Posters: topic B8

P 140 Modecular Design: Experimental and Computational P 141

A 3D-QSAR STUDY OF THE BIOLOGICAL ACTIVITY OF δ-OPIOID ANTAGONIST

Ki-eun Kim,[1] Seonggu Ro,[2,*] and **Chang-Ju Yoon**,[1,*] from [1]Department of Chemistry, The Catholic University of Korea, Pucheon 420-743; [2]Biotech Research Institute, LG Chemical Ltd. / Research Park, Taejon 305-380, Korea.

The δ-opioid receptor antagonist H-Dmt-Tic-OH (2,6-dimethyl-L-tyrosyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) exhibits extraordinary δ-opioid receptor binding characteristics [Ki^{δ} = 0.022 nM; Ki^{μ}/Ki^{δ} = 150,000] and antagonism (pA_2 = 8.2; Ke = 5.7 nM). Their analogs have been studied by Salvadori et al. (J. Med. Chem. **1997**, *40*, 3100.). We have carried out 3D-QSAR studies of these anatagonists to identify 3D-arrangement of pharmacophores for their activity at the δ-opioid receptor using CATALYST program. The selected hypothesis show a nice correlation (r = 0.95) and can predict activities of other analogs which are not included in the training set.

Since the H-Dmt-Tic-OH analogs are highly constrained and thus, demonstrate clear conformational preferences, we have searched the best fitting conformers to the hypothesis to obtain the conformation required for the antagonistic activity at the δ-opioid receptor. In the resulting conformer, two aromatic rings are in a close proximity. Interestingly, the conformation is similar to the model suggested from the structural studies of cyclic peptide opioids that are highly active at the δ-receptor (Ro et al. *Peptide Science* 1995, 3, 157). These results indicate that agonists and anatagonists of δ-opioid receptor may adopt similar conformations when they bind to the receptor.

Additionally, we have carried out 3D-search using database including known δ-receptor selective aginists and antagonists. The selected hypotheses picked up most of the known compounds. Thus, we believe that our studies will be highly useful for the discovery of drugs related to the δ-opioid receptor.

MOLECULAR DYNAMICS SIMULATION OF BETA AMYLOID PEPTIDE FRAGMENTS IN WATER AND ORGANIC SOLVENTS

Gergo Kiss[a], Tamás Körtvélyesi[b], Botond Penke[a]
[a]Department of Medical Chemistry, University of Szeged, Hungary, [b]Department of Physical Chemistry, University of Szeged, Hungary

Deposition of the 39- to 42-amino-acid β-amyloid peptide (βA) in the brain of Alzheimer's disease patients is the most charasteristic feature of this dementia of the central nervous system. Because of the poor solubility of these peptides in water, in most cases they are studied in organic solvents such dimethyl-sulfoxide (DMSO) and trifluoro-ethanol (TFA). Computer simulations were done in these solvents and in water to find correlation between the measured and the theoretically calculated conformations in water.

In our work we performed molecular dynamics simulations on the 25-35 fragment of the βA peptide, which acts the same way in biological investigations, to see the conformational changes when moving from water to TFA or DMSO. The calculations were performed with the GROMACS 2.0 software package in explicit solvent models. Pure water, DMSO and TFA was used, an the 25%, 50% and 75% water mixture of the organic solvents. 1 ns trajectories were generated by a modified GROMOS87 and a GROMOS96 force fields by 2 fs integration steps at 300 K and 1 bar (NPT ensemble). PDB's 1AML NMR structure was used as a starting point for the simulations.

Preliminary results show correlation with measurements. Conformational changes in organic solvents are also suggestive.

P 142 P 143

COMPUTATIONAL STUDY ON CONFORMATION OF OLIGOPEPTIDES PREPARED FROM α,α-DISUBSTITUTED AMINO ACIDS

Masaaki Kurihara[a], Masakazu Tanaka[b], Makoto Oba[b], Hiroshi Suemune[b], and Naoki Miyata[a], [a]Division of Organic Chemistry, National Institute of Health Sciences, Tokyo 158-8501, Japan, [b]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Recently the conformation of peptides constituted by non-proteinogenic amino acids (β-amino acid, α,α-disubstituted amino acid, etc.) has received considerable attention. α,α-Disubstituted amino acids have two alkyl substituents at the α-position of normal α-amino acids and are conformationally restricted. To predict the conformation of these peptides presents an interesting challenge. We report the conformational analysis of oligopeptides prepared from α,α-disubstituted amino acids (isovaline, diethylglycine, butylethylglycine) by computational study. Conformational energy computations on oligopeptides of α,α-disubstituted amino acids were performed using molecular mechanics. Conformational search calculations were carried out by the Monte Carlo method of MacroModel® (ver. 6.5, Schrodinger, Inc.). When AMBER* was used as the force field, the global minimum energy conformations were found to be 3_{10}-helix. These results are in agreement with their conformational properties in the solid state determined by X-ray crystallographic analysis. In the case of the MMFF force field the global minimum energy conformations of diethylglycine and butylethylglycine peptides are planar structures, which are in agreement with their conformations in solution.

References

M. Tanaka, N. Imawaka, M. Kurihara, H. Suemune, *Helv. Chim. Acta.*, **82**, 485-510 (1999); M. Kurihara, M. Tanaka, N. Imawaka, H. Suemune, N. Miyata, *JCPE Journal*, **11**, 185-190 (1999)

THE STRUCTURAL REQUIREMENTS IN THE TURN STRUCTURE OF SIKVAV FOR BIOLOGICAL ACTIVITY

Sándor Lovas[a], Tamás Körtvélyesi[a,b], Hynda Kleinman[c], Ferenc Ötvös[a,d], Richard F. Murphy[a], [a]Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178, U.S.A.,[b]Department of Physical Chemistry, József Attila University, Szeged, Hungary, [c]National Institute of Dental Research, NIH, Bethesda, MD 21244, U.S.A.,[d]Biological Research Center, Szeged, Hungary

The SIKVAV fragment of the α1 chain of Laminin promotes neurite outgrowth, tumor growth and angiogenesis. To further characterize this peptide, two series of analogs were synthesized: each residue was substituted individually with L-Ala (1) or with its D-enantiomer (2). Structures of peptides were simulated in 2 ns Molecular Dynamics (MD) in the presence of SPC/E water using the GROMACS 1.6 package. Secondary structures of peptides in trajectories were determined by the DSSP method. The biological activity of peptides in promoting neurite outgrowth of PC12 cells was assessed.

The L-Ala replacement was accommodated only in position one; AIKVAV had the same biological activity as had the parent peptide. Removal of the side chains of the other residues had a detrimental effect on the biological activity. In the series with D-amino acid replacements, only analogs with D-Ser^1, D-Val^6 and D-Ala^5 had activity, albeit smaller than that of the SIKVAV.

After the start of each MD simulation from an extended conformation, structures of biologically active analogs folded to unique conformations with different degrees of β-turns and β-bends centered around residues 3 and 4. The different substitutions changed differently the β-turn/β-bend character of the structures. Some of the inactive analogs also had a high degree of β-bend conformation. For example, in SIKAAV K^3 and A^4, respectively, were in a β-bend for 73% and 77% of the time. These results suggest that not only the backbone fold but the side chains at positions 2 to 6 and the L configuration at positions 2, 3 and 4 are important for the biological activity.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

Posters: topic B8

P 144 Modecular Design: Experimental and Computational P 145

RATIONAL DESIGN OF INHIBITORS OF MAMMALIAN RIBONUCLEASE REDUCTASE

Dale F. Mierke,[a] Maria Pellegrini,[a] Sebastian Liehr,[b] Alison L. Fisher,[b] Paul B. Laub,[b] Barry S. Cooperman[b]

[a]Department of Molecular Pharmacology, Division of Biology and Medicine, Brown University, Providence, RI 02912; [b]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

Mammalian ribonuclease reductase (mRR) catalyses the radical desoxygenation of ribonucleotides to 2' deoxyribonucleotides, which is the rate-determining step in *de novo* DNA synthesis. As such, it is a potential target for cancer intervention. The enzyme is a dimer of two subunits R1 and R2, whose association is critical for the activity of the enzyme. This association of the subunits takes place via the C-terminal residues of mR2, and can be inhibited by peptides mimicking the C-terminal sequence of mR2. The linear heptapeptide Ac-Phe-Thr-Leu-Asp-Ala-Asp-Phe, corresponding to the seven amino acids at the mR2 C-terminus, was found to have the minimum length necessary for full inhibitory activity. Here we describe the structural features of the mRR-inhibitor Ac-Phe-cyclo[Glu-Leu-Asp-Lys]-Asp-Phe-OH while bound to the R1 subunit as determined by transferred NOEs.

Structural refinement employing an ensemble-based, full-relaxation matrix approach results in two structures varying in the conformations of Phe^1 and the cyclic lactam side chains of Glu^2 and Lys^5. The remainder of the molecule, both backbone and side chains, are extremely well-defined, with an RMSD of 0.54 Å. A comparison with the bound structure of the linear peptide, Ac-Phe-Thr-Leu-Asp-Ala-Asp-Phe-OH, provides unique insight into the requirements for binding to mRR, which will be critical for further inhibitor development.

MODELING OF SGCI, A SERINE PROTEASE INHIBITOR

Zoltán Mucsi[a], Árpád Bódi[b], László Gráf[b], András Perczel[b] András Patthy[c] and György Orosz[d]

[a]Department of Organic Chemistry and [b]Biochemistry, Eötvös University, Budapest, [c]Agricultural Biotechnology Centre, Gödöll_, [d]Research Group of Peptide Chemistry, Hung. Acad. Sci., Budapest, Hungary

SGCI, a recently discovered proteinase inhibitor form *Schistocerca gregaria*, is a member of the PMP-D2 type of canonical inhibitors. Modification of P_1 and P_1' positions of the chymotrypsin inhibitor yielded a derivative which exhibited strong trypsin inhibition.

Based on the available NMR structural information in the modeling of this compound we attempted to identify the minimal three-dimensional part of the inhibitor necessary for efficient binding.

As a minimal requirement, the close neighborhood of P_1 site was built into the structure with the two β-sheet parts found in the close neighborhood of the P_1 site. This compound was found to be a good substrate of chymotrypsin. After identification of the two main cleavage positions, one at the P_1 position and other is at a phenylalanine in a stabilizing β-sheet, the model compound was further modified to prevent the cleavage at the stabilizing β-sheet portion of the molecule.

Since the inhibitor activity of this modified fragment was still not high enough, a larger part of the molecule was synthesized including the P_4 position of the original inhibitor.

Convergent peptide synthetic details, the biological data of the compounds prepared will be presented.

P 146 P 147

QUANTUM MECHANICAL APPROACH TO DESCRIBE AND PREDICT FOLDING UNITS OF PEPTIDES AND PROTEINS

András Perczel

Department of Organic Chemistry; L. Eötvös University, 112 Budapest P.O.B.32, H-1518, HUNGARY, Telephone: 36-1-209-0555 Fax: 36-1-209-0602, E-mail: perczel@para.chem.elte.hu,

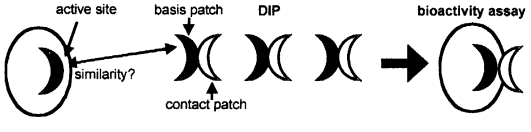
Backbone building units of the different folds observed in proteins are rather similar for most of the 20 natural amino acids. During the past several years a large number of quantum mechanical computations at different levels of theory have been carried out on several amino acid diamide derivatives (PCO–NHCHR–CONHQ). Major conformational properties of amino acid diamids could be denoted from the topological analysis of $E=E(\phi,\psi,\chi_1,\chi_2)$ hypersurface. Previous *ab initio* structure determinations have provide a handful information relevant to this topic by analysing the HCO-L-Xxx-NH_2 type models. Not surprisingly, the first model compounds studied were themselves the simplest residues; R =H [glycine], $-CH_3$ [alanine], $-CH(CH_3)_2$ [valine], $-CH_2SH$ [cysteine], $-CH_2OH$ [serine], $-CH(CH_3)OH$ [threonine], phenylalanine $-CH_2-C_6H_5$ etc. These studies attempted to describe many conformational and energetic properties of protein folding units. Interesting features of the structural building units of the right-handed helix and that of the poly proline II structures were revealed. The optimised molecular structures were compared with data taken from a non-homologous data set of X-ray determined proteins incorporating over 150000 amino acid residues. Selected conformers of longer models were also the subject of *ab initio* studies exploring some conformers of tri–, tetra–, penta–, hexa– and even hepta-amide systems (PCO–$[NHCHR–CO]_n$–NHQ where $2 \leq n \leq 6$). All these studies provided energetic and structural information to support spectroscopy (e.g. IR, NMR), diffraction studies, folding investigations and even protein structure predictions.

COMPUTER-AIDED IDENTIFICATION OF SHORT PEPTIDES BINDING TO PREDEFINED PROTEIN SURFACE PATCHES USING THE „DICTIONARY OF INTERFACES IN PROTEINS" AND SUBSEQUENT EVALUATION OF THEIR BIOACTIVITY

Ulrich Reineke[a], Robert Preißner[b], Andrean Goede[b], Lothar Germeroth[a], Jens Schneider-Mergener[a,c] and Cornelius Frömmel[b]

[a]Jerini Bio Tools GmbH, Rudower Chaussee 29, 12489 Berlin; Medizinische Fakultät der Humboldt-Universität (Charité), [b]Institut für Biochemie and [c]Institut für Medizinische Immunologie, Monbijoustraße 2A, 10117 Berlin, Germany

The identification of molecules binding to a predefined protein surface area and thereby inhibiting binding to the naturally occuring ligand is the first step in drug lead development. This can most successfully be achieved by high-throughput screening of small organic molecule libraries, scans of overlapping peptides derived from the ligand and/or receptor sequences or by computer algorithms evaluating virtual libraries. Here we present a novel method for the latter approach based on the „Dictionary of Interfaces in Proteins" (DIP) [1] which is a database derived from a representative selection of three-dimensional protein structures. These proteins are divided into secondary structural elements (SSEs) named basis patches and paired with their adjacent SSEs (contact patches) from the protein context. Subsequently, the predefined surface patch for which a ligand has to be identified is compared with the basis patches from DIP by a 3-D superposition procedure and ranked by matching parameters. The resulting contact patch in turn is expected to bind to the predefined surface patch of the protein for which a ligand has to be identified. The practicability of this approach is proven for the identification of inhibitors for protein kinase A (PKA) binding to the substrate binding site. Seven out of eight peptides selected by the described procedure inhibited PKA as shown in a phosphorylation assay, among them the shortest being tetrameric peptides.



[1]Preißner, R., Goede, A., Frömmel, C. *J. Mol. Biol.* **1998**, *280*, 535. [2]German patent application, *Deutsches Patent- und Markenamt* **2000**, DE198 31 758 A 1

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

Posters: topic B8

P 148 Modecular Design: Experimental and Computational P 149

STUDIES ON THE STRUCTURE-FUNCTION RELATIONSHIPS OF CYCLIC RGD PEPTIDES.

Athanassios Stavrakoudis, Democritos Tsoukatos, Maria Sakarellos-Daitsiotis, Constantinos Sakarellos and Vasillios Tsikaris.
Department of Chemistry, University of Ioannina, GR-45110, Ioannina, Greece

The inhibition of platelet aggregation and fibrinogen-receptor binding is the objective of many studies. Several RGD containing peptides exhibit anti-aggregatory properties. Numerous conformational studies using ^{1}H-NMR spectroscopy and theoretical calculations have been carried out on cyclic and linear RGD analogues in order to elucidate the conformation-activity relationships. In this work we investigate the stucture/activity relationships of several RGD peptide analogues. The peptides :

1) Ac-Arg-Cys-Asp-Cys-NH_2, 2) Ac-Arg-Cys-Asp-Cys-Arg-NH_2,

3) Ac-Asp-Cys-Arg-Cys-Asp-NH_2, 4) Pro-Arg-Cys-Asp-Cys-Lys-NH_2,

5) Pro-Ser-Lys-Cys-Asp-Cys-Arg-NH_2 6) Ac-Arg-Gly-D-Asp-NH_2

7) Ac-Arg-Gly-Asp-NH_2,

were studied with molecular dynamics in order to get information on conformational properties. Some of these peptides showed inhibitory activity in the micromolar scale. Peptide 7 was modeled for comparison reasons. Standard molecular dynamics trajectories of 2.2 ns length were produced using the Amber95 pameteres and analyzed with respect of their backbone and side chain geometry and energy. Solvation effects were modeled implicitly with the Ooi/Scheraga SASA method (Solvent Accessible Surface Area). The peptides' structures seem to be governed by electrostatic interactions between the side chain polar groups, although hydrogen bonding interactions between these groups generally not observed.

CONFORMATIONAL STUDY OF PEPTIDES CONTAINING α-ETHYLATED α,α-DISUBSTITUTED AMINO ACID: (*S*)-BUTYLETHYLGLYCINE

Masakazu Tanaka[a], Naoto Imawaka[a], Makoto Oba[a], Masaaki Kurihara[b], Hiroshi Suemune[a], [a]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan; [b]Division of Organic Chemistry, National Institute of Health Sciences, Tokyo 158-8501, Japan.

α,α-Disubsituted amino acids and their peptides have attracted considerable attention since these amino acids and peptides show unique biological activities and very stable secondary structures. The conformation of the peptides prepared from achiral α,α-disubstituted amino acids such as 2-aminobutyric acid: Aib, dialkylglycine has been studied extensively because the achiral α,α-disubstituted amino acids could be easily prepared. The property of Aib is known to be a 3_{10}-helical structure, and that of dialkylglycine such as diethylglycine and dipropylglycine is proved to be a fully planar C_5-conformation. Recent development of asymmetric reaction enables the peptide chemists to synthesize several chiral α,α-disubstituted amino acids, and the properties of α-methylated α,α-disubstituted amino acids are known to be 3_{10}-helical structure.
We studied the conformation of peptides prepared from a chiral α-ethylated α,α-disubstituted amino acid; (*S*)-*butylethylglycine* (= 2-*amino*-2-*ethylhexanoic acid*: Beg). The homopeptides containing (*S*)-Beg (up to hexapeptide) were prepared by using solution-phase methods employing an ethyl ester as the *C*-terminal protection and a trifluoroacetyl group as the *N*-terminal protection. The heteropentapeptides containing (*S*)-Beg as a guest molecule in the Aib sequence were also prepared in the similar manner. The conformations of these peptides in the solid state were studied using X-ray crystallographic analysis, and those in solution were studied using IR, ^{1}H NMR, and CD spectra. The dominant conformation of homopeptides containing (*S*)-Beg was a fully planar C_5-structure, and that of heteropeptides containing (*S*)-Beg and Aib was a 3_{10}-helical structure.

P 150 P 151

MOLECULAR MODELLING OF MEMBRANE PROTEINS AND RATIONAL DESIGN OF SELECTIVE K^+ CHANNEL BLOCKERS

Michael Thormann, Miquel Pons, Ernest Giralt
Departament de Química Orgànica, Universitat de Barcelona, Spain.

Membrane proteins, among them K^+ channels, are of outstanding biochemical, physiological, pharmacological, and medicinal importance. They play pivotal roles in the control of a variety of physiological processes of vertebrates and invertebrates.

Malfunction or derangement of K^+ channels are involved in a couple of disease states and can lead to death. Selective K^+ channel blockers are promising for example as antiarrhythmic agents or the treatment of type II diabetes.

Naturally occurring modulators are, for example, scorpion toxins, venom peptides of about 35 amino acids and known 3D-structure, that block K^+ flux through the channel by occluding the pore. The exhibit very distinct affinities and selectivities for different K^+ channel subtypes but they are too large for pharmacological applications. Currently applied small antagonists, on the other hand, lack specificity.

The first results of the rational drug design of small, highly selective K^+ channel blockers is described here following four main lines:
(1) Molecular modelling in explicit membrane environment of homolog K^+ channels based on the recent X-ray crystal structure of KcsA [1],
(2) Refinement of modelled K^+ channel structures using extensive scorpion toxin inhibition data,
(3) Docking of scorpion toxins and well-known small antagonists to K^+ channels to get detailed interaction maps of possible binding sites for rational design of small inhibitors with high affinity,
(4) Docking of entire structural databases to find unique binding sites on each channel subtype to tailor the small inhibitors for selectivity.

[1] D. A. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, R. MacKinnon; Science 280 (1998) 69.

RATIONAL ENGINEERING OF A CD4 MINI-PROTEIN WITH NATIVE-LIKE AFFINITY FOR GP120 AND INHIBITING HIV-1 ENTRY

Claudio Vita[1], Loïc Martin[1], Olivier Combe[1], André Ménez[1], Elodie Prieto[2], Christian Roumestand[2], Dorothée Missé[3], Arnaud Dupuy D'Angeac[3] & Francisco Veas[3]; [1]Département d'Ingénierie et d'Etudes des Protéines, CEA Saclay, 91190 Gif-sur-Yvette; [2]Centre de Biologie Structurale, Faculté de Pharmacie, 34060 Montpellier; [3]Laboratoire d'Immunologie Rétrovirale et Moléculaire, IRD/CNRS, 34094 Montpellier, France

Interaction of HIV-1 envelope glycoprotein gp120 with cellular CD4 represents the first step of viral entry into target cells, and involves a large (800 Å^2) and complex interaction surface. In spite of such difficulty, we have reproduced the core of the CD4 binding site in a 27 amino acid three-disulfide mini-protein system, functioning as structural scaffold. Mini-protein structural and functional mimicry was then improved by 100-fold, to a 0.4 μM affinity, by incorporating *five mutations, suggested by an NMR structural analysis and Ala-scanning* approach. The three-dimensional structure of the improved mini-CD4 was then solved by NMR, and revealed a close structural similarity between the backbone and side chains conformation of its putative binding site and that of CD4, explaining the effect of mutations introduced. Modeling the interaction of the mini-protein with gp120 structure suggested other mutations that, once incorporated, produced a further 100-fold increase in gp120 binding affinity to a 2 *nM* IC_{50}, identical to that shown by native sCD4. The optimized mini-protein efficiently inhibited HIV-1 infection of CD4+ cells. The present work demonstrates that stable natural mini-proteins can act as structural scaffolds to reproduce the core of protein-protein interaction surfaces, allowing further refinement of the introduced binding affinity by recursive cycles of structure-function analysis. Such engineered mini-proteins represent unique tools in biology, biotechnology and medical sciences, but may also represent fundamental steps facilitating the conversion of a protein functional epitope into a classical pharmaceutical.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

Posters: topic B9

P 152

Structural Studies

P 153

MODIFICATION OF THE CONFORMATIONAL EQUILIBRIUM OF THE N-TERMINAL LOOP OF APAMIN BY WEAK ELECTROSTATIC INTERACTIONS

André Aumelas[a], Dung Le-Nguyen[b], Léo Barry[b]
[a]Centre de Biochimie Structurale, UMR 5048, U414 INSERM, Université Montpellier 1, Faculté de Pharmacie, 15 avenue Charles Flahault 34060 Montpellier Cedex 02, France; [b]INSERM U 376, CHU Arnaud de Villeneuve, 371, rue du Doyen Gaston Giraud, 34295 Montpellier, Cedex 05 France

The solution structure of the bee venom neurotoxin apamin (18 residues and two disulphide bridges) has been determined at pH 2.0 by nuclear magnetic resonance and mainly consists of a beta-turn (residues 2-5) and of an alpha-helix (residues 9-17) *(Pease & Wemmer, Biochemistry (1988) 157, 269-274)*. It has been shown that the deprotonation of the Glu^7 side chain induces a dramatic downfield shift and an unexpected line broadening of the amide proton signals of Glu^7 and Asn^2, although no major structural change occur for the global structure of apamin. This downfield shift is partially reverse above pH 6 *(Dempsey, Biochemistry (1986) 25, 3904-3911)*.
In this communication, we will present NMR data of apamin as a function of pH and temperature showing that these two amide signals display a non linear temperature coefficient and that their line broadening was due to the conformational equilibrium of the 1-7 loop. Moreover, at 263 K, the Glu^7 and Asn^2 amide signals corresponding to two conformations in slow exchange were observed. Due to the close proximity of the α-amino group and of the Glu^7 carboxylate group, electrostatic interactions were hypothesised to occur between these two groups. In order to confirm this hypothesis, the Ac-apamin and the [Gln^7]-apamin analogues, in which the N-terminal positive charge and the Glu^7 negative charge were removed one by one, respectively, were synthesised. NMR measurements at different pH showed that Gln^7 and Asn^2 amide signals were insensitive to pH for the [Gln^7]-apamin analogue, whereas linear temperature coefficients were measured for the Glu^7 and Asn^2 amide signals for the N-terminal acetylated analogue. Based on the study of these two analogues, we concluded that electrostatic interactions involving the α-amino group and the side chain carboxylate group are able to slow down the conformational equilibrium of the 1-7 loop. Nevertheless, these localised weak electrostatic interactions do not interfere on the global structure of apamin.

SOLUTION STRUCTURE AND ACTIVITY OF THE SYNTHETIC MEDITERRANEAN MUSSEL DEFENSIN (MGD-1)

André Aumelas[a], Yin-Shan Yang[a], Guillaume Mitta[b], Alain Chavanieu[a], Jean-Frédéric Sanchez[a], Bernard Calas[a], Philippe Roch[b]
[a]Centre de Biochimie Structurale, UMR 5048, U414 INSERM, Université Montpellier 1, Faculté de Pharmacie, 15 avenue Charles Flahault 34060 Montpellier Cedex 02, France; [b]Défense et Résistance chez les Invertébrés Marins (DRIM), UMR 219 Université Montpellier 2, CC80 Place Eugène Bataillon 34095 Montpellier Cedex 5, France.

MGD-1 is an antibacterial peptide of 39 residues that was isolated from the edible mediterranean mussel *Mytilus Galloprovincialis (Hubert et al., Eur. J. Biochem. (1996) 240, 302-306)*. MGD-1 sequence is characterised by its high contain in cysteine (20.5%, four disulphide bridges), glycine (20.5%), arginine (12.8%) and by the presence of a hydroxylated tryptophane residue on the C2 position of the indole ring. The three-dimensional structure of MGD-1 was unknown. Based on the alignment with the arthropod defensin A, whose structure mainly consists of the Cystine-Stabilized alpha-beta motif (CSαβ motif) *(Cornet et al., Structure (1995) 3, 435-48)*, MGD-1 presents a sequence identity of 38%, including 5 out of the 8 cysteine residues. Therefore, it was hypothesised that MGD-1 structure could also contain the CSαβ motif frequently observed in scorpion toxins and plants defensins. In order to confirm this hypothetical structure, MGD-1 was synthesised by solid-phase synthesis with a non modified tryptophane residue. The antibacterial activity of the synthetic MGD-1 was similar to that of the native peptide. The MGD-1 three-dimensional solution structure as determined by NMR appeared highly constrained and mainly consists of the canonical CSαβ motif (C4-C25, C10-C33 and C14-C35 disulphide bonds). These three disulphide bonds are located in the hydrophobic core of the molecule whereas the fourth disulphide bond (C21-C37), which is specific of the MGD-1 structure, was found solvent exposed. Finally, probably due to constraints induced by the C4-C25 disulphide bond, the C4-P5 amide bond was found to adopt the unusual cis conformation.
Although MGD-1 and defensin A structures mainly consist of the CSαβ motif, they significantly differ by the length of the loop between the two first cysteines of the sequence (5 and 12 residues, respectively). Finally, based on the location of hydrophobic and positively charged residues the respective antibacterial activities of MGD-1 and defensin A will be discussed.

P 154

STRUCTURE ELUCIDATION OF THE FIRST Y5 RECEPTOR SELECTIVE AGONIST OF NEUROPEPTIDE Y

Reto Bader[a,b], Chiara Cabrele[b,c], Michael Langer[a], Oliver Zerbe[b], Annette G. Beck-Sickinger[a,b]
[a]Institute of Biochemistry, University of Leipzig, D 04103 Leipzig, [b]Department of Applied Biosciences, ETH Zürich, CH 8057 Zürich, [c]MPI for Biochemistry, D 82152 Martinsried

The first Y_5-receptor selective analog of neuropeptide Y (NPY), [Ala^{31}, Aib^{32}]-NPY, has been developed and biologically characterized. By using competition binding assays on Y-receptors selectively expressing cell lines, the affinity was determined to be 6 nM at the Y_5-receptor, > 500 nM at the Y_1- and Y_2-receptors, and > 1000 nM at the Y_4-receptor. It is well established that the C-terminal part of NPY represents the interaction site with the Y-receptors, and that amino acid exchange is poorly tolerated in the region 33-36; therefore, we induced a conformational change within the peptide region that mediates the receptor binding by the introduction of the β-turn-inducing dipeptide Ala-Aib into positions 31-32 of NPY and of some NPY/PP chimera. The [Ala^{31}, Aib^{32}]-modified peptides showed high selectivity for the Y_5-receptor; furthermore, *in vitro* and *in vivo* studies clearly proved their NPY receptor agonism as well as stimulation of food intake[1].
The solution structure of [Ala^{31}, Aib^{32}]-pNPY was investigated by CD and 2D-NMR. By comparison with the NMR structure of human NPY (hNPY) determined by Monks and co-workers[2], a significant conformational change of the C-terminal fragment 28-36 was observed: while in the native peptide the α-helix extends up to residue 36, the α-helical motif of [Ala^{31}, Aib^{32}]-pNPY ends with a 3_{10}-helical turn between residues Ile^{28} and Ala^{31}, followed by a flexible C-terminus. In the light of the binding properties of the NPY analog, this structural motif seems to be the key for Y_5-receptor selectivity.

[1]*C. Cabrele, M. Langer, R. Bader, H. Wieland, H. Doods, O. Zerbe, A.G. Beck-Sickinger (2000), submitted.*
[2]*Monks, S.A., Karagianis, G., Howlett, G.J., and Norton, R.S. (1996), J. Biomol. NMR 8, 379-390.*

P 155

COMPARATIVE CONFORMATIONAL STUDIES OF TOAC-LABELED ANGIOTENSIN II AND BRADYKININ ANALOGUES.

[a]Simone R. Barbosa, [b]Fábio Casallanovo, [a]Eduardo M. Cilli, [a]Antonio C. M. Paiva, [b]Shirley Schreier and [a]Clovis R. Nakaie.
[a]Dept. of Biophysics, Universidade Federal de São Paulo; [b]Dept. of Biochemistry, Chemistry Institute, Universidade de São Paulo, Brazil.

Biological assays of AII and BK analogues containing the amino acid-type TOAC spin label (2,2,6,6-tetramethylpiperidine-N-oxyl-4-amine-4-carboxylic acid) introduced by this group in the peptide chemistry field with two sequential strategies [(a) *Braz. J. Med. Biol. Res.* (1981) *14*, 173 and (b) *J. Am. Chem. Soc.* (1993) *115*, 11042] showed that only the N-terminally labeled derivatives ($Toac^1$-AII and $Toac^0$-BK) maintained partially the native activity of these two vasoactive peptides. Otherwise the internally labeled analogues ($Toac^3$-AII and $Toac^3$-BK) were devoid of activity. A comparative conformation-biological activity investigation with these paramagnetic peptides varying the pH of the medium and the amount of the secondary structure-inducing TFE was carried out applying the electron paramagnetic resonance (EPR) and circular dichroism (CD) methods. In general, AII, BK and their spin labeled analogues are composed of extended conformers but trending to acquire progressively a slightly more folded structures as the pH increases. Irrespective of the medium, the rotational correlation time (τ) of AII and BK active analogues were systematically lower than that of the inactive compounds (~ 3 x 10^{-10} and ~ 9 x10^{-10} s, respectively). In contrast to the pH effect, a more relevant conformational variations in both classes of peptides were observed by increasing TFE concentration. At 100% TFE solution, the percentage of α-helix structure for the biologically actives AII and $Toac^1$-AII increased up to near 25% and 15%, respectively. Contrariwise, for BK analogues this increase in α-helix content (about 25%) at higher TFE concentration was only attained by the inactive derivative ($Toac^3$-BK). In this conditions BK and its active $Toac^0$-BK analogue presented folded conformations but of not defined characteristics. Most relevant and stressing for the first time a direct structure-biological potency relationship for these class of paramagnetic peptides, the present data showed that regardless the medium, the conformational features of AII and BK turn out to be more closely paralleled only by their active spin labeled analogues.
Supported by the Brazilian FAPESP, CNPq and CAPES scientific agencies.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

THE COMPACT THREE-DIMENSIONAL STRUCTURE OF THE 21-RESIDUE CYCLIC ANTIBIOTIC PEPTIDE, MICROCIN J25

Alain Blond[a], Michel Cheminant[a], Isabelle Ségalas[b], Jean Péduzzi, Michel Barthélémy[a], Raul Salomón[c], Felipe Moreno[d], Sylvie Rebuffat[a]

[a] Laboratoire de Chimie des Substances Naturelles, ESA 8041 CNRS, Muséum National d'Histoire Naturelle, 63 rue Buffon 75005 Paris, FRANCE; [b] Laboratoire de RMN, UPRES A 6014 CNRS, IFRMP 23 INSERM, Université de Rouen, FRANCE, [c] Departamento de Biochímica de la Nutrición, INSIBIO, Universidad Nacional de Tucumán, ARGENTINA; [d] Unidad de Genética Molecular, Hospital Ramón y Cajal, Madrid, SPAIN.

Of the potent antibiotic peptides termed microcins and ribosomally synthesized by enteric bacteria, microcin J25 (Mcc J25) is the single cyclic representative. Its primary structure, *cyclo*(-Val[1] Gly Ile Gly Thr Pro Ile Ser Phe Tyr Gly Gly Gly Ala Gly His Val Pro Glu Tyr[20] Phe-), has been previously determined [1]. Its mechanism of action has been hypothesized to involve cell filamentation[2] and the cyclic form shown to be necessary for the antibiotic activity[1].

We thus investigated the three-dimensional structure of Mcc J25 by 2D-NMR spectroscopy and molecular modeling. The NMR conformational parameters, NOEs, chemical shift deviations (CSD), $^3J_{NHC\alpha H}$ coupling constants, NH/ND exchange rates and thermal coefficients of amide protons ($\Delta\delta/\Delta T$), were measured in methanol solution. The typical NOE pattern accompanied by the presence of large coupling constant values in the regions 8-10 and 16-19, were indicative of a two-stranded β-sheet, including a β-bulge. The region 11-15, which contains four out of the six glycines, formed a β-hairpin. A total of 213 NOE-derived interproton distance constraints inferred from 119 intraresidual, 44 sequential and 50 medium and long-range NOEs, together with 9 Φ dihedral angle restraints were used as input for calculation of 3D structures in the program X-PLOR, consistent with the NMR data. Mcc J25 adopts a highly compact structure, the cyclic backbone being folded back on itself and braced by several hydrogen bonds. The Gly[11]-Gly[15] β-hairpin comes very close to residues 1 to 5 which form a less structured loop. It is reasonable to think that this compact structure strongly contributes to both the marked stability of Mcc J25, which appears resistant to most of the proteases, and to its antibiotic activity.

1. Blond, A, Péduzzi, J., Goulard, C., Chiuchiolo, M.J. Barthélémy, M., Prigent, Y., Salomon, R.A., Farias, R.N., Moreno, F. Rebuffat, S., Eur. J. Biochem.: 259, 747~755, 1999.
2. Salomon, R.A., Farias, R.N., J. Bacteriol.: 174, 7428~7435, 1992.

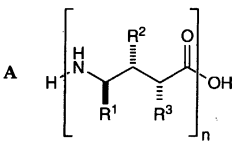
SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF γ-PEPTIDES

Meinrad Brenner, Dieter Seebach*

Eidgenössische Technische Hochschule, Laboratorium für Organische Chemie, Universitätstrasse 16, CH-8092 Zürich, Switzerland

Oligomers consisting of β-amino acids have attracted great attention. These so called β-peptides form stable secondary structures even at short chain length [1]. A variety of different structures has been characterized in solution and in the solid state. Recently, interesting biological properties of β-peptides have been observed and linked to their predictable secondary structures. Encouraged by these results we started investigations towards the synthesis and structural characterization of γ-peptides. We wondered whether the incorporation of an additional CH_2 group in each amino acid still allows the formation of stable secondary structures.

In independent work S. Hanessian and we have already reported the formation of a 2.6_{14} helical structure of γ^4-peptides in solution [2]. Herein the synthesis of γ-peptides **A** and the enantioselective preparation of the corresponding γ-amino acids are described.



The secondary structures of the γ-peptides in solution and in the solid state have been examined by CD-Spectroscopy, NMR-techniques and X-ray crystal-structure analysis.

[1] D. Seebach, J.L. Matthews, *Chem. Commun.*, **1997**, 2015.
[2] a) T. Hintermann, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 983. b) S. Hanessian, X. Luo, R. Schaum, S. Michnick, *J. Am. Chem. Soc.* **1998**, *120*, 8569.

P 158 P 159

(*E*)-α,β-DEHYDROPEPTIDE-RELATED COMPOUDS: STEREOELECTRONIC PROPERTIES FROM INFRARED AND THEORETICAL STUDIES

Malgorzata A. Broda, Barbara Rzeszotarska, Z. Kubica
Department of Organic Chemistry, University of Opole, Opole, Poland

α,β-Dehydroamino acids have a double bond between the C^α and C^β atoms and thus chirality gets lost and (*Z*)/(*E*) isomerism appears. Both (*Z*) and (*E*)-forms occur in nature and are being used for conscious peptide modification. The prototypical molecule of the family is dehydroalanine (ΔAla). The simplest (*Z*) and (*E*) natural representatives are (*Z*)-dehydrobutyrine [(*Z*)-ΔAbu] and (*E*)-dehydrobutyrine [(*E*)-ΔAbu], respectively. In peptide modification, the most commonly applied are (*Z*) and (*E*)-dehydrophenylalanine [(*Z*)- and (*E*)-ΔPhe]. Due to some difficulties in preparing the (*E*)-isomers, their conformational behavior is however insufficiently recognized.

We obtained Ac-(*E*)-ΔAbu-NHMe [1] and Ac-(*E*)-ΔPhe-NHMe [2] as the simple compounds related to (*E*)-α,β-dehydropeptides and using FTIR spectroscopy as well as quantum-chemical calculations compared their stereoelectronic properties with those of the respective (*Z*)-isomers, reference saturated compounds and the ΔAla parent, all examined previously [3]. The FTIR measurements were performed in dichloromethane (DCM) and acetonitrile (ACN) in the region of mode ν_s(N–H), amide I, amide II and $\nu_s(C^\alpha{=}C^\beta)$. The geometry and electron density, calculated by the DFT-based method at the B3LYP/6-31G* level, were employed to support spectroscopic interpretation and gain some deeper insight into molecules. Discussion of charge distribution was based upon analyses of Natural Bond Orbitals (NBO).

The sample compounds, like all the hitherto investigated Ac-ΔXaa-NHMe, adopt C_5 conformation in both solvents. While only the single C_5 structure was observed for Ac-ΔXaa-NHMe, two C_5 conformers exist for each (*E*)-compound in DCM solution: the C_5 H-bonded conformer that is characteristic of the ΔAla prototype, and the C_5 warped conformer(s) that is characteristic of Ac-(*Z*)-ΔXaa-NHMe. The C_5 hydrogen bond of the (*E*)-isomers, unlike the H-bond in Ac-ΔAla-NHMe, is disrupted by ACN. The most stable conformer *in vacuo* of the (*E*)-dehydroamides has $\Phi \approx 180°$ and $\Psi \approx 160°$. The geometry of both peptide units flanking the $C^\alpha{=}C^\beta$ bond and their electron density distribution in the (*E*)-compounds significantly differ from each other.

1. M.A.Broda, B.Rzeszotarska, L.Sme_ka, G.Pietrzy_ski, *J.Pept.Res.* **52** (1998) 72-79.
2. Z.Kubica, T.Ko_lecki, B.Rzeszotarska, *Chem.Pharm.Bull.* **48** (2000) in press.
3. M.A.Broda, B.Rzeszotarska, L.Sme_ka, M.Rospenk, *J.Pept.Res.* **50** (1997) 342-351.

SOLUTION STRUCTURE OF NEUROPEPTIDE γ AS DETERMINATED BY CD SPECTROSCOPY AND 2D NMR IN CONJUNCTION WITH THEORETICAL METHODS

Krzysztof Brzozowski[a], Sylwia Motowidło[a], Anna Łęgowska[a], Elwira Gwizdała[a], Adam Liwo[a], Jerzy Silberring[b], Krzysztof Rolka[a]

[a]Faculty of Chemistry, Uniwersity of Gdańsk, Sobieskiego 18, PL 80-952 Gdańsk, Poland; [b]Faculty of Chemistry Jagiellonian Uniwersity, Ingardena 3, PL 30-060 Kraków, Poland

Isolated from rabbit's intestines neuropeptide γ (NPγ) exhibits agonistic activity towards NK-2 tachykinin receptor. It is the biggest member of tachykinin family peptides and its C-terminal decapeptide is identical with the amino acid sequence of other agonist of NK-2 – neurokinin A. The amino-acid sequence of NPγ is as follows: Asp[1]-Ala-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met[21]-NH_2

In this paper we report the solution structure of this peptide in phosphate buffer, in the presence of SDS micelles and TFE solutions (CD investigations) and in DMSO-d_6 using 2D NMR technique in conjunction with three different theoretical approaches.

1) Global conformational search of the peptide studied using the EDMC method with the ECEPP/3 force field and subsequent calculation of statistical weights of the obtained conformations by fitting the theoretical NOESY spectra and vicinal coupling constants $^3J_{NH\text{-}C\alpha}$ to the experimental ones.

2) As above, but using the *Simulated Annealing* (SA) method to generate the conformational ensemble.

3) Using restrained molecular dynamics (with interproton distances calculated from NOE intensities) to obtain conformations that satisfy experimental data. The SA method was used as implemented in the DYANA and X-PLOR programm..

The results obtained with these methods were compared and also discussed in relation to the relevant data published for other agonists of NK-2 tachykinin receptor.

Acknowledgements:
This work was supported by the Polish Scientific Research Council - KBN (Research Grant N_o. PB 463/T09/97/13). The calculations were carried out in the Academic Computer Center in Gdańsk (TASK) and in Interdisciplinary Center for Mathematical Modeling (ICM) in Warsaw.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

P 160

Structural Studies

P 161

Interactions of bovine PP3 and its 119–135 C-terminal peptide with phospholipids. A monolayer and infrared study.

Sylvie Campagna[a], Nicole VanMau[b], Fréderic Heitz[b] and Jean-Luc Gaillard[a].
[a]Laboratoire des BioSciences de l'Aliment, UA INRA, Université H. Poincaré - Nancy 1, France ; [b]CRBM-CNRS (UPR 1086), Montpellier, France.

Component PP3, a phosphoglycoprotein from bovine milk with unknown function, displays in its C-terminal region (residues 99 to 135) a putative amphipathic α-helix which is a typical structural feature for lipid binding. In order to advance the knowledge of its possible functions, the behaviour of component PP3 and of a synthetic peptide from the 119-135 C-terminal domain were investigated at lipid-water interfaces. Penetration measurements into monolayers composed of various phospholipids indicated that the lipid-protein (or peptide) interactions were the highest with anionic phospholipids in a gel state (dipalmitoylphosphatidylglycerol, DPPG). Interestingly, the critical insertion pressure, above which PP3 no longer inserts, was higher than the value of biological membrane pressure. Moreover, we showed that, for the protein fraction, the hydrophobic and electrostatic interactions were involved in its insertion into the lipid film while the peptide-lipid interactions appeared to mainly arise from electrostatic interactions. The peptide, as shown by Fourier transform infrared spectra study, was in a β-type conformational state in aqueous solution and in the presence of anionic phospholipids (DPPG). In contrast, the protein adopted, in aqueous solution, an α-helical conformation which remained the dominant conformational state in the presence of DPPG although the apparition of β-structure was detected.

A MINIMALISTIC APPROACH TO UNDERSTAND THE MECHANISMS OF ACTION OF HEMOLYTIC AND ANTIBACTERIAL AMPHIPATHIC (LK) PEPTIDES: STRUCTURES AND ACTIVITIES ON VARYING TOPOLOGY.

Sabine Castano[ab], Bernard Desbat[b] and Jean Dufourcq[a]
[a]Centre de Recherche P.Pascal, CNRS, 33600 Pessac; [b]Laboratoire de Physico-Chimie Moléculaire, Université Bordeaux I, 33400 Talence, France.

Amphipathic peptides composed of L and K residues mimick most features of natural cytotoxic peptides: they are potent hemolytic and antibacterial compounds, they permeabilize and enter the cells, they bind nucleic acids, they efficiently destabilize membranes and lipid bilayers.

Here 15 residue-long peptides were designed to vary charge topologies and secondary structures: KLLKLLLKLLLKLLK (**1**), LKLLLLKLLKLKLLK (**2**), LLLLLLLLLLKKKKK (**3**), $(KL)_7K$ (**4**) and $(KL_2)_5K$ (**5**). Peptide **1** should give an ideal amphipathic α-helix, peptide **2** has the same composition but no amphipathy, peptide **3** has a di-bloc sequence, peptides **4** and **5** have respectively a two- and three-fold periodicity with 1/1 and 2/1 L/K ratios.

All peptides form films at the air-water interface but their stability and structure vary. *In situ* FT-IR and PMIRRAS spectroscopy indicates that peptide **1** folds into a pure α-helix, peptides **2** and **4** form pure β-sheets while peptide **3** displays spectra of both structures. The same conclusions hold when bound to monolayers of phosphatidylcholine, except for peptide **3** which folds in a pure α-helix. Calculated spectra allow to conclude that: all the peptides remain mainly flat-oriented compared to the interface without deep perturbation of zwitterionic phospholipids.

The lipids affinities, obtained both from monolayer expansion and from peptide fluorescence changes when insertion into lipid vesicles, strongly vary according to hydrophobicity and amphipathicity. The lytic activities measured on lipid vesicles and erythrocytes vary with the same hierarchy. Using the fraction of peptide bound allows to get the peptide efficiency to destabilize membranes. It correlates quantitatively with the total hydrophobic moment and the molecular area of the peptides and peptide **4** has a lytic efficiency higher than peptide 1.

Binding is the first discriminating step for activity, it controls the invasion of the outer membrane leaflet by the peptides lying flat at the interface whatever their topology. Here they form rafts depending on their self-association or lipid-domain formation in the bound state and all the peptides induce leakage and lysis whatever their secondary structure and charge topology.

P 162

CONFORMATION AND ION CHANNEL PROPERTIES OF A PENTAMERIC PEPTIDE TEMPLATE.

Laurent Chaloin[a], Emmanuelle Dé[b], Annie Heitz[c], Jean Méry[a], Gérard Molle[b], Frédéric Heitz[a]
[a]CRBM, 34293 Montpellier Cedex 5, France. [b]UMR 6522 Université de Rouen, 76821 Mont-Saint-Aignan, France. [c]CBS, Faculté de Pharmacie, 34060 Montpellier Cedex, France.

The structural and ion channel properties of a linear primary amphipathic peptide and its corresponding pentameric template were compared. The sequence of the peptide is derived from an association between a signal peptide (SP) and a nuclear localization sequence (NLS). In order to enhance the helical content and introduce a fluorescent probe in the SP sequence, a Trp residue has been introduced into a position which was selected using the AGADIR algorithm leading to a His to Trp substitution (Ac-M-G-L-G-L-W-L-L-V-A^{10}-A-A-L-Q-G-A-K-K-K-R^{20}-K-V-NH-CH_2-CH_2-SH). The template was synthesized by a covalent linking through the C-terminal SH group of five peptides to a cyclic peptide built of twenty residues and containing five iodoacetylated lysines regularly distributed, every i, i+4 residue. CD and FTIR measurements revealed that the parent peptide (without Trp) was unordered in water, while the mutant peptide (with Trp) was partly α-helical. This helical content, which increased with the pentameric template, was probably due to the interstrand hydrophobic interactions. NMR investigations showed that the helical part is located in the hydrophobic SP sequence. Both, the free and assembled peptides interacted with neutral and negatively charged phospholipids with, however, a higher affinity for the latter. In addition, fluorescence measurements indicated that the Trp residue was embedded in the hydrophobic domains. When incorporated into artificial lipid bilayers, the mutant peptide and its pentameric template induced single channel events with similar amplitudes of 70 pS in 1M KCl but with different behaviors. With the free peptide, typical fluctuations, with successive openings and closings, agree with different states of peptide aggregation. In contrast, the template presented stepwise events without closure, characteristic of an intramolecular channel constituted by these five monomers.

P 163

STRUCTURE OF A FUNCTIONALLY IMPORTANT DOMAIN IN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR STUDIED WITH MONOCLONAL ANTIBODIES TO α (181-192) SYNTHETIC PEPTIDES.

Frédéric Coutrot[a], Michel Marraud[a], Manh Thong Cung[a], Régis Vanderesse[a], Elena Lykhmus[b], Marina Skok[b], Sergey Voitenko[c], Larisa Voitenko[c], Vladimir Skok[c], Socrates Tzartos[d], Theodore Tsouloufis[d], Maria Sakarellos-Daitsiotis[e], Dimitrios Krikorian[d]
[a]LCPM, UMR-CNRS 7568, ENSIC-INPL, Nancy, France. [b]Palladin Institute of Biochemistry, Kiev, Ukraine. [c]Bogomoletz Institute of Physiology, Kiev, Ukraine. [d]Hellenic Pasteur Institute, Athens, Greece. [e]Department of Chemistry, University of Ioannina, Greece.

Neuronal nicotinic acetylcholine receptors (AChRs) consist of two acetylcholine (ACh)-binding α subunits and three structural β subunits. By nos sont en course β subunits have been identified; α3 and α5 subunits are homologous, however, in contrast to α3 subunit, α5 one seems to bind ACh poorly.
The aim of the present work was to compare the structure of a protein domain close to ACh-binding site in α3 and α5 AChR subunits. For this purpose, monoclonal antibodies (mAbs) have been produced against synthetic peptides corresponding to the 181-192 fragment of these subunits, which contain Tyr-190 and Cys-192 critical for ACh binding. MAbs distinguished among the peptides of homologous α subunits, bound native AChR and blocked ACh-induced currents in the neurones of rat superior cervical ganglia. Therefore, the structure of the peptide recognized by the mAb was close to that of the native receptor fragment.
To study the structure of α3 and α5 peptides bound to corresponding mAbs, the affinity constants of the mAb binding with several substituted peptide analogues synthesized by solid-phase procedures were calculated from the data of competitive ELISA. In addition, the structural analysis has been performed by 2D ^{1}H-NMR spectroscopy (COSY, NOESY and TOCSY) in association with field gradients when necessary. The appearance of TR-NOESY correlation in the presence of the mAb denoted the peptide-Ab affinity and revealed the most closely recognized sequences. The structure of mAb-bound peptides and some of the analogues has been studied by TR-NOESY with the elimination of water signal. The geometrical constraints, mainly short interproton distances, resulting from the above NMR, experiments have been used for solving the conformation of the bound ligands by using constrained dynamics simulations.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

Posters: topic B9

P 164

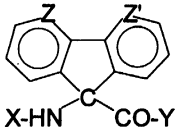
Structural Studies

SYNTHESIS AND CONFORMATION OF DAF PEPTIDES

Marco Crisma[a], Fernando Formaggio[a], Claudio Toniolo[a], Michel Wakselman[b], Jean-Paul Mazaleyrat[b]

[a]Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova, Italy; [b]SIRCOB, Bât. Lavoisier, University of Versailles - St Quentin, 78000 Versailles, France

The conformationally constrained $C^{\alpha,\alpha}$-symmetrically disubstituted Gly residues are among the simplest and most widely used structural units in the construction of peptides with a predetermined secondary structure. In this connection, *inter alia* we have previously shown that the tendency of 9-aminofluorene-9-carboxylic acid (Afc) peptides to give folded/helical structures is rather low. In particular, when Afc is N-terminal, the fully-extended C_5 structure appears to dominate. Here, we present the synthesis of terminally protected peptides from its diaza-analogue 9-amino-4,5-diazafluorene-9-carboxylic acid (Daf) and the results of a conformational study of Ala/Daf peptides to the nonamer level.



Z = CH; Z' = CH : **Afc**
Z = N; Z' = N : **Daf**

Peptide	X	Y
1	Boc	Ala-OMe
2	Boc-Ala	OMe
3	Boc-Ala	Ala-OMe
4	Boc-Ala-Daf-Ala-Ala	Ala-OMe
5	Boc-(Ala-Daf-Ala)$_2$-Ala	Ala-OMe

Coupling of H-Ala-OMe at the C-terminus of Daf was performed by using the acylazide method applied to Boc-Daf-$NHNH_2$. In addition, the mixed anhydride method was used for the coupling of Boc-Ala-OH to H-Daf-OMe and N-deprotected **1**. Finally, fragment condensations of the C- and N-deprotected derivatives of **3** by the EDC/HOBt method led first to **4** and subsequently to **5**. Our IR absorption, ^1H NMR, and X-ray diffraction study indicates that the conformational preferences of Daf peptides match rather closely those of the related Afc peptides.

P 165

THE COMPREHENSIVE ANALYSIS OF THE THIRD HELIX OF ANTENNAPEDIA HOMEODOMAIN AND ITS DERIVATIVES

András Czajlik[a], Eszter Meskó[b], Botond Penke[c] and András Perczel[a]

a, Department of Organic Chemistry, Eötvös Loránd University Budapest, Hungary; b, Department of Organic Chemistry, József Attila University Szeged, Hungary;

c, Department of Medical Chemistry, Szentgyörgyi Albert Medical School, Szeged, Hungary;

The homeodomain transcription factors bind to DNA through a specific sequence of 60 amino acids, the homeodomain, composed of three α-helices, with one β-turn between the second and the third helices. Scientists have recently reported, that a 16-amino acid long peptide corresponding to the third helix of the homeodomain of Antennapedia, the Drosophila transcription factor, translocates through plasma membranes, and is eventually conveyed to the nucleus of cells. The peptide, called penetratin can be used as a vehicle for the cellular delivery of oligonucleotides, and oligopeptides. The mechanism of this translocation is unclear, therefore we considered the determination of the dimensional structure important. NMR spectra have been recorded in the solvent-mixtures of water and partially deuterated trifluor-ethanol. While, the aqueous solution simulates the extracellular matrix, the trifluor-ethanol (TFE) solution simulates the membran environment. We concluded that the third helix of antennapedia homeodomain is poorly structured in water, but adopts an amphipatic α-helical structure in the low dielectric environment. This is further supported by the recently recorded CD spectra. We have also examined two mutant peptides, which do not have any biological effect. Similarly to penetratin both mutant sequences fold into an amphipatic α-helical structure in TFE. The overall fold look rather similar although minor differences were observed. Therefore, it looks as if the mechanism of translocation is not influenced by structural behaviour. Molecule dynamical, semi-empirical, ab initio calculations are now performed to have a better understanding of the conformational features of the peptides.

P 166

CONFORMATIONAL STUDIES ON GLYCOPEPTIDE FRAGMENTS OF HUMAN MYELIN OLIGODENDROCYTE GLYCOPROTEIN.

Annamaria D'Ursi,[a] Alfonso Carotenuto,[a] Elena Nardi,[b] Eloise Mastrangelo,[b] Anna Maria Papini,[b] and Paolo Rovero,[a] from [a]Dipartimento di Scienze Farmaceutiche, Università di Salerno, I-84084, Fisciano, Italy; [b]Dipartimento di Chimica Organica "Ugo Schiff", Università di Firenze, I-50131 Firenze, Italy.

Myelin oligodendrocyte glycoprotein (MOG), a minor myelin component, is an important central-nervous system-specific target autoantigen for primary demyelination in autoimmune diseases like multiple sclerosis (MS). The native structure of MOG presents a glycosylation site at position 31 (Asn^{31}). It has been recently described that glycosylation of a MOG peptide epitope improved the detection of specific autoantibodies in sera of MS patients [Mazzucco et al., Bioorg. Med. Chem. Lett. 9: 167, 1999]. It is well known that post-translational modifications such as glycosylation affect the antigenic properties of protein antigens. Accordingly, the aim of this study is the analysis of the structural role of the glycosyl moiety, using the synthetic peptide MOG(30-50) as a model. This peptide contains both the native glycosylation site of MOG (Asn^{31}) and the immunodominant portion of the protein (residues 37-46). We have synthesized the peptide MOG(30-50) and the corresponding glycopeptide bearing the β-D-glucopyranosyl (β-D-Glc) moiety linked to Asn residue at position 31.

We describe here a conformational analysis of these two peptides by NMR spectroscopy and molecular modeling methods. A whole set of 1D and 2D protonic NMR data were recorded in several environmental conditions with the aim of finding a solvent endowed with biologically significant properties, but also able to favor ordered conformers over extended and/or disordered ones. During the past few years it has been shown that the conformational freedom of short linear peptides in solution can be limited by the use of solvent mixtures of viscosity higher than that of pure water, but comparable to that of the interface cellular membrane/biological fluids. Accordingly the quantitative analysis of NMR data recorded in water and mixtures of water/dimethylsulfoxide (DMSO) and water/hexafluoracetone (HFA) evidences a significant propensity of [Asn^{31}(N-β-D-Glc)]MOG(30-50) to assume an ordered conformation, while in the same conditions the non glycosylated peptide displays more disordered conformational characteristics. Interestingly, these results support the hypothesis that the glycosydic moiety plays a crucial role in imposing structural constraints and in stabilizing the immunological active conformation of this peptide.

P 167

MODELING OF LEBETIN PEPTIDES WHICH INHIBIT PLATELET AGGREGATION : MOLECULAR DYNAMICS AND MOLECULAR MECHANICS STUDIES.

Mohamed Fathallah, Imed Regaya, Hervé Rochat, Jean-Marc Sabatier, Kamel Mabrouk, Laboratoire de Biochimie - Ingeniérie des Proteines CNRS UMR 6560, Faculté de Médecine Secteur Nord, Bd Pierre Dramard, 13916 Marseille Cedex 20, France.

Lebetin, from *Vipera lebetina* venom (1,2), is a new class of platelet aggregation inhibitors devoids of Arg-Gly-Asp motif. It contains peptide isoforms: lebetin 1 (α and β, 13 and 12 residues) and lebetin 2 (37 and 38 residues). Deletion of Aspartyl in position 2 resulted in analog sL1γ(Asn3-Lys-Pro-Pro-Lys-Lys-Gly-Pro-Pro-Asn-Gly_{13}) exhibiting the most potent inhibitory activity. To map the functional amino acid residues as well as to assess the effect of peptide cyclization on anti-aggregation activity, twenty sL1γ derived peptides were chemically synthesized and characterized. Among the peptides, several L-Ala substituted analogs were found to retain anti-aggregation activity.

The conformational behavior of these peptides was studied by both molecular mechanics (MM) and molecular dynamics (MD) simulations. MM allows to build up hypothetical, most favored conformations of the peptides. The initial conformations were first investigated by MM, and the low energy conformations were further studied by MD simulation.

The results will be discussed on the basis of the preferred peptide conformations, and the effects of structural constraints (cyclization) on the stability of these peptides.

(This work is supported by MENRT n°99H0241 and Cellpep grant).

1- R. Barbouche, N. Marrakchi, P. Mansuelle, M. Krifi, E. Fenouillet, H. Rochat and M. El Ayeb. FEBS lett.1996, 392, 6-10.

2- R. Barbouche, N. Marrakchi, K. Mabrouk, M. N. Krifi, E. *Fenouillet, M. El Ayeb* and H. Rochat.Toxicon 1998, 36, 1939-1947.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

Posters: topic B9

P 168

Structural Studies

P 169

SOLUTION STRUCTURE OF SMALL SERINE PROTEINASE INHIBITORS: A COMPARATIVE STUDY

Zoltán Gáspári[a], András Patthy[b], László Gráf[c] & András Perczel[a]
[a]Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary; [b]Agricultural Biotechnology Center, Gödöll_, Hungary; [c]Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary

The solution structure of two wild type and one mutant small serine proteinase inhibitors was determined by homonuclear NMR spectroscopy. The molecules belong to a recently established family of small (~4KDa) protein proteinase inhibitors isolated from locust species. All family members with known structure share a highly similar global fold of three antiparalel beta-strands and three conserved disulphide bridges. These molecules exhibit a very interesing alteration in primary sequence in connection with their specificity towards trypsin- and chymotrypsin-like proteases. The structure of wild type and mutant *Schistocerca gregaria* chymotrypsin inhibitor (SGCI, the mutant is a potent inhibitor of trypsin) and *Schistocerca gregaria* trypsin inhibitor (SGTI) is described in full detail. The high number of distance restraints allows us to discuss the small but significant differences in the atomic structure of SGCI and its mutant, as well as comparison with SGTI and family members already described in the literature. Enzyme-ligand interaction simulations and implications for the mechanism of action are presented.

NEW PEPTIDE IMMUNOMODULATORS VILON AND THYMOGEN, CHEMICAL AND BIOPHYSICAL ASPECTS OF THEIR ACTION IN THE WATER MEDIUM

Evgeny Grigoriev[a], Igor Kotchnev[b], Tatiana Kudriavtseva[a], Alexey Grigoriev[b], Vladimir Khavinson[a], [a]St. Petersburg Institute of Bioregulation and Gerontology, Russia; [b]St. Petersburg State University, Russia.

Peptide compounds reveal the greatest immunomodulating potential. However, the mechanism of their action has not been thoroughly studied yet. We carried out investigations in this field on the model of a widely known dipeptide Thymogen® (glutamyl-tryptophan) and a recently obtained dipeptide Vilon® (lysil-glutamic acid). The main purpose of these investigations consisted in the study of the correlation between the chemical structure of the peptides (primary structure), their potential and most probable conformations, spectral characteristics in far and near infrared (IR) and the property to reveal biological activity. Vilon, Thymogen and a number of compared dipeptides were synthesised according to the classical methods of peptide synthesis in a solution. Thereby, over 99% purity of the dipeptides was obtained. The structures were confirmed by the data of amino acid and elementary analysis and the spectra and were thoroughly characterised. Conformations for the ionised and non-ionised forms were calculated by "PC Model" programme. Far IR-spectrum (20-600 cm^{-1}) was obtained on the Fourier-Spectrometer. Temperature dependencies of a water-absorption strip for the dipeptide solutions were measured by 5180 cm^{-1}. According to the calculated data for Vilon, which manifested the greatest biological activity in a number of medical tests (methods and results are presented), the cyclic form was the most energetically stable conformation for the ionised peptide form. This form was obtained by means of the approximation of α- and ε- amino groups of lysine and α- and γ- carboxyl groups of glutamic acid. Thymogen molecules were unlikely to possess similar cyclic forms. In the far IR characteristic strips for the studied compounds, specific to each concrete structure and connected with the oscillatory energy for the whole structure were registered. Spectral characteristics of the amino acids in a mixture and their dipeptides differed essentially. Temperature dependencies of a water-absorption strip by 5180 cm^{-1} for Vilon and Thymogen solutions significantly differed from the data on the mixture of individual amino acids. This distinction was not observed for other studied dipeptides. Thus, a new mechanism of peptide action was revealed on the basis of the obtained experimental data. At this stage the distant discontinuous transfer of signals from peptide molecules to receptors in the water medium was proved possible.

P 170

SQUASH TRYPSIN INHIBITORS FROM *MOMORDICA COCHINCHINENSIS* CONTAIN AN ATYPICAL MACROCYCLIC STRUCTURE

Jean-François Hernandez[a], Jean Gagnon[a], Laurent Chiche[b], Tuyet Mai Nguyen[c], Jean-Pierre Andrieu[a], Annie Heitz[b], Thai Trinh Hong[c], Trân Châu Pham[c], Dung Le Nguyen[d].
[a]Institut de Biologie Structurale Jean-Pierre Ebel, Grenoble, France; [b]Centre de Biochimie Structurale, Faculté de Pharmacie, Montpellier, France; [c]Centre de Biotechnologie, Université Nationale du Vietnam, Hanoï, Vietnam; [d]INSERM U376, CHU Arnaud de Villeneuve, Montpellier, France.

Three trypsin inhibitors (TIs), that belong to the squash family, have been isolated from the seeds of *Momordica cochinchinensis* (MCo) using gel filtration, ion exchange chromatography and reversed-phase high performance liquid chromatography. Their sequences could be determined only after proteolytic cleavages. In the case of MCoTI-I, MCoTI-II, and their isoforms resulting from an Asp-Gly peptide bond rearrangement, we showed that their polypeptide backbones are cyclic, a structure that had never been described in squash TIs. They contain 34 amino acid residues with three disulfide bridges, for molecular masses of 3453 and 3480.7 respectively as determined by electrospray mass spectrometry. They are the largest of the known macrocyclic peptides containing disulfide bridges. Their sequences show strong homology with the other squash TIs, suggesting a similar 3-dimensional structure and an analogous mechanism of action. MCoTI-II was homology modeled from the crystal structure of the complex between bovine trypsin and CMTI-I. The model indicates that the linker joining the two termini does not impose significant geometrical constraints. Although the importance of cyclisation is not clear, it might confer additional stability and resistance to proteases. A third species, MCoTI-III, was also characterized as containing 30 amino acid residues for a molecular mass of 3379. This minor component possesses a linear peptide backbone, as usually observed in the squash family, and its N-terminus was shown to consist of a pyroglutamyl residue. Considering their high stability, MCoTIs represent interesting candidates for drug design, either changing their specificity of inhibition, or using their structure as natural scaffolds bearing new binding activities.

P 171

Contribution to the study of the role of two target peptides from Pr P^C helix 1 in the hydrophilic seeding of prion aggregates.

Françoise Jacquemotte [1], Eugene Shakhnovich [3] Mike Morrissey [3], Bart Devreese.[2] Jozef Van Beeumen.[2].

1. Département des Substances Naturelles, Institut Meurice, Haute Ecole Lucia de Brouckère, av.Gryzon, B-1070 Bruxelles, Belgium
2. Laboratorium voor Eitwitbiochemie en Eitwitengineering, Universitcit Gent, K.L. Ledeganckstraat, B-9000 Gent, Belgium
3. Division of Engineering and Applied Sciences, and Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA02138

PrP^{sc} is the pathogenic prion form responsible of prion infectivity.
It is a misfolded isoform of the naturally occuring prion protein (PrP) and is generally accepted to be the important agent in a class of mammalian neurodegenerative disorders (Creutzfeldt-Jakob disease in humans, scrapie in sheep, bovine spongiform encephalopathy in cows).
PrP^C (prion protein) helix 1 (residues 144-153) is characterized by a highly unusual sequence, described by Morrissey and Shakhnovich, that may play a pivotal role in prion infectivity.
The short peptides of PrP^C helix 1 DWED (Asp-Trp-Glu-Asp) and DWEDRYYR (Asp-Trp-Glu-Asp-Arg-Tyr-Tyr-Arg) were prepared by solid phase peptide synthesis using Fmoc-chemistry. The sequences and molecular masses of the peptides were verified by electrospray mass spectrometry.
The aim of this work is to examine the possibility of aggregation of these two peptides.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd

Posters: topic B9

P 172 Structural Studies P 173

DECONVOLUTION OF CD CURVES IN A 32-BIT WINDOWS ENVIRONMENT VIA CCA+ ENCOMPASSING CD SPECTRA FROM PEPTIDES TO PROTEINS

Imre Jákli[a], András Perczel[a], Gábor Tusnády[b]
[a]Department of Organic Chemistry, Loránd Eötvös University, Hungary, H-1117 Budapest, Pázmány Péter Sétány 1/A.
[b]Mathematical Institute of the Hungarian Academy of Sciences, Hungary, H-1053 Budapest, Reáltanoda u. 13-15.

Determination of secondary structure is still one of the most important question for a protein chemist. Qualitative analysis of CD spectra was the first applied tool for the secondary structure determination. Beside the dominant modern NMR and X-ray techniques, CD spectroscopy is still remained a valuable tool. We present here applications of the 32 bit version of the CCA+ program. Beside the larger workspace some new features are introduced: the spectrum import module has improved, the new Jasco format is included, the handling of long filenames is fixed, the rather complicated spectrum converter has been removed. The program incorporates the LINCOMB method, which approximates the measured CD curves with the linear combination of pre-defined "base-curves". These "base-curves" - selected via X-ray and NMR data - are assigned as typical spectra representing the secondary structure elements of peptides and proteins. An alternative and a possibly more powerful approach is also built in, when such an assumption is not made, and the set of recorded CD spectra are directly deconvoluted. The CCA+ (Convex Constraint Algorithm Plus) eliminates the weakness of the previous method. It extracts the "base-curves" only from the measured CD spectra. The deconvolution process determines and gives the contributions of the "base-curves" in the individual CD spectrum. This method doesn't depend on any X-ray or NMR data, this way it provides a tool for quantitative use of CD spectroscopy for secondary structure determination. We present here an extended CD spectra "database", which could help to use the program for different molecules and conditions. This database can be useful especially for peptide and protein chemists, since it contains CD spectra of proteins in water, cyclic peptides in TFE, TFA and water and small protein fragments. The program is available free of charge via internet: http://www2.chem.elte.hu/protein/programs/cca

CONFORMATIONAL CHANGES IN THE REGION 17-24 OF SOLUBLE Aβ(1-42) CAUSE AMYLOID FORMATION

Katharina Janek,[a] Sven Rothemund,[a] Klaus Gast,[b] Michael Beyermann,[a] Josef Zipper,[a] Heinz Fabian,[b] Michael Bienert,[a] and Eberhard Krause,[a]
[a]Institute of Molecular Pharmacology, Alfred-Kowalke-Str.4, D-10315 Berlin, Germany; [b]Max-Delbrück-Center of Molecular Medicine, Berlin, Germany

A critical event in Alzheimer's disease is the conformational transition of Aβ peptides from their soluble forms into disease-associated β-sheet rich conformers. We report for the first time using the full-length peptide, Aβ(1-42), the location of the region which causes the conformational switch into a β-sheet structure. Because of the high tendency to form aggregated β-sheets at µM to mM concentration a monomeric α-helical structure of Aβ(1-42) was stabilized by trifluoroethanol. Using a D-amino acid replacement set we studied the site-specific destabilization of the soluble structure of Aβ(1-42). Substitution of amino acids by their D-enantiomers induces a local destabilization of secondary structures without changing other properties of a peptide. Thus, this approach allowed the separation of conformational from hydrophobic effects. Although two separated helical domains of Aβ(1-42) (I: 11-24; II: 28-36) were determined by NMR spectroscopy, only the destabilization of the region comprising position 17 to 24 causes a transition to a β-sheet structure. The conformational α-to-β switch is directly accompanied by an aggregation process yielding to amyloid fibrils.

P 174 P 175

COMPUTATIONAL AND NMR STUDY OF MULTIPLE ANTIGENIC GLYCOPEPTIDES WITH Tn ANTIGENS

Jan Jezek, Jirí Vondrásek, Jan Sejbal[a], Jirí Velek, Tomás Trnka[a], Pavel Veprek
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo námestí 2, 166 10 Prague 6, Czech Republic; [a]Department of Organic Chemistry, Faculty of Natural Sciences, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic

Multiple antigenic peptides (MAPs) and multiple antigenic glycopeptides (MAGs) containing oligolysine core, both with (MAGs) and without (MAPs) dimeric Tn antigen, i.e. Lys_2-Lys-β-Ala-NH_2 **(I)**, [Ac-Lys(Ac)]$_2$-Lys-β-Ala-NH_2 **(II)**, (Lys-γ-Abu)$_2$-Lys-β-Ala-NH_2 **(III)**, [Ac-Lys(Ac)-γ-Abu]$_2$-Lys-β-Ala-NH_2 **(IV)**, (Ac-γ-Abu)$_4$-Lys_2-Lys-β-Ala-NH_2 **(V)**, [Ac-γ-Abu-Lys(Ac-γ-Abu)-γ-Abu]$_2$-Lys-β-Ala-NH_2 **(VI)**, [Ac-(Tn)$_2$-γ-Abu]$_4$-Lys_2-Lys-β-Ala-NH_2 **(VII)** and [Ac-(Tn)$_2$-γ-Abu]$_4$-(Lys-γ-Abu)$_2$-Lys-β-Ala-NH_2 **(VIII)**, were prepared by SPPS using Fmoc chemistry. Biological activities of these compounds were object of correlation studies with their conformational states studied by NMR, molecular modelling and molecular dynamics (MD) calculations.
We have studied de novo created generations of dendrimers **I-VIII** in which only the last generation bears Tn antigens (compounds **VII,VIII**). MD simulation was selected to describe their conformational properties and prevailing conformational states in vacuo, DMSO and water solutions. The conformational behavior of dendrimers is an issue of debate giving rise to question if the end groups in dendrimers are pointing outwards or if they are severely backfolded. We show (both experimentally and theoretically) that conformations of the studied dendrimers are flexible in water and DMSO and that there is no predominating conformational state exposing the terminal dimeric Tn antigen in ordered manner for these generations of dendrimers. This result is in contrast to MD simulation in vacuo conditions where all dendrimers show tightly packed core with numerous hydrogen bonds.

Biophysical Characterisation of Cationic Peptide–DNA Interaction: Model Studies to Understand DNA Condensation

Michael Keller, Monika Preuss, Mirjam Tecle, Imran Shah, Toshiaki Tagawa and Andrew D. Miller

Imperial College Genetic Therapies Centre, Department of Chemistry Imperial College, South Kensington, London SW7 2AY, United Kingdom.

Cationic peptides containing the positively charged Lys and Arg residues are known to bind DNA *via* charge–charge interactions and display an important role in compacting DNA. Here, the interaction of three highly positively charged peptides of different origin and length with double stranded dodecameric, palindromic *oligo*-DNA is investigated. Fluorescence studies using dansylated peptides demonstrate that peptide–DNA interaction is accompanied by an increase in fluorescence intensity and a blueshift from 540 to 528 nm until stoichiometric charge/charge ratios are reached. CD spectroscopy revealed important impact on the intensity and nature of the Cotton effects when titrating *oligo*-DNA with charged peptides. This is compatible with a conformational adaptation of the DNA structure upon binding to cationic peptides. Isothermal calorimeter studies were used to define the binding energies involved.
These experiments serve as model studies to understand how cationic species such as short peptides and polyamines are able to compact the volume of DNA. Such condensing processes are observed in the nucleus of cells *in vivo*, where highly positively charged histone proteins are responsible to compact DNA to a fraction of its volume (1:10000) as found during uncoiled stages of DNA replication.

Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd