15%) a structure close to that of the cystatin was hypothesised. In order to determine its three-dimensional structure, ProS of protegrin-3 was overexpressed in *E. coli* by using the Tag-His/thrombin site/ProS construct. This protein was purified on nickel column and after thrombin cleavage ProS was obtained and characterised by mass spectroscopy. By using this strategy unlabelled and uniformly 15N labelled ProS was obtained on the 10 mg scale and its structural study was undertaken by using CD, IRFT and NMR spectroscopies. The full assignment of the 2D homonuclear and 3D heteronuclear spectra is in progress. Together, preliminary results are indicative that the ProS three-dimensional structure roughly consists of 75% of beta strand and 25 % of alpha helix, which is in agreement with secondary structure predictions.

The knowledge of the three dimensional structure of the widely encountered cathelicidin motif would be helpful to understand the interactions between the ProS and protegrin-3 that are responsible for the inactivation of protegrin-3. Such interactions could be further used in the design of small antibacterial peptides of therapeutic interest.

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AMIDE SURROGATES FROM α-AMINOXY ACIDS. THE ψ[CO-NH-O], ψ[CR=N-O] AND ψ[CH_2-NH-O] LINKS

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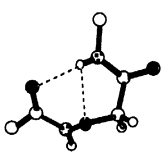
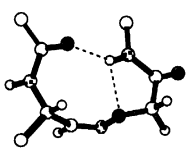
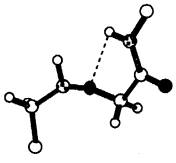
The α-aminoxy acids NH_2-O-CHR-CO_2H are easily obtained as pure enantiomers from the α-amino acids, and can be N-coupled to a peptide carboxyl to give the ψ[CO-NH-O] amidoxy link, or to a peptide aldehyde to give the ψ[CH=N-O] aldoxime link. The latter may be further reduced into the ψ[CH_2-NH-O] reduced amidoxy link. IR, NMR and X-ray diffraction experiments on model dipeptides show that the ψ[CO-NH-O] link gives rise to a γ-like turn, a very stable folded structure with a bifurcate hydrogen bond closing an 8-membered pseudocycle. The ψ[CH=N-O] link essentially occurs in the *trans* conformation when acidic conditions are strictly avoided after oxime formation, and induces extended conformations. The *cis* ratio increases up to 70% upon acidic treatment, and this form stabilizes a β-like turn with a bifurcate hydrogen bond closing an 11-membered cycle. Reduction of the ψ[CH=N-O] imine bond with $NaBH_3CN$ gives the ψ[CH_2-NH-O] link which retains the NH to O hydrogen bond closing a 5-membered cycle.



ψ[CO-NH-O]



cis-ψ[CH=N-O]



ψ[CH_2-NH-O]

The ψ[CR=N-O] ketoxime link (R = Me, nPr) has been prepared by treatment of the mixed anhydride with a Grignard reagent, and coupling of the aminoxy terminus on the resulting ketone. It experiences the same *trans*/*cis* isomerization in acidic conditions as the aldoxime one, and exhibits quite similar conformational properties.

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INTRAMOLECULAR CIDEP EFFECTS IN PEPTIDES

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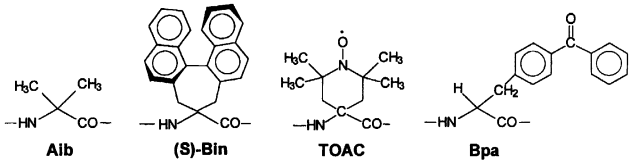
Two hexapeptides [Boc-Bpa-Aib-Ala-TOAC-$(Ala)_2$-O*t*Bu and Boc-(S)Bin-Ala-Aib-TOAC-$(Ala)_2$-O*t*Bu], each bearing one photoactive α-amino acid (Bin or Bpa) and one nitroxide-containing TOAC residue, have been synthesized and fully characterized. FT-IR absorption measurements indicate that a 3_{10}-helical conformation is adopted by these peptides in a structure-supporting solvent. As two amino acid units separate the photoactive residue from TOAC in the peptide sequences, the two moieties face each other at a distance of about 6 Å after one complete turn of the ternary helix.



Irradiation by a light pulse from an excimer laser populates the excited states localized on the chromophores. An intramolecular interaction between the singlet (Bin) or triplet (Bin and Bpa) excited states and the doublet state of the TOAC nitroxide makes a spin-selective decay pathway possible that produces transient spin polarization. In addition, in order to determine whether the intramolecular exchange interaction occurs through-bond or through-space, we have prepared linear and cyclic TOAC-Bin dipeptide units. A CIDEP (chemically induced dynamic electron polarization) study revealed that a through-space intramolecular interaction is operative. The observation of spin polarization makes the two helical hexapeptides suitable models to test the possibility of application of this novel technique to conformational studies of peptides in solution.

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ARRAYS OF SYNTHETIC WW PROTEIN DOMAINS

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Here we report on the application of the SPOT-Technique to construct arrays of synthetic WW domains which were then employed for the examination of this structural motif. WW domains are of about 40aa length and contain a three-stranded anti-parallel beta-sheet. They bind short proline-rich peptides, are involved in a variety of cellular processes and have been found to play a role in many human diseases. We constructed arrays with hundreds of WW variants, including natural and non-natural amino acid substitutions. The arrays were then screened for binding to a variety of peptide targets. In one case we constructed a complete substitutional analysis of the hYAP WW domain and screened it with the natural binding peptide GTPPPPYTVG (WBP1). The binding pattern is in agreement with results from other investigators obtained by site-directed mutagenesis, X-ray and NMR. The degree of tolerance towards amino acid substitutions at every position in the WW sequence allows the identification of unstructured regions and highly structured regions e.g. the beta-sheet. Furthermore, key residues and patterns of non-allowed substitutions can be seen. The results obtained by the array assay were verified applying BIAcore measurements with a selected subset of sequences which we synthesized and purified with traditional solid-phase peptide synthesis methods.

PROLYL–AROMATIC AND AROMATIC–PROLYL LOCAL INTERACTIONS IN POLYPEPTIDES

Gergely Tóth, Richard. F. Murphy, Sándor Lovas, Biomedical Sciences Department, Creighton University, Omaha, NE, USA

The weakly polar interactions between the side–chain aromatic rings and hydrogens of backbone amides (Ar–HN) and of CH_n (CH–π) in polypeptides are known to further stabilize secondary structures and to form local structures. Such local structures in polypeptide fragments containing Zaa–Pro or Pro–Zaa where Zaa is Phe, Tyr or Trp were characterized in this study. Thus, 560 non–redundant proteins from the Brookheaven Protein Database, with less than 25% similarity, were searched for CH–π and Ar–HN interactions in Xaa–Zaa–Pro–Yaa and Xaa–Pro–Zaa–Yaa sequences where Xaa and Yaa could be any residue. In Zaa–Pro fragments, the relative frequency of CH–π interactions and Ar–HN interactions, respectively, was 29.29% and 9.29%, while in Pro–Zaa fragments 23.25% and 3.83%. 4.71% of the Pro–Zaa fragments contained both interactions, while no Zaa –Pro fragments had both. The protein fragments containing Ar–HN and/or CH–π interaction were clustered on the basis of similarity of selected torsion angles. The analyses of the clusters revealed that the conformations of protein fragments containing Ar–HN interactions did not depend on the amino acid sequence but on the χ^1_{Zaa} and ϕ_{Zaa} torsion angles. The conformation of Pro–Zaa fragments containing either CH–π or both CH–π and Ar–HN interactions resulted in 8 and 6 clusters, respectively. These clusters were defined by torsion angles χ^1_{Zaa}, ϕ_{Zaa}, $\omega_{Zaa-Pro}$ and ψ_{Pro}. Zaa–Pro fragments with CH–π interactions yielded 4 clusters, the conformation of which depended only on ψ^1_{Zaa}, ϕ_{Zaa}, $\omega_{Zaa-Pro}$ torsion angles. The occurrence of Ar–HN and/or CH–π interactions almost doubled in turns and bends but was much less frequent in random coil and β–sheet structures compared to fragments without any Ar–HN or CH–π interactions.

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CHARACTERIZATION OF THE CONFORMATIONS OF POLY–PEPTIDES CONTAINING AROMATIC(i)–AMIDE(i+1, i+2) INTERACTIONS

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Weakly polar interactions between the side–chain aromatic rings and hydrogens of backbone amides (Ar–HN) may be found in different types of protein secondary structures. No unique conformations, which could be responsible for the formation of Ar–HN interactions, have been identified. In the search for such conformations, four 4 ns molecular dynamics (MD) simulations were performed using three different low energy conformations from simulated annealing [1] and one extended conformation of the model tripeptide Ac–Phe–Gly–Gly–NH–CH_3 as starting structures. The trajectories were generated with the GROMACS 1.6 package using SPC/E water. The Ar(i)–HN(i+1) interactions were four times more frequent than were Ar(i)–HN(i+2) interactions. Half of the conformations with Ar(i)–HN(i+2) interactions also contained an Ar(i)–HN(i+1) interaction. The solvent access surface area (SASA) of Phe side–chains and of the amide groups of Gly^2 and Gly^3 involved in Ar–HN interactions was significantly smaller than in residues not involved in such interactions. The number of hydrogen bonds between the solvent and Gly^2 and Gly^3 amide groups was 20% lower in conformations with Ar–HN interactions. The decrease in SASA and in the number of hydrogen bonds further supports the assumption that Ar–HN interactions stabilize folded structures of polypeptides. For each trajectory, structures which contained either Ar(i)–HN(i+1), Ar(i)–HN(i+2) or both interactions were clustered on the basis of similarity of selected torsion angles. The most representative conformations from the largest clusters matched well with the conformations of Phe–Gly–Gly protein fragments containing Ar–HN interactions from the Protein Brookheaven Database. Furthermore, the conformation of the Zaa–Xaa and Zaa–Xaa–Yaa (Zaa = Phe, Tyr or Trp; Xaa or Yaa = any residue) protein fragments containing Ar–HN interactions can be described by the clusters identified from MD simulations. Thus, in polypeptides Ar(i)–HN(i+1) interactions can be characterized by two pairs of χ^1_{Zaa}, ψ_{Zaa} torsion angle regions and Ar(i)–HN(i+2) interactions by three different sets of χ^1_{Zaa}, ψ_{Zaa}, ϕ_{Xaa}, ψ_{Xaa}, torsion angle regions in which two sets also include the conformations of Ar(i)–(i+1 and i+2) interactions.

1. G. Tóth, R.F. Murphy, S. Lovas, *Internet Journal of Chemistry*, **1999**, 2. 5.

A SLOW RATE EXCHANGE PROCESS ALLOWS THE DETECTION OF BOTH CARBONYL (C=O) AND HYDROXYL (–OH) ^{17}O NUCLEAR MAGNETIC RESONANCES OF THE CARBOXYLIC GROUP.

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It is well known that, due to the tautomerism of the carboxylic group, which is a very fast exchange process in the NMR time scale, only a single resonance is detected in the ^{17}O NMR spectrum for both oxygens (–C=O, –OH) in any carboxylic acid. On the contrary, both frequencies of the –OH stretching (3000-3400 cm^{-1}) and the –C=O stretching (1700-1750 cm^{-1}), in the IR spectrum, are rather easily detected. This means that the exchange rate is very slow in the IR time scale and becomes even slower when the –OH group is hydrogen bonded. In this study, we now report, for the first time, on the detection of both the carbonyl (–C=O) and the hydroxyl (–OH) ^{17}O nuclear magnetic resonances of the carboxylic group of a Tyr derivative by ^{17}O-NMR spectroscopy in DMSO-d_6 solutions. Two resonances are observed for the carboxylic oxygens: (i) the carbonyl resonance at 340 ppm and (ii) the hydroxyl resonance at 175 ppm. These resonance values are similar to the carbonyl oxygen of the amide and the hydroxyl oxygen of the alcohols, respectively. The origine of this exciting finding is attributed to the participation of the carboxylic hydrogen in an intramolecular hydrogen bonded interaction.

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TWO DIMENSIONAL GRADIENT ENHANCED CONFORMATIONAL ANALYSIS OF ANGIOTENSIN ANALOGUES BY THE USE OF NMR SPECTROSCOPY

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Conformational aspects of the pressor hormone angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, AII) and four of its analogues ([Cys(S-tB)^5]AII, [Sar^1,Cys(S-tB)^5]AII, [Cys(S-tB)^5,Leu^8]AII and [Sar^1,Cys(S-tB)^5,Leu^8]AII) have been investigated by the use of Nuclear Overhauser effect (NOE) gradient enhanced NMR spectroscopy. 2D ^{1}H,^{1}H NOESY experiments demonstrate significant NOEs between TyrH^{3,5}-HisHα, HisHα-PheNH, TyrNH-CysNH, CysNH-HisNH, and TyrHα-CysNH. These results indicate the presence of folded conformations of angiotensin II in aqueous solution. The important characteristic of the backbone and side chain conformation of the hormone and its analogues is the proximity of the side chain aromatic rings (formation of a ring clustering), the potential formation of a turn centered at Pro^7 and a βII-turn (Val^3-Tyr^4-Cys^5-His^6) which seems to be a motif among the five residues. This finding of proximity of the histidine, phenylalanine and tyrosine side chains might be relevant to the receptor-bound conformation of angiotensin II. The X-ray structure of the angiotensin II-Fab complex indicates many common characteristics with the observed secondary structural elements of angiotensin II and its analogues.

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NMR STRUTURES OF THE NOVEL CONOTOXIN EVIA FROM *CONUS ERMINEUS*

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Conotoxin-EVIA, a novel peptide from *Conus ermineus* venom preferentially acts on neuronal voltage-dependant sodium channels. It consists of 32 residues including six cysteines (DDCIKOYGFCSLPILKNGLCCSGACVGVCADL). This toxin, which is used by *Conus ermineus* for rapid immobilization of the prey, induces seizures on mice and tetanic paralysis in fish. Electrophysiological data lead us to conclude that this toxin is an excitotoxin in vertebrate systems which preferentially targets neuronal voltage-sensitive sodium channels. Synthetic EVIA was obtained in a scale of about 10-20mg by solid phase peptide synthesis using Fmoc-chemistry and behaved biologically as the natural conotoxin.

NMR data were recorded for a 2 mM aqueous solution at ^{1}H = 500 MHz, 10°C and pH = 3. Conotoxin EVIA is characterized by two slow exchanging conformers on the NMR chemical shift time scale due to a *cis*-Proline 13 / *trans*-Proline 13 equilibrium in a *ca* 1:1 ratio. The NMR structure of both conformers of conotoxin EVIA will be presented.

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SOLUTION STRUCTURE OF OLIGOPEPTIDES - COPPER(II) COMPLEXES BY CIRCULAR DICHROISM SPECTROSCOPY

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Our earlier combined potentiometric and spectroscopic studies on the solution equlibria of the copper(II) ion and various hexapeptide containing systems revealed that CD measurements in the copper(II) *d-d* transition region are useful for the determination of the coordinating donor groups of the ligand molecules[1]. It suggested us to try to perform these measurements in the systems containing copper(II) and glycine-L-alanine tripeptides of various sequences, like Gly-Gly-Ala, Gly-Ala-Gly, Ala-Gly-Gly and Ala-Ala-Ala. On the basis of potentiometric and spectrophotometric titrations performed at the same time in the same solution we calculated the molar CD for the individual complex species, which did not necessarily exist separately and in 100% in solutions. These results allowed us to assign the CD spectra to two types of the species with the compositions of $CuLH_{-1}$ and $CuLH_{-2}$ 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. 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 chromphore and the stability of the metal complexes, and are additive: the sum of the CD spectra of the Gly-Gly-Ala, Gly-Ala-Gly and Ala-Gly-Gly complexes equals to the spectra of Ala-Ala-Ala complexes.

After this first step with the series of glycine-L-alanine peptides (no or non-coordinating side chains) further investigations are planned with more complex systems. Glycine, L-alanine and L-histidine will be combined in the tripeptide sequences, since L-histidine moiety plays an exceptional role from the coordination chemistry point of view of peptides and proteins, which is due to the ability of the imidazol nitrogen(s) in the amino acid side chain, to serve as a primary binding site - an anchor - for most metal ions occurring in biological systems. Such kind of systematic studies will lead us to more detailed information of the binding mode of the ligands to the metal ions either in the small molecular model compounds or in the active centres of a number of metalloproteins with different physiological activities.

1. B. Gyurcsik, I. Vosekalna and E. Larsen, *Acta Chem. Scand.*, **51**, 49-58 (1997).

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ANTIAMOEBIN – STRUCTURE AND FUNCTION STUDIES ON A PEPTAIBOL MEMBRANE CHANNEL

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The peptaibols (polypeptides which contain a large number of Aib residues and a C-terminal alcohol group) generally act as antibiotics as a result of their membrane-modifying properties. We have determined the crystal structure of one peptaibol, antiamoebin (AAM) (Snook & Wallace, *Acta Cryst.* D55:1539-1545, 1999), and shown it to be helical with a significant bend in the middle. In addition, our NMR studies (Galbraith et al, in prep) have shown that the structures of AAM in solution and in crystals are very similar. Circular dichroism studies (Snook et al, *Structure* 6:783-792, 1998) have demonstrated that the AAM structure in the crystal is essentially identical to its structure in phospholipid vesicles. As a result of these and functional studies which indicate that the active membrane-channel forming complex may be an octomer (Duclohier, Snook & Wallace, *BBA* 1415:255-260,1998), we have used molecular modelling techniques to produce a proposed structure for the membrane channel form (Wallace et al, *16th APS Symposium Proc.*, 2000). Furthermore, a database of the known peptaibol sequences and structures has been compiled (http://www.cryst.bbk.ac.uk/peptaibol), and provides the basis for homology modelling of other peptaibols (Chugh & Wallace, in prep) and detailed nformation on a series of related molecules from which functional roles of various residues can be deduced. This combination of structural and functional studies has therefore indicated that the simple peptaibol molecules are good naturally-occurring model systems for studying the structure and function of ion channels.

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The comparative conformational study of the chemotactic peptide For-Met-Leu-Phe-OMe and its analogues incorporating α-α disubstituted amino acids at position 2.

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The tripeptides Formyl-Methionyl-X-Phenylalanyl-OMe (with X is respectively the α-α disubstituted amino acid α-aminoisobutyric Aib, The amino acid 1-cyclopentanecarboxylic Acc5, and 1-cyclohexanecarboxylic Acc6), are active analogues of chemotactic peptide Formyl-Methionyl-Leucyl-Phenylalanyl-OMe (FMLPOMe), known by it ability to induce release lysosomal enzyme. The Acc6 analogue is largely more active than the parent peptide FMLPOMe, whereas Aib and Acc5 analogues are less active. The comparative conformational study of these peptides by the method of exploring of conformational hypersurfaces PEPSEA shows a flexible structure for the parent peptide, and a tendency to the β turn structure for the three other conformationally constrained peptides. The difference in activity between the Acc6, Acc5, and Aib suggests a significant role of amid NH group at position 2 of the backbone in the interaction with the receptor.

Key words: Chemotactic peptide, Conformation, α-α disubstituted amino acid, Intramolecular hydrogen bond.

STRUCTURAL BEHAVIOUR OF BETA-AMYLOID FRAGMENTS

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Alzheimer's disease (AD) is the most common neurodegenerative disease in elderly people. One of the principal characteristic of these disease is the occurrence of senile plaques that consist largely of 39-43 amino acids peptide called Aβ or βA4 derived by proteolytyic cleavage from amyloid precursor protein (APP).
Similar deposits have been detected in aged monkeys, dogs but rarely have been found in rats and mice. The sequence of Aβ of mice is about 96% similar to the human Aβ.
The amino acid sequences of human and mice Aβ(1-43) peptide are as follows:
Human: DAEFR^{5}HDSGY^{10}EVH^{13}HQKLVFFAEDVGSNKGAIIGLMVGGVVIAT^{43}
Mice: -------G----------F-------R----------
The synthetic Aβ can fold as a random coil, α-helical and β-sheet structure in solution. The α-helix conformation is very soluble, whereas the β-sheet conformation is oligomeric and neurotoxic and eventually precipitates as an amyloid.
We synthesised different fragments of human and mice Aβ (fragments of N-terminal part like: 1-6, 1-10, 1-16, 1-20 and fragments of C-terminal part like: 21-28, 31-40) and used the circular dichroism (CD) for studying formation secondary structures by these peptides in environmental variables (solvent: water, trifluoroethanol; pH, temperature, chalotropic agents: urea, guanidine hydrochloride) and effect of the aging on the CD spectra. Based on CD evidence we located a region responsible for a conformational transition and we compared human and mice Aβ peptides conformation behaviour. Additionally, we would present our preliminary studies of influence of different metal cations (Cu^{2+}, Zn^{2+}) for conformational stability of Aβ fragments.

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SYNTHESIS, MOLECULAR STRUCTURE AND CONFORMATION STUDY OF DIPEPTIDES CONTAINING PIPECOLIC ACID OR ITS α-AZA-ANALOGUE

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Despite the obvious importance of pipecolic acid in natural products and a variety of biopeptides, surprisingly, in contrast to Pro (AzPro) derivatives, little is known about its conformational behaviour. On the basis of the minimal dipeptide backbone R-CO-Xaa-Yaa-NHR' able to accomodate a β-turn with Xaa(i+1) and Yaa(i+2) as corner residues, we studied the propensity of the pipecolic residue Pip and its α-aza-analogue AzPip to favour β-turn formation while Ala, the simplest coded chiral residue, was selected as the other residue in the dipeptide sequence. We looked at the influence of both the LL or DL chiral sequences and the i+1 or i+2 position of Pip (AzPip) on the occurrence of a β-turn folded structure.
First, the piperidine ring, when present in the dipeptide sequences, is characterized by a *chair* conformation with the 2-secondary amide function being *axial* (H^{α}*equatorial*) as the result of the pseudoallylic strain which tends to minimize the steric conflicts between the 1- and 2-substituents by choosing the 1,2 diaxial over diequatorial orientation.
When Pip occupies the i+1 position, whatever the homo LL or hetero DL chiral sequence and the chemical nature of R= tBu, OtBu and R'= Me, iPr in RCO-Pip-Ala-NHR', a unique population of unstructured conformers is observed in solution (DCM, DMSO). In that case, the pseudoallylic constraint prevails for the piperidine ring by imposing a φ angle value (≈±120°) which precludes any β-turn formation regardless to the *cis* or *trans* isomerization of the N-terminal amide group (Piv, Boc).
When Pip is in i+2, the conformational preferences are highly dependent on the chiral sequence. For the homochiral Boc-L-Ala-L-Pip-NHR', two rather equal populations (45/55) of conformers according to the *cis/trans* disposition of their middle tertiary amide function are observed either in DCM or DMSO. The entire population of *cis*-conformers is folded according to a βVI-turn while all *trans*-conformers are unfolded. Most of the conformers (80%) with the heterochiral sequence Boc-D-Ala-L-Pip-NHR' have a *trans* middle amide function and about 65% of them are β-folded. The rest of the conformers (20%) with a *cis* middle amide function are unstructured.
When substituting AzPip for Pip, RCO-AzPip-Ala-NHiPr (R= tBu or OtBu) is characterized by two populations of stereoisomers in DMSO, whereas only one is observed for the Boc-Ala-AzPip-NHiPr pseudodipeptide. In this latter case, nearly all of the conformers are βVI-folded as encountered in their crystal molecular structure.

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CONFORMATIONAL STUDIES OF THE ALZHEIMER AMYLOID Aβ(1–16) PEPTIDE BY CD AND NMR SPECTROSCOPY

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Alzheimer disease is characterized by amyloid deposits within the brain tissue. A 4 kDa peptide, known as amyloid β-peptide (Aβ(1-42)), is the major component of amyloid plaques. Aβ(1-42) is generated by proteolytic cleavage of the amyloid precursor protein (APP). In brain, this peptide can fold either into a soluble monomeric form, with α-helix or random coil structure, or into a less soluble form with a β-sheet conformation, which easily aggregates and is predominantly present in amyloid deposits. The Aβ(1-16) peptide is the APP amino acid sequence within the cleavage sites of α- and β-secretases. As we hypothesized that it could be a key-peptide in the neurodegenerative processes, conformational studies of Aβ(1-16) were undertaken by CD and NMR spectroscopy. CD experiments showed a predominantly helical conformation of Aβ(1-16) in trifluoroethanol (TFE) / water mixtures 30:70 to 95:5, while a β-hairpin structure was suggested in phosphate buffer. The absence of peptide aggregation was verified in both media, for concentrations between 5 μM and 15 mM. Complete ^{1}H and ^{13}C NMR assignments were obtained in TFE-*d3*/H_2O (8:2) solution, from homonuclear COSY-DQF, TOCSY, and heteonuclear ^{1}H-^{13}C HSQC and HMBC experiments. Conformational NMR parameters, such as NOEs, $^{3}J_{NH C\alpha H}$ couplings, chemical shift deviations, isotopic NH/ND exchange rates and thermal coefficients of amide protons were obtained. The whole data, involving particularly the typical NOE pattern of dNN(i,i+1), dαN(i,i+n), with 1<n<4, and dαβ(i,i+3), argue for a helical conformation of Aβ(1-16) peptide in this membrane mimicking medium.