Poster presentations

Topic D

D1 - Peptide and protein interactions with DNA
D2 - Structural studies, spectroscopy and molecular modelling
D3 - Peptide interactions with membranes and in signal transduction
D4 - Peptide and protein folding
D5 - Peptides in drug design
D6 - The structure of peptides and their interaction with biomolecules

P D1 - Direct complex formation between DNA and peptides derived from HIV-1 Tat protein in the preparation of simple non-viral gene delivery system

S. V. Burov[1], A. V. Pavlotzkaya[1], M. V. Leko[1], M. Y. Dorosh[1], I. B. Ditkovskaya[1], E. B. Dizhe[2], I. A. Ignatovich[2], S. V. Orlov[2], A. P. Perevozchikov[2]

1. Institute of Macromolecular Compounds, Academy of Sciences, St.-Petersburg - Russian Federation
2. Institute of Experimental Medicine, Academy of Medical Sciences, St. Petersburg - Russian Federation

The design of efficient delivery vehicles for "therapeutic" gene transfer into somatic tissues and organs is of fundamental importance for progress in gene therapy of different human pathologies. Non-viral delivery tools despite their relatively low efficiency, are characterized by negligible immunogenicity and low toxicity as compared to gene transfer using recombinant viruses. An approach based on receptor-mediated endocytosis of DNA complexes with different polycations and ligands of specific surface receptors [1] has some advantages for the directed gene transfer into cells and sometimes, for prolonged maintenance of delivered gene constructs.
In the course of simple non-viral gene delivery system development we have compared transfection efficiency using DNA complexes with several peptides: amphipathic one, oligolysine analogue (OA), hybrid RGD-OA structure, Tat (47-57) fragment and control cationic peptides of different nature. The correspondent complex formation was determined by retardation of DNA migration in 1% agarose gel, permitted to estimate optimal components mass ratio for each case. Recently, it was shown that Tat (47-57) is responsible for Tat transfer following nuclear translocation into human cells [2]. There are numerous examples of Tat (47-57) and related peptides application for gene transfer into mammalian cells in vitro, using oligonucleotide-peptide conjugate formation. Our preliminary studies of non-covalent DNA/cationic peptide complexes and their application for the delivery of "suicide" HSV-1 thymidine kinase gene into human hepatoma cells HepG2 have prompted us to investigate the possibility of similar Tat (47-57) usage for the foreign DNA delivery. The experiments on HepG2 cells was demonstrated an increased efficacy of β-galactosidase expression vector transfer when the charge of DNA/Tat (47-57) complex was changed to positive, with optimal peptide/DNA mass ratio 3:1. Further enhancement of peptide contents resulted in decreased efficacy of bacterial reporter gene delivery. The permeability of complex via cell membrane have been additionally checked using Tat (47-57) fragment bearing N-terminal fluorescent label. It was found that transfer potency is dependent on the presence of Ca^{2+} ions in cell culture medium. The control experiments using biologically active fragments of natural hormones containing several arginine and lysine residues allowed to discuss the role of peptide structural features in complex formation and its subsequent penetration via cellular membrane. These results in conjunction with literature data permits us to formulate a hypothesis regarding the mechanism of DNA/Tat (47-57) constructions delivery. It was shown that Tat derived peptides directly interacts with DNA producing a stable non-covalent complexes and represents a promising means for foreign DNA transfer into human cells.

References
[1] Cristiano, R., Roth, J. *J. Mol. Med.*, (1995), 73, 479-486.
[2] Schwarze, S., Dowdy, S. *TiPS*, (2000), 21, 45-48.

P D2 - Synthesis of amphipatic α-helical peptides modified with lipophilic fragments and their interaction with DNA and erythrocites.

I. Gurjanov[1], E. Vlasova[1], V. Korol'kov[1], G. Vlasov[1], A. Kiselev[2], E. Lesina[2], V. Baranov[2], E. Chirarzhina[3], V. Vorob'ev[3]

1. Institute of Macromolecular Compounds, Russian Academy of Sciences - Russian Federation
2. Institute of Obsterics and Ginecology, Russian Academy of Medical Sciences - Russian Federation
3. Institute of Cytology, Russian Academy of Sciences - Russian Federation

A goal of gene therapy is treatment of a variety deseases, which are caused by changes in genes. For effective gene therapy corresponding gene must be delivered into nucleus of cell and expressed there. Between the carriers of DNA a short basic amphipatic peptides are most promising. They can provide binding to DNA, cell targeting, membrane destabilisation and nuclear localisation. An important for membrane destabilisation and perturbation is helicity and hydrophobicity of the peptides.
A serie of amphipatic peptides containing fragments of lysine, histidine, glutamic acid, tryptophane, tyrosine, cysteine was synthesized. Peptides were modified with different quantity of residues of capric acid. Membrane activity of peptides and their ability to form complexes with DNA were investigated. Heptadecapeptide containing four lipophilic residues of capric acid in position 5, 8, 12 and 15 has strongest hemolytic activity. Geneicosapeptide containing residues of capric acid in position 2, 7, 12, 17 had on the contrary low membrane activity. Not modified peptide having high level of helicity was the most unactive in this assay.
Particularities of synthesis, binding and transfection ability of peptides in complexes with DNA will be discussed.

P D3 - A peptide selected from a phage display library selectively recognizes modified nucleosides containing anticodon domain of yeast tRNA(Phe)

P. Mucha[1], A. Szyk[1], P. Rekowski[1], R. Guenther[2], P. A. Weiss[3], P. F. Agris[2]

1. University of Gdansk, Department of Chemistry - Poland
2. North Carolina State University, Department of Molecular and Structural Biochemistry - U.S.A.
3. Xeragon AG, Zurich - Switzerland

While it is known that modified nucleosides influence RNA structure, the modified nucleoside determinants of protein discrimination between modified and unmodified RNA have not been elucidated. Affinities of phage display selected peptides for the anticodon domain of yeast $tRNA^{Phe}$ (ASL^{Phe}) having three naturally-occurring modified nucleosides (Cm_{32}, Gm_{34}, m^5C_{40}) were characterized by fluorescence quenching of the peptides' tryptophans. Peptide t^F2 (Ser[1]-Ile-Ser-Pro-Trp[5]-Gly-Phe-Ser-Gly-Leu[10]-Leu-Arg-Trp-Ser-Tyr[15]) had the most specific and highest affinity ($K_d = 1.3 \pm 0.4$ μM) for the triply modified ASL^{Phe} used in phage selection as compared to unmodified ASL^{Phe} ($K_d = 70.1 \pm 12.3$ μM). However, affinity for a doubly modified molecule (ASL^{Phe}-Cm_{32},Gm_{34}) that formed a duplex instead of a hairpin was dramatically reduced (K_d>500 μM). CD spectra showed that peptide t^F2 had a tendency to adopt β–sheet structure in aqueous solution. Substitutions $Ala^{4,6,9}$ that influenced the secondary structure of the peptide, 25-fold reduced peptide's binding affinity for triply modified ASL^{Phe}. Thus, modifications contribute identity elements in peptide recognition of the anticodon domain and the peptide's β–sheet structure is a significant factor in the RNA-peptide recognition.

Acknowledgements: this work was supported by the Foundation for Polish Science post-doctoral fellowship and the Polish State Committee for Scientific Research, grant 1219/T09/2001/20 to P.M.

P D4 - Chiral peptide nucleic acids: design, synthesis, optical purity and hybridization properties

S. Sforza[1], R. Corradini[1], T. Tedeschi[1], A. Dossena[1], R. Marchelli[1]

1. Dept. of Organic and Industrial Chemistry, Università di Parma, Parma - Italy

Peptide nucleic acids (PNAs) are DNA mimic pseudopeptide, first described by Nielsen and co-workers in 1991, [1] which have attracted a great interest because of their highly specific and very stable DNA binding.[2] Due to their outstanding behavior, PNAs are currently used in a plethora of biological applications which require specific DNA recognition, such as point mutations, genetic probing by *in situ* hybridisation, PCR clamping, inhibition of transcription and of translation and many others. Since their discovery, many modifications of the original PNA monomeric units have been reported, in order to improve the stability of the binding to the complementary nucleic acids and/or the specificity of the complexation.[3,4] Among all the proposed modifications, one of the most interesting is the introduction of a stereogenic centre on the α carbon of the aminoethylglycine unit.[5] In the present communication the effect of this modification will be discussed in comparison with "regular" achiral PNAs and with other chiral PNAs having an ornithine-based backbone.[6] The synthetic challenge of chiral PNA will be introduced and discussed, particularly as far as the preservation of the optical purity is concerned. It will be shown that the optical purity, particularly in the case of ornithine-based chiral PNAs, may have important consequences on the binding properties of PNAs.[7] Mechanistic aspects of the racemization occurring during solid phase synthesis of chiral PNAs will be discussed in comparison with peptide synthesis. As a matter of fact, the binding abilities of chiral PNAs can be finely tuned by an accurate choice of the amino acid to be used as chiral synthon, of the position of the strand where the chiral monomers have to be placed and of the number of chiral monomers. Chiral PNAs containing three adjacent D-lysine-based chiral monomers placed in the middle of a PNA strand ("chiral box") were demonstrated to have very interesting properties in terms of specificity, mismatch discrimination and direction control. In fact, PNAs bearing a D-lysine "chiral box" bind to complementary DNA exclusively in the antiparallel mode and also the presence of a point mutation usually strongly inhibit PNA-DNA duplex formation [8]. Therefore, this kind of PNAs can be useful as extremely specific genetic probes. Some examples of diagnostic applications using chiral PNA probes by a biosensor (BiaCore), microarrays and chromatography will be presented and the results will be discussed also in perspective of future applications (cystic fibrosis diagnosis, OGM determination in food).

References
[1] P.E. Nielsen, M. Egholm, R. M. Berg, O. Buchardt, *Science*, 1991, *254*, 1497-1500.
[2] For an extensive review, see: E. Uhlmann, A. Peyman, G. Breipohl, D.W. Will, *Angew. Chem. Int. Ed.* 1998, *37*, 2796-2893.
[3] K.L. Duheolm, P.E. Nielsen, *New J. Chem.*, 1997, *21*, 19-31.
[4] B. Falkiewicz, *Acta Chem. Polon.*, 1999, *46*, 509-529.
[5] A. Puschl, S. Sforza, G. Haaima, O. Dahl, P.E. Nielsen, *Tetr. Lett.* 1998, *39*, 4707-4710.
[6] R. Corradini, S. Sforza, A. Dossena, G. Palla, R. Rocchi, F. Filira, F. Nastri, R. Marchelli, *J.Chem. Soc., Perkin Trans. 1*, 2001, 2690-2696.
[7] S. Sforza, G. Galaverna, A. Dossena, R. Corradini, R. Marchelli, *Chirality*, 2002, in press.
[8] S. Sforza, R. Corradini, S. Ghirardi, A. Dossena, R. Marchelli, *Eur. J. Org. Chem.*, 2000, 2905-2913.

P D5 - Using capillary electrophoresis to study methylation effect on RNA - peptide interaction

A. Szyk[1], P. Mucha[1], P. Rekowski[1], R. Guenther[2], P. F. Agris[2]

1. University of Gdansk, Department of Chemistry - Poland
2. North Carolina State University, Department of Molecular and Structural Biochemistry - U.S.A.

Posttranscriptional and posttranslational modifications of RNAs and proteins are not well understood mechanisms of gene expression regulation. Methylation is the simplest modification known. Modified residue (nucleoside or amino acid)-dependent RNA-protein interaction is difficult to study because of inherent problems in isolation, chemical synthesis and characterization of interaction between functional biomolecules. We have used a new technique, capillary electrophoresis (CE) to study methylation-dependent RNA-peptide interaction. Two examples of methylation-dependent RNA-peptide interactions have been studied. Peptide t^F2 (Ser[1]-Ile-Ser-Pro-Trp-Gly-Phe-Ser-Gly-Leu-Leu-Arg-Trp-Ser-Tyr[15]), the most populated ligand selected from a random phage display library specifically binds a triply modified anticodon domain of yeast $tRNA^{Phe}$, ASL^{Phe}-$Cm_{32},Gm_{34},m^5C_{40}$. To study an effect of protein methylation, TAR-Tat HIV-1 system has been chosen. Interaction of TAR with an arginine rich region (49-57) (ARR) of Tat is mediated by the single arginine residue Arg52. This residue forms a specific network of hydrogen bonds with the bulge region of TAR. One may conclude that distortion of the guanidynium group structure of the Arg52 by methylation should modify hydrogen bonds pattern and decrease of ARR affinity for TAR. Under CE conditions applied a methylation-dependent binding affinity increasing (for peptide t^F2-ASL^{Phe}-$Cm_{32},Gm_{34},m^5C_{40}$) and abolishing (for TAR-[$Arg(Me)_2^{52}$]Tat(49-57)) of RNA-peptide interaction have been observed. The results revealed critical role of methylation for RNA-peptide interactions studied and usefulness of CE to study modification-dependent intermolecular interactions of biomolecules.

Acknowledgment: this work was supported by the Polish State Committee for Scientific Research, grant 1409/T09A/2001/21

P D6 - Starburst carbon chain polymer protein conjugates: adjustment of immunogenicity and search of the possibility of DNA delivery

G. Vlasov[1], G. Pankova[1], O. Charenko[1], N. Antonov[1], V. Baranov[2], A. Baranov[2], P. Glazkov[2], A. Kiselev[2], O. Ostapenko[2], E. Lesina[2]

1. Institute of Macromolecular Compounds, Russian Academy of Sciences - Russian Federation
2. Institute of Obsterics and Ginecology, Russian Academy of Medical Sciences - Russian Federation

In the last decade, polymer protein conjugates such as conjugates of transferrin or insulin with polylysine or polyethyleneimine were used for DNA delivery. However, proteins are high immunologenic. Therefore it is desirable to supress high immunogen stimulation effect of protein moiety of DNA-protein complexes. In order to study the possibility of regulation of immune properties of polymer protein conjugates synthesis of starburst carbon chain polymer protein conjugates (SCPPCs) [1], based on the bovine serum albumin modified by carbon chain polymers coupled via a single bond to the protein and containing as a model compound bradykinin in the polymer moiety was carried out. It was shown that there is a possible by alteration of the chemical structure of carbon chain polymer moiety of SCPPC to enhance (polymethacrylic acid as a polymer moiety) or suppress [poly(N-vinylpyrrolidone) or poly(N-vinyl-2-methylimidazole) as polymer moieties] of antibody elicitation against low molecular weight hapten (bradykinin) introduced in the polymer moiety, as well as against protein (BSA) moiety of conjugates. This may appear a useful tool in preparation of semi-synthetic immunogenes for production of specific antibodies for immunological analysis or creation of artificial vaccines when immunogenicity of low molecular weight hapten have to be enhanced but immunogenicity of protein carrier have to be suppressed. The data received may be peculiarly useful for preparation of polymer protein carriers for DNA delivery when immunogenicity of protein moiety have to be suppressed.
As an illustration sturburst carbon chain polymer transferrin conjugates containing poly(N-vinyl-2-methylimidazole) as well as copolymers of N-vinyl-2-methylimidazole with acrylamide coupled via a single bond to the protein were prepared. Influence of quantities of polymer chains grafted to transferrin, their chemical structure as well as their MW on the SCPPC – DNA complex formation (retardation assay), transfection and expression were studied.

Reference
[1] Vlasov, G. P.; Illarionova, N. G.; Izvarina, N. L.; Denisov, I. G. *Macromol. Chem., Suppl.* 1985, 9, 239-249

P D7 - Lysine dendrimers and their starburst polymer derivatives as carriers for the DNA delivery

G. Vlasov[1], V. Korol'kov[1], G. Pankova[1], N. Bajanova[1], I. Tarasenko[1], V. Baranov[2], A. Baranov[2], A. Kiselev[2], P. Glazkov[2], O. Ostapenko[2], E. Lesina[2]

1. Institute of Macromolecular Compounds, Russian Academy of Sciences - Russian Federation
2. Institute of Obstetrics and Ginecology, Russian Academy of Medical Sciences - Russian Federation

In the past decade, the development of gene therapy technology has focused on the design of new nonviral carriers for gene delivery. Among them complexes of polyamidoamine (PAMAM) dendrimers with DNA have shown to have rather interesting perspectives. However PAMAM dendrimers consist of unnatural buildings blocks and as a result its usage in vivo can create some problems. In connection with it biodegradable dendrimers built from natural amino acids might be more perspective.
Two lysine dendrimers (D1 and D2) and their starburst polymer derivatives were synthesized and investigated. One of them (D2) contained two lipophilic palmitoyl fragments at its C- terminal end. The dendrimer D1 was transformed into two starburst polymer derivatives. The first starburst polymer derivative of D1, polylysine derivative, was prepared by polymerization of N-carboxyanhydride of N-epsilon-carbobenzoxy lysine on dendrimer D1 amino groups. The second starburst polymer derivative of D1, poly(N-vinylimidazole) derivative, was prepared in two stage. At first stage the amino groups of D1 were modified by dimethylimidate of 2,2'-azobisisobutyric acid. Dendrimer D1 macroinitiator prepared at first stage was used then for polymerization of N-vinylimidazole with formation of starburst poly(N-vinylimidazole) dendrimer D1.
The agarose gel retardation assay has shown that dendrimers D1, 2 and starburst polymer derivatives of D1 form complexes with plazmid DNA (pCMV-nlsLacZ) at the relationship 1 : 1. It was shown that the density of D2- DNA complex was much higher than that of D1-DNA. This results in practically complete exclusion of ethidium bromide intercalation and decrease in fluorescence intensities already at the relationship dendrimer (D2)- DNA 4 : 1.
Peculiarities of transfection and expression of complexes of D1, 2 and starburst polymer derivatives of D1 with DNA in cells of the human epithelial carcinoma (HeLa) and the mouse mioblast (C2C12) cells will be discussed.

P D8 - Investigation of the conformational properties of the cyanide bound human myoglobin by FTIR spectroscopy

J. I. Al-Mustafa[1]

1. Jordan University of Science and Technology, Irbid - Jordan

Cyanide poisoning affects virtually all body tissues by attaching itself to the ubiquitous metalloenzymes and proteins rendering them inactive. In this work, the binding of the cyanide ion to the iron atom of the oxygen carrying protein myoglobin (Mb) has been investigated by Fourier Transform Infrared (FTIR) spectroscopy. The FTIR spectra of myoglobin ligated with the various isotopic forms of the cyanide ion reveal a weak asymmetric isotope sensitive band. In potassium phosphate buffer at pH of 7.4, the band was observed at 2126 cm^{-1} for Mb-$^{12}C^{14}N$. This band shifts to 2095 cm^{-1} for Mb-$^{12}C^{15}N$ and to 2080 cm^{-1} for Mb-$^{13}C^{14}N$. The observed asymmetry suggest that the measured bands are produced by the overlap of several C-N stretching peaks [1]. Applying mathematical resolution enhancement procedures such as Fourier self deconvolution [3], maximum likelihood deconvolution [4] and maximum entropy deconvolution [5]) and peak fitting, we were able to deconvolve the C-N stretching vibrations into 5 self-consistent peaks. In a potassium phosphate buffer at pH of 7.4, the 5 bands were observed at 2119, 2124, 2126, 2129 and 2135 cm^{-1} for natural abundance Mb-CN. These bands shift to 2088, 2092, 2095, 2098 and 2101 cm^{-1} for Mb-$^{12}C^{15}N$ and to 2074, 2077, 2081, 2085 and 2089 cm^{-1} for Mb-$^{13}C^{14}N$. All of the observed shifts are consistent with the values calculated on the basis of the simple harmonic oscillator (i.e., ~44 cm^{-1} for the Mb-$^{13}C^{14}N$ and ~33 cm^{-1} for the Mb-$^{12}C^{15}N$). This behavior provides strong evidence that these bands arise from a variation in the binding properties of the CN^- ion to the heme iron caused by, most probably, the protein conformational motion. The frequencies of the bands are independent on the pH (3.5-10.5) of the medium. However, slight variations in the relative intensities of the bands were observed. This result supports the presence of 5 rapidly interconverting conformational states for Mb-CN that might play a crucial role in facilitating the oxygen transport by the protein.

References
[1] S. Yoshikawa, D. O'Keefe, W.S. Caughey, *J. Biol. Chem.* 260 (1985), 3518-3528
[2] J. R. Morrey, *Anal. Chem.* 40 (1968), 905-914
[3] P. R. Griffiths, G. L. Pariente, *Trends Anal. Chem.* 5 (1986), 209-215
[4] L. U. Lucy, Astron. J., 79 (1974), 745-765
[5] B. R. Frieden, *J. Opt. Soc. Am.* 73 (1983), 927-938.

P D9 - Homology modelling of thimet oligopeptidase (EP24.15)

R. J. Anderson[1], A. Smith[2], K. Carr[1]

1. University of Sunderland - United Kingdom
2. Baker Medical Research Institute - Australia

Thimet oligopeptidase (EC 3.4.24.15, EP 24.15) is a soluble, zinc-dependent, thiol-activated metalloendopeptidase belonging to the thermolysin-like family of oligopeptidases and incorporates the classic HEXXH motif at its catalytic site. It hydrolyses and inactivates a wide range of oligopeptides, including neurotensin, bradykinin, gonadotrophin releasing hormone (GnRH) and substance P, and is involved in the production of met- and leu-enkephalins from their precursor peptides.[1]

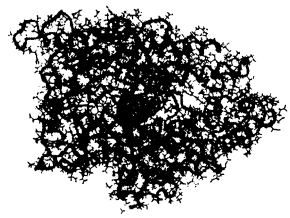


Fig. 1 - Homology Model of Thimet Oligopeptidase (EP24.15) with the inhibitor cFP.

Very little is known about the conformation of the catalytic site of EP 24.15 and work to date has focussed on the structural requirements of this enzyme for its substrates and inhibitors. Recently, the crystal structure of a closely related enzyme, neurolysin (EP 24.16) became available.[2] This enzyme has great similarity to EP24.15 in its structural requirements for substrates [3] and inhibitors and provides a good basis for homology mapping of EP 24.15.

Using Homology within the Insight environment (Accelrys, CA, USA), we have carried out homology mapping of EP24.15 using EP24.16 as the base protein. There is great structural homology between these enzymes and the computer generated model shows close structural similarity as expected. We have modelled the binding of the most studied EP24.15 inhibitor, *N*-[1-(*R, S*)-carboxy-3-phenylpropyl]-Ala -Ala-Tyr-*p*-aminobenzoate (cFP) and to both enzymes to evaluate the differences and further refine the model and will report the results of these studies.

References
[1] C.N. Shrimpton, and A.I. Smith., *J. Pept. Sci.*, 2000, 6, 251-263; A.I. Smith, C.N. Shrimpton, U.M. Norman, I.J. Clarke, A.J. Wolfson, and R.A. Lew., *Biochem. Soc. Trans.*, 2000, 28, 430-434.
[2] C.K. Brown, K. Madauss, W. Lian, M.R. Beck, W.D. Tolbert, D.W. Rodgers., *Proc. Natl. Acad. Sci., U.S.A.*, 2001, 98, 3127-3132.
[3] V. Oliveira, M. Campos, R.L. Melo, E.S. Ferro, A.C.M. Camargo, M.A. Juliano, and L. Juliano., *Biochem.*, 2001, 40, 4417-4425

P D10 - Histidine in proteins. Quantum chemical calculations related to catalytic mechanism

T. Beke[1], P. Hudáky[1], A. Perczel[1]

1. Eötvös Loránd University, Department of Organic Chemistry - Hungary

The conformational properties of histidine were analysed in the view of its catalytic power in enzymes, using the complete conformational library of N-formyl-L-histidinamide (see. P. Hudáky et. al. J. of Mol. Struct. (THEOCHEM) 2002. in press.). Histidine takes an active part in catalytic mechanisms of enzymes being both proton acceptor and donor. Accepting a proton the neutral histidine (Fig. 1) acquires a positive charge (Fig. 2). Although a part of the positive charge is often transferred to the surrounding residues (through H-bonds) the conformational characteristics of neutral and charged histidine moieties can be evaluated by investigating the conformational space of N-formyl-L-histidinamide. It was determined, that the protonation of N-formyl-L-histidinamide can induce conformational changes, due to the different potential energy hypersurface (PEHS) of the two forms. For the neutral tautomers the most favoured conformer is one of the γ-turns, while that for the positively charged form is the building unit of the left handed α-helix. In the present study these possible conformational changes – some of them are called the ring-flip mechanism (Haddad K.C., Sudmeier J.L., Sanford D.G., Bachovchin W.W., Meeting Abstract, Biochemistry 40 (29): 75 JUL. 24. 2001.) – are evaluated in the view of protonation processes of catalytic histidine residues in enzymes.

Fig. 1 - A neutral tautomer of N-formyl-L-histidineamide. Lightgrey represents negative to neutral potentials while darkgrey signals positive potentials on the surface

Fig. 2 - Positively charged form of N-formyl-L-histidinamide. Definition of darkness is given by Fig. 1.

P D11 - Discrete conformational states of short linear peptides in solution: experimental evidence of a new concept

N. Biris[1], A. Stavrakoudis[1], A. S. Politou[2], E. Mikros[3], M. Daitsiotis-Sakarellos[1], C. Sakarellos[1], V. Tsikaris[1]

1. University of Ioannina, Department of Chemistry - Greece
2. University of Ioannina, School of Medicine - Greece
3. University of Athens, Department of Pharmacy - Greece

According to the general belief short linear peptides are present in solution as an ensemble of rapidly interconverting conformations. The structures of such peptides derived from 1H-NMR spectroscopy are believed to represent only population-weighted averages over all conformers of the ensemble. To our knowledge, no experimental detection of discrete conformational states has been reported to date, even at the very low temperatures employed in many studies of the peptides.

In the recent past we provided experimental evidence for slow conformational exchange of Boc-[17O]Tyr(2,6-diClBzl)-OH in DMSO-d6 solution [1]. Following a new approach, we have further been able to identify discrete conformations of short linear peptides, which are stabilized by hydrogen bonding and undergo slow exchange with their extended structures at temperatures as high as room temperature and even higher. The novel information deduced from this study can be summarized as follows: (i) short linear peptides can adopt in DMSO-d6 stable conformations which vary in a way critically dependent on the reconstitution conditions used before their dissolution in DMSO-d6 and undergo very slow conformational exchange, (ii) the species normally observed by NMR represent only fast in the NMR time scale local conformational changes and not an overall average structure of the peptide, (iii) depending on the nature of the stabilizing interactions intramolecular hydrogen bonding can persist even at very high temperatures and (iv) DMSO-d6 seems to be a suitable solvent for inducing slow exchanges between different conformers. The reported study provides experimental evidence that short linear peptides are not the completely structureless entities that are so often thought to be. Furthermore our findings formulate a novel conceptual and experimental framework for the detailed study of short linear peptide conformations in solution.

References
[1] Tsikaris, V., Moussis, V., Sakarellos-Daitsiotis, M., Sakarellos, C., *Tetrahedron Lett.*, 2000, 41, 8651.

P D12 - A new 3_{10}-helicoid peptide inducer: applications to the main immunogenic region of the acetylcholine receptor

N. Biris[1], S. Mammonas[1], M. Sakarellos – Daitsiotis[1], C. Sakarellos[1], V. Tsikaris[1]

1. University of Ioannina, Department of Chemistry - Greece

One of the major problems in studying peptides that constitute parts of a protein is their conformational changes compared to their initial conformation within the protein. A great effort is made in developing peptide scaffolds that induce and stabilize discrete secondary structures [1]. In previous studies we have shown that sequential oligopeptide carriers Ac-(Lys-Aib-Gly)n-OH(SOCn) adopt a distorted 3_{10}-helical structure [2]. With the aim to induce helical structures, the peptide sequence –Lys(Ac)–Aib–Gly was incorporated in the C–terminal part of a series of analogues of the main immunogenic region of the acetylcholine receptor MIR(67-76) Trp-Asn-Pro-Ala-Aso-Tyr-Gly-Gly-Ile-Lys. The MIR analogues adopt a β-turn type I in the N-terminus and random structures in the C-terminus [3]. ^{1}H NMR spectroscopy and molecular dynamics experiments showed that when the –Lys(Ac)–Aib–Gly sequence is incorporated in the C–terminal part of peptides induces a 3_{10} helical structure along the peptide backbone.

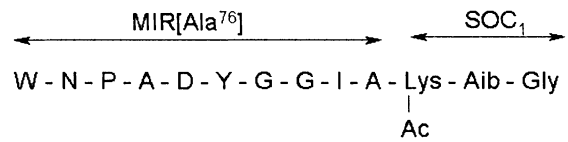


Fig. 1 - Incorporation of the -Lys(Ac)-Aib-Gly scaffold to MIR[Ala76]

References
[1] Ashley, S., Sherman, C., Sherman, J., *Biorganic and Medicinal Chemistry*, 1999, 7, 23.
[2] Tsikaris, V., Sakarellos, C., Sakarellos-Daitsiotis, M., Orlewski, P., Marraud, M., Cung, M.T., Vatzaki, E., Tzartos, S., *Int. J. Biol. Macromol.*, 1996, 19, 195.
[3] Tsikaris, V., Sakarellos, C., Cung, M.T., Marraud, M., Sakarellos-Daitsiotis, M., *Biopolymers*, 1995, 38, 291.

P D13 - Thermodynamics of a protein catenane

J. W. Blankenship[1], P. E. Dawson[1]

1. The Scripps Research Institute - U.S.A

Topological linking provides a unique way to engineer the structure and activity of organic compounds in supramolecular chemistry. Using a variety of strategies, multiple pseudorotaxanes, rotaxanes, and catenanes have been synthesized from organic scaffolds. Recently, protein domains have been added to the list with the creation of a backbone cyclized protein catenane based around the p53 tet domain [1]. Four linear domains fold and then undergo cyclization by the aid of native chemical ligation, forming a 'dimer' of catenanes in place of the linear tetramer. As might be expected, the structural contributions greatly stabilize the protein against thermal and chemical denaturation. To dissect this stabilization, and to better understand the resulting changes in protein behavior, we have prepared a dimeric form of the p53 tet domain based on the M340E/L344K dimer [2] and formed a catenane that does not associate with itself or other linear p53 peptides. Introduction of the linker did not greatly destabilize the linear peptide relative to the original dimer and the formation of the catenane proceeded on the same timescale as that of the tetrameric p53 catenane. Using the p53 dimer system, we found that the process of forming a catenane stabilized the dimer considerably – raising the T_m from 31° C to 83° C – similar to the difference betweeen the linear dimer and the WT tetramer. Topological linking therefore provides a powerful and unique tool for the stabilization of peptides and protein domains.

References
[1] Yan, L.Z, Dawson, P.E. Design and Synthesis of a Protein Catenane. *Angew. Chem. Int. Ed.* 40, pp. 3625-3627, 2001.
[2] Davison, T.S., Nie, X., Ma, W., Lin, Y., Kay, C., Benchimol, S., Arrowsmith, C.H. Structure and Functionality of a Designed p53 Dimer. *J. Mol. Biol.* 307, pp 605-617, 2001.

P D14 - A structure-activity study of [D-ALA^8]-dynorphin-(1-13)-peptide amide. Synthesis of analogues with modified amino acids in positions 0,1,3,4,7

I. Bobrova[1], M. Goodman[2], R. N. DeHaven[2], J. D. Daubert[2], L. Johansson[3], P. Karsnas[3], S. Burov[4], L. Terenius[5]

1. Institute of Organic Synthesis, Riga - Latvia
2. Department of Chemistry and Biochemistry, University of California, San Diego - U.S.A.
3. Malardalens Hogskola - Sweden
4. Institute of Macromolecular Compounds, St.Peterburg - Russian Federation
5. Experimental Alcohol and Drug Addiction Research, CMM, Karolinska Institute, Stockholm - Sweden

Dynorphin A, (Dyn A) a 17-amino acid peptide, is postulated to be an endogenous ligand for the κ opioid receptor. Dyn A(1-13) exhibits a similar receptor binding profile to Dyn A, therefore Dyn(1-13)NH_2 has been chosen as the parent structure for developing analogues. A series of dynorphin analogues was synthesized with the following modifications: 1) Tyr^1 was replaced by Tyr(Allyl), Fmoc-Tyr(Allyl); 2) N-terminal extention with Lys(Alloc), Fmoc-Lys(Alloc); 3) exchange of Gly^3 for Lys(Alloc); 4) Lys^{7}was substituted with Lys(Alloc); 5) replacement of two or three amino acid residues. The analogues were synthesized by solid-phase methodology using Fmoc-amino acid pentofluorophenyl esters. Side chains were protected with Boc- for Lys and Mtr- for Arg. Cleavage of the peptide from the solid support and deprotection of side chains was performed simultaneously in trifluoroacetic acid with scavengers. The resulting peptides were purified by RP-HPLC and characterised by mass spectrometry. The purified dynorphin analogues were assayed *in vitro* for their μ-,δ-,κ- and ORL-1 binding activity. The new dynorphin analogues synthesized were κ-selective. Incorporation of Lys(Alloc) in position 3 and 7 resulted in a compounds, which showed the highest κ-activity (Ki=0,25-0,30 nM). Replacement of Tyr^1 by Tyr(Allyl), Fmoc-Tyr(Allyl) and extention with Lys(Alloc) afforded an analogues which was essentially inactive at the μ,δ-type of opioid receptor, but exhibited modest κ-activity.
Most of the compounds exhibited modest activity at the ORL-1 receptor, as well.

P D15 - Computational docking and opioidmimetics: investigation of δ-opioid agonist and antagonist interactions

S. D. Bryant[1], S. Salvadori[2], R. Guerrini[2], G. Balboni[3], L. H. Lazarus[4]

1. Niehs, Lcbra - U.S.A
2. University of Ferrara - Italy
3. University of Cagliary - Italy
4. NIEHS - U.S.A.

Opioidmimetics with disparate bioactivities containing the pharmacophores 2,6-dimethyltyrosine (Dmt) and 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) were used to evaluate agonist and antagonist ligand interactions via molecular modeling and docking with the δ-opioid receptor. H-Dmt-Tic-1H-benzimidazole-2-yl (Bid) (1), H-Dmt-Tic-NH-CH_2-Bid (2), H-Dmt-Tic-Gly-NH-Bzl (5), δ-opioid receptor agonists (pEC50 = 7.3, 9.9, 8.5; respectively), and H-Dmt-Tic-NH-CH_2-CH_2-Bid (3), H-Dmt-Tic-Gly-NH-CH_2-Bid (4), and H-Dmt-Tic-Gly-NH-CH_2-Bzl (6), δ-opioid receptor antagonists (pA_2=8.3, 9.0, 9.3, respectively) were modeled based on the X-ray crystal structure of N,N(Me)$_2$-Dmt-Tic-OH (δ-antagonist) and extensive conformational searching of the C-terminal modifications. Four unique low energy conformers of each ligand were selected for docking with the δ-opioid receptor. The receptor was modeled based on the x-ray structure of the G-protein coupled receptor bovine rhodopsin using molecular dynamics simulations. The conformers characterized by agonist bioactivities displayed binding in two regions of the receptor. The first mechanism involved interactions between receptor residues Arg192, His301, His274, Tyr129 and Dmt with the Bid and Bzl groups in the aromatic pocket defined by Phe222, Trp274, Tyr308, Phe218, Phe270. The second mechanism involved interactions between Dmt and Asp128, Asp95, Asn131 and Gln105 with Tic near Trp274 and Bid or Bzl in the aromatic region. In contrast, low energy conformers of the antagonist derivatives displayed interactions in only one region of the receptor; similar to the first mechanism described for the agonist analogues. Although other studies have suggested that there is not a direct interaction between Asp128 and opioid ligands, this study implies that δ-agonists containing the Dmt-Tic pharmacophore may interact with residues in the region of Asp128 to activate the δ-opioid receptor.

P D16 - Will the N-terminus of human growth hormone releasing hormone (GHRH) adopt a polyproline (PPII) helix?

L. Cervini[1], C. Donaldson[1], S. C. Koerber[1], W. Vale[1], J. Rivier[1]

1. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037 - U.S.A

Over the past several years, attempts have been made to constrain the secondary structure of hGHRH and other members of the secretin/glucagon family. It is thought that the bioactive conformation of these peptide hormones is preferentially helical over the length of the molecules. Restricting these large peptides to a rigid bioactive conformation has met with difficulty and not been achieved. The method of choice that proved to be partially successful has been to form covalent lactam bonds between the side chains of residues at positions where salt bridges are likely to form and stabilize α-helical conformation. However, other factors limit the number of lactams that can be present in one molecule, such that more than one lactam bridge per peptide is not well tolerated by the receptor. The stability of the PPII helix lends itself to potential application in these molecules. The proline residue itself is a small scaffold, restricting the backbone conformation of the peptide. Sequential placement of prolines at i to i + 3 spacing is equivalent to the polyproline helices seen in the PP fold. We tested the hypothesis with four diproline analogs of [MeTyr^1,Ala^{15},Nle^{27}]hGHRH(1-29)-NH_2, paralleling the equivalent Glu-Lys lactam bridged analogs at positions 5-8, 9-12, 12-15 and 16-19. The lactam analogs are equipotent to or more potent than hGHRH(1-40)-OH in stimulating the release of GH in a rat pituitary cell culture assay. Alanine replacements at the bridging positions are also tolerated, which indicates that the side chain functionalities are not message sites for the receptor. Therefore, it was hypothesized that proline substitutions would be tolerated and hopefully would stabilize a preferred bioactive conformation. However, the analogs proved to be non-potent *in vitro*. Circular dichroism studies showed that the analogs adopt a helical conformation albeit at a higher concentration of TFE than that seen for the corresponding lactam analogs.

P D17 - Conformational studies of a new tetraamine-functionalized Tyr^3-Octreotate derivative (Demotate)

P. Cordopatis[1], P. Sotiriou[1], A. S. Galanis[1], G. A. Spyroulias[1], B. Nock[2], A. Nikolopoulou[2], E. Chiotellis[3], T. Maina[2]

1. University of Patras / Department of Pharmacy - Greece
2. NCSR "Demokritos" - Greece
3. University of Thessaloniki / Department of Pharmacy - Greece

Demotate is a new tetraamine-functionalized [Tyr3]octreotate derivative that binds 99mTc with high efficiency under mild conditions. The resulting radioligand [99mTc]-Demotate is stable for at least 6 h after labeling. The affinity of the unlabeled peptide to somatostatin receptors is high (IC50=0.13 nM) and comparable to that of [Tyr3]octreotate or [Tyr3]octreotide, as demonstrated by competition binding experiments in rat brain cortex or AR42J cell membrane preparations.
In order to understand the structure-function relationship of this biologically interesting compound, the role of the tetraamine chelator in the peptide structure and the binding affinity of the molecule towards 99mTc, conformational analysis of the peptide [DPhe1-Cys2-Tyr3-DTrp4-Lys5-Thr6-Cys7-Thr8 (disulfide bridged)] with and without the tetraamine group has been performed in solution through 1H NMR Spectroscopy. Assignment of each individual residue has been performed through 2D TOCSY spectra and dipolar inter-proton connectivities have been identified through NOESY maps recorded with a variety of mixing time. The solution structure of the peptide [Tyr3]octreotate has been determined through the DYANA program. High resolution models (low target function and rmsd values between the 20 models which comprise the family of structures) have been calculated using 213 NOE-derived constrains (155 meaningful; 19.4 per residue). Its structure presents great similarities with the analogous Sandostatin (Octreotide) molecule reported in bibliography. The C-terminal skeleton comprised by Cys7 and Thr8 has been found to form a turn-like structure (Figure 1).
NMR analysis and preliminary structure calculation has revealed that the attachment of the tetraamine chelator at the N-terminal of the [Tyr3]octreotate (Demotate) seems to impose some new structural features on the molecule. Distortion of the structure has been observed not only for the characteristic C-terminal turn, which is expected to be close in space to the tetraamine ligand of the parent molecule, but also for the peptide backbone.

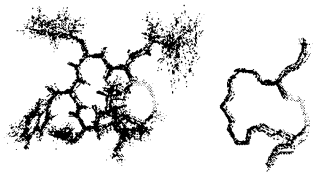


Fig. 1 - NMR solution structures of [Tyr3]octreotate with all atoms (left) and only backbone atoms (right)

P D18 - ^{1}H-NMR spectroscopy to probe the specific interactions of sense-antisense peptide epitopes and mAbs deriving from the La/SSB autoantigen.

M. Cung[1], C. Hemmerlin[1], M. Papamattheou[2], V. Tsikaris[3], M. Sakarellos-Daitsiotis[3], C. Sakarellos[3], E. Dotsika[2], A. G. Tzioufas[5], H. M. Moutsopoulos[5]

1. LCPM, UMR 7568 CNRS-INPL, Nancy - France
2. Dept. Parasitology, Hellenic Pasteur Institute, Athens - Greece
3. Dept. of Chemistry, Univ. of Ioannina, Ioannina - Greece
4. Dept. of Pathophysiology, National Univ. of Athens, Athens - Greece

The humoral autoimmune response in patients with Sjogrens Syndrome (SS) and Systemic Lupus Erythematosus (SLE) is directed against the La/SSB autoantigen, one of the protein particles of the Ro/La RNP complex, and more particularly against four distinct regions (145-164, 289-308, 301-318 and 349-364). According to the molecular recognition approach, translation of two complementary RNA strands into proteins generates pairs of peptides, which could bind to each other with high specificity and affinity. Anti-sense complementary peptides (cpep) corresponding to the epitopes 289-308 and 349-364 (pep) of La/SSB were synthesized and used for studying the idiotypic/anti-idiotypic network. We have found that anti-idiotypic Abs bind close or within the paratopic binding site of the idiotypic Abs. Moreover, using the cpep 289-308 we have been able to unmask the anti-La/SSB response in "false" negative sera.
The aim of this study is to shed more light on the specific linkage of the sense and anti-sense peptide epitopes of La/SSB, as well as on the interactions of anti-idiotypic Abs to the anti-sense epitopes.

pep289-308	H_2N-Ala-Asn-Asn-Gly-Asn-Leu-Gln-Leu-Arg-Asn-Lys-Glu-Val-Thr-Trp-Glu-Val-Leu-Glu-Gly
cpep289-308	Cys-Ile-Ile-Thr-Val-Glu-Leu-Glu-Pro-Val-Phe-Phe-His-Ser-Pro-Phe-Tyr-Glu-Phe-Ser-NH_2
pep349-364	H_2N-Gly-Ser-Gly-Lys-Gly-Lys-Val-Gln-Phe-Gln-Gly-Lys-Lys-Thr-Lys-Phe
cpep349-364	Pro-Arg-Thr-Phe-Ser-Phe-Tyr-Leu-Lys-Leu-Ala-Leu-Phe-Arg-Phe-Lys-NH_2

The preceding pairs of complementary peptides were incubated at 40°C for 30 min and the complex formation of each pair is under investigation by electrospray ionization mass spectrometry (ESI-MS). ^{1}H-NMR spectroscopy is also in progress to probe the specific pairing of pep289-308/cpep289-308, as well as of anti-cpep(289-308)mAbs to cpep(289-308).

P D19 - *Ab initio* chemical shielding calculations of alanine oligopeptide models

A. Czajlik[1], A. Perczel[1]

1. Eötvös Loránd University, Department of Organic Chemistry - Hungary

Chemical shielding anisotropy tensors have been determined for the all atoms of the backbone homoconformers and β-turn and polyprolin-like conformers of For-L-$(Ala)_n$-NH_2 (n=1-8) using the GIAO-RHF formalism with the 3-21G(d), 6-31+G(d) and the 6-311++G(d,p) basis sets. Two-dimensional chemical shift plots have been generated using all nuclei of all residues. We have found, that nine regions, corresponding to major conformational cluster could be separated by means of $^1H^{\alpha}$–$^{13}C^{\alpha}$ and $^1H^{\alpha}$–$^{13}C^{\beta}$ chemical shift-chemical shift plots. This fact provides a basis for the quantitative identification of conformers from NMR shifts data. Comparing the oligo-Ala(s) of different length, we concluded that the length of the main chain does not influence significantly the chemical shift values. Both the N- and C-terminal groups and residues show a remarkable chemical shifting effect, called capping effect. However this capping effect also depends on the type of backbone conformations. To some surprise the $^1H^{\alpha}$ chemical shifts of single stranded β-sheet $((\beta_L)_n)$ and $(\alpha_L)_n$ type backbone conformers are rather similar. In large β-turn-type structures that consist of antiparalel β-sheets, the $^1H\alpha$ chemical shift values are much higher, than those in a single stranded β-sheet $((\beta_L)_n)$ backbone conformers. Now the computed chemical shift differences between helices and β-sheets agree well with the experimental observations.

P D20 - Structural analysis of the antisigma protein FlgM

G. D'Auria[1], L. Falcigno[1], R. Oliva[1], G. Flores[1], M. Vacatello[1], M. Bertero[2], A. Galizzi[2], L. Paolillo[1]

1. Dept. of Chemistry - University of Naples "Federico II" - Italy
2. Dip. di Genetica e Microbiologia - Università di Pavia - Italy

FlgM protein regulates the process of flagellum biosynthesis in both gram-positive and gram-negative bacteria. It prevents late flagellar gene transcription by binding the specific σ factors that direct the RNA polymerase to specific promoter sequences. FlgM is itself regulated in response to the assembly of an incomplete flagellum known as hook-basal body intermediate structure. Upon completion of the hook-basal body structure, FlgM is exported through this structure out of the cell. Inhibition of sigma dependent transcription is relieved and genes required for the later assembly stages are expressed, allowing completion of the flagellar organelle. [1] As the flagellum is a crucial structure for infection ability of pathogenic bacteria, the molecular based knowledge of FlgM regulatory mechanism may open alternative strategies to control bacteria infection.
It was recently shown that FlgM in *Salmonella Typhimurium* can bind both to free σ^{28}, to prevent it from interacting with core RNA polymerase (RNAP), and to σ^{28}/RNAP to destabilise the complex. [2] It was also shown that the *Salmonella* FlgM C-terminal part becomes α-helix structured when bound to its target σ^{28}. [3]
Very recently it was identified, overproduced and functionally characterized FlgM in *Bacillus Subtilis*. [4] This protein (9.8 Kda) was shown to form a stable complex with the σ^D factor. Partial proteolysis experiments suggest that FlgM is organised in two regions: the N-terminal (residues 1-51) and the C-terminal (residues 65-84) moieties, which appear resistant to proteolitic cleavage. The two structural motifs are connected by a flexible loop, easily accessible to cleavage. In order to investigate the FlgM/σ^D interaction, the binding and transcription inhibition properties of the N-terminal and C-terminal fragments were separately tested. [5] The results suggest that the C-terminal region of FlgM, corresponding to a 36 residue polypeptide, is responsible of the σ^D binding and of the inhibitory activity.
Here we report on the NMR analysis of the overexpressed N- and C-terminal fragments of FlgM from *Bacillus Subtilis*. Our data show that the N- fragment is, at physiological pH values, in a random conformation. The N-terminal part of FlgM is hypothesised to be essential for flagella-specific export, and the lack of structure observed may be important for this function. The conformational characterization of the FlgM C-terminal fragment, will supply the elements useful to understand the molecular mechanisms of FlgM activity.

References
[1] Chilcott G.S. and Hughes K.T., *Microb. and Mol. Biol.Rev.*, 64, 694, 2000.
[2] Chadsey M.S., Hughes K.T. *J. Mol. Biol.*, 306, 915-929, 2001.
[3] Daughdrill G.W. et al., *Nat. Struct. Biol.*, 4,285-91, 1997.
[4] Bertero M., Galizzi A. et al. *J. Biol. Chem.* 274, 12103-107, 1999.
[5] Bertero M., J. Sclaefer, Callegari M., Ceciliani F. & Galizzi A. *submitted*, 2002.

P D21 - Temperature dependence of backbone nanosecond fluctuations from ^{15}N and ^{13}C NMR relaxation

V. A. Daragan[1], D. Idiyatullin[1], K. H. Mayo[1]

1. University of Minnesota, Department of Biochemistry, Minneappolis ,55455 - U.S.A

NMR relaxation-derived spectral density functions F(w) = 2wJ(w), where J(w) is the spectral density function, provide a novel model-independent way to visualize and analyze internal motional correlation time distributions for protein/peptide backbone and side-chains. These functions have been determined for CH and NH backbone bonds from the measurements of ^{13}C and ^{15}N NMR relaxation parameters at 3 magnetic fields for 56 residue immunoglobulin-binding domain of streptococcal protein G over a temperature range of 5 to 60 degrees. New method of deconvolution of internal motions and overall tumbling has been developed. Large contributions of nanosecond time scale motions (0.5 – 2 ns) into spectral density functions have been found for all residues. Activation energies of slow motion correlation times have been determined which are close to the activation energy of overall tumbling correlation times. Changes in energy landscape (correlation time activation energies) were found at high temperatures and it can be interpreted as a prelude to the melting transition.

P D22 - Structural and thermodynamic studies of diastereomeric β-hairpin mimetics

M. Erdélyi[1], V. Langer[2], A. Karlén[3], A. Gogoll[1]

1. Dept of Organic Chemistry, Uppsala University - Sweden
2. Dept of Environmental Inorg. Chem, Chalmers University of Technology - Sweden
3. Dept of Medicinal Chemistry, Uppsala University - Sweden

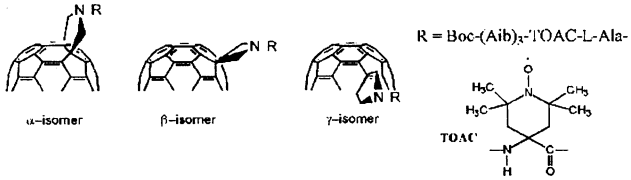
Two diastereomers of a model β-hairpin peptide mimetic were synthesized and studied with a combination of experimental (NMR, CD, IR, MS, X-ray) and computational methods (Monte Carlo/Molecular Mechanics calculation).
The secondary structure stabilising effects of hydrophobic interaction and hydrogen bonding were investigated. The comparison of the extent of folded hairpin population in non-competitive, polar aprotic and polar protic solvents illustrated the critical role of intramolecular hydrogen bonding on hairpin stability. Investigation of ^{1}H NMR melting curves of the diastereomeric compounds in a variety of solvents allowed a quantitative evaluation of the role of the hydrophobic effect and its relative importance compared to hydrogen bonding on secondary structure stabilisation. The comparison of thermodynamic constants of the two diastereomeric compounds in the same solvent allowed a quantitative evaluation of the hydrophobic effect. Significantly higher thermodynamic stability of the S,S-diastereomer compared to R,S-diastereomer in DMSO solution suggests that the hydrophobic effect might have significant influence on hairpin stability in polar solvents. Similar behaviour of the diastereomers in methanol solution indicates that in such a small model system the effect of a competing solvent dominates over weak hydrophobic forces.

P D23 - Interaction between the TOAC nitroxyl radical and the photoexcited [C70] fullerene triplet chromophore covalently linked to a peptide template

F. Formaggio[1], C. Toniolo[1], F. Della Negra[2], M. Maggini[2], G. Scorrano[2], M. Mazzoni[3], A. Toffoletti[3], C. Corvaja[3]

1. Biopolymer Research Centre, CNR, Dept. of Organic Chemistry, University of Padova, 35131 Padova - Italy
2. CMRO-CNR, Dept. of Organic Chemistry, University of Padova, 35131 Padova - Italy
3. Dept. of Physical Chemistry, University of Padova, 35131 Padova - Italy

Recently, peptides double labelled with a triplet precursor chromophore and with the C^{α}-tetrasubstituted, cyclic α-amino acid TOAC have been examined by time-resolved electron paramagnetic resonance (TR-EPR) by laser excitation [1]. The interaction between the photoexcited chromophore and the TOAC nitroxyl free radical has been studied through the spin polarisation effects on the radical spectral lines. Spin polarisation occurs because the quenching of the excited state takes places through the formation of an intramolecular radical triplet pair (RTP) intermediate.



RTP can exist in the metastable quartet state (S = 3/2) obtained by coupling of the triplet electron spin (S=1) with the radical unpaired electron spin (S=1/2). The TR-EPR spectrum of the quartet state has been observed and characterized in a number of compounds where a nitroxyl radical is covalently linked to a [C_{60}]fullerene derivative, including a molecular system consisting of a TOAC-containing peptide and [C_{60}]fullerene. In this work we synthesized and separated three isomeric compounds containing a TOAC-based peptide chain and [C_{70}]fullerene at the same sequence positions, but having the [C_{70}]fullerene covalently linked at three different 6,6 junctions (α-, β-, and γ-isomers). The sequence of the compounds examined is Boc-$(Aib)_3$-TOAC-L-Ala-pyrrolidin-[C_{70}]fullerene. The aim of this study was to test the effect on the radical triplet interaction of placing the chromophoric [C_{70}]fullerene at different relative orientations with respect to the TOAC radical. The α-, β-, and γ-isomeric [C_{70}]fullerene derivatives are indeed found to behave quite differently and, in particular, only for the γ-isomer the typical spectrum of the RTP in the excited quartet state is observed.

References
[1] C. Corvaja, E. Sartori, A. Toffoletti, F. Formaggio, M. Crisma, and C. Toniolo, *Biopolymers (Pept. Sci.)* 2000, *55*, 486-495.

P D24 - Internal dynamics of small serine protease inhibitors from the desert locust, *schistocerca gregaria*

Z. Gáspári[1], B. Szenthe[2], L. Gráf[2], A. Perczel[1]

1. Department of Organic Chemistry, Eötvös Loránd University - Hungary
2. Department of Biochemistry, Eötvös Loránd University - Hungary

The solution structure of the small serine protease inhibitors SGCI (*Schistocerca gregaria* chymotrypsin inhibitor) and SGTI (*Schistocerca gregaria* chymotrypsin inhibitor) has been determined previously by NMR spectroscopy [1]. While SGCI is a potent inhibitor of bovine chymotrypsin, SGTI exhibits weak and less specific inhibitory activity against mammalian enzymes. However, SGTI is a considerable more effective inhibitor of arthropodal (crayfish and shrimp) trypsins [2].
NMR proton-deuterium exchange experiments together with FT-IR measumerents revealed that the amide protons in SGTI are exchanging significantly more slowly than in SGCI. This observation led us to the hypothesis that the internal dynamics of the two molecules is considerably different. To test this, the SGTI-SGCI dimer (the two molecules are transcribed from a single mRNA) was expressed using N^{15} labelling. In paralel, molecular dynamics simulations were performed for both molecules with different programs and protocols. The results obtained suggest that the two closely related inhibitors exhibit different internal motions. Our presentation describes and compares the output of experimental and theoretical approaches used.

Reference
[1] Gáspári et al, *Eur J Biochem*, in press; Peptides 2000: 473-474
[2] Gráf et al, *Arch Biochem Biophys*, in press

P D25 - Study of chromatin peptides structure by mass/mass spectroscopy

G. L. Gianfranceschi[1], F. Giavarini[2], R. Bruni[3], I. Calzuola[1], L. De Angelis[2], G. Koch[1], V. Marsili[1]

1. Dep. Biologia Cellulare e Molecolare. Università di Perugia - Italy
2. Ist. Scienze Farmacologiche. Università di Milano - Italy
3. Dep. Informatica e Sistemistica. Università La Sapienza Roma - Italy

Low molecular weight peptides involved in the control of cell growth and differentiation have been isolated from the chromatin of animal and vegetal tissues [1]. The final steps of peptide purification lead to a fraction composed of a family of co-purified peptides with similar amino acid composition and sequence. Some predominant biochemical characteristics of this peptide fraction have been demonstrated: 1) molecular weight of about 1000 Da; 2) blocked N-terminus probably constituted by pyroglutamic acid; 3) predominant presence of glutamic acid, aspartic acid, serine, glycine and alanine; 4) possible serine phosphorylation. Further structural information have been obtained by mass spectrometry analysis (FAB+ and electron spray). By this technique it is possible to know the exact molecular weight of a peptide and also the molecular weight of its breakdown products. So for a single peptide of low molecular weight in some cases we can determine by mass spectrometry the exact sequence. For a mixture of peptides, the design of the sequences is more complicated, however some information can be obtained to try to design a molecular model. The sequences of a series of peptides designed on the basis of combined information arisen from biochemical and mass spectrometry analisys have been obtained [2]:

1) pyroGlu-Ala-Glu-Ser-Asn
2) PyroGlu-Ala-Gly-Glu-Ser-Glu-Asp
3) pyroGlu-Ala Gly- Glu-Glu-Ser-Asn
4) pyroGlu-Ala Gly-Glu-Glu-Glu-Glu-Ser-Asn
5) pyroGlu-Asp-Asp-Ser-Asp-Glu-Glu-Asn
6) pyroGlu-Val-Ala-Asp-Ser-Asp-Gln-Asn

These acidic structures show several biochemical peculiarities and biological effects very similar to these observed in the native peptides: a) serine phosphorylation by kinase CKII; b) binding to DNA "in vitro" in the presence of divalent cations with consequent control of transcription; c) stimulation of specific differentiation pathway in PC12 and HL60 cells [3]. However the synthetic peptides do not exert the potent control of cell proliferation demonstrated by the native fraction. Recently some experimental evidences support the hypothesis that the inhibition of cell proliferation is related to a peptide sequence of molecular weight 572 which is a sharp peak present in the mass spectrum of the peptide native fraction. Consequently the ion [MH+]=572 has been subjected to mass/mass spectroscopy. To analyse this spectrum the study of an automatic computing program is in progress. The availability of a computing program that automatically provides the possible amino acid combinations of a peptide structure and of its breakdown products, represents a winning key for the achievement of peptide sequence from the analysis of mass/mass spectrum. The molecular models designed from the analysis of the mass/mass spectrum of the ion [MH+]=572 will be synthesised and tested on the proliferation of several cell lines.

References:
[1] L.Mancinelli, F.Chillmi, E.Cardellini, V.Marsili, F.Giavarini, L.De Angelis, G.Lugaro & G.L.Gianfranceschi. *Biol. Chem.* 380, 31-40 (1999)
[2] G.L.Gianfranceschi, A.Czerwinski, A.Angiolillo, V.Marsili, E.Castigli, L.Mancinelli, A.Miano, M.Bramucci & D.Amici. *Peptides* 15,7-13 (1994)
[3] V.Marsili, A.Angiolillo & G.L.Gianfranceschi *Cell. Mol. Biol.* 41, 515.525 (1995)

P D26 - Modeling local conformational effects on the reactivity of widespread basic cleavage sites.

R. Improta[1], L. Esposito[1], R. Oliva[2]

1. Istituto di Biostrutture e BioImmagini CNR, Via Mezzocannone 6, 80134 Napoli - Italy
2. Diparimento di Chimica, Università "Federico II", Complesso Universitario Monte S. Angelo, V. Cintia , 80126 Napoli - Italy

Cleavage of the peptide bond is a key event in the chemistry of biological systems. Proteolytic cleavage at monobasic [1] and multibasic [2] sites is the most common processing mechanism to release mature peptide sequences from their larger biosynthetic precursors. Not every potential basic cleavage site in precursors is however recognized by the corresponding convertases. The recognition is usually related to the substrate secondary and tertiary structures, affecting the accessibility and the shape complementarity of the cleavage sites with respect to the processing enzymes. The effect of different conformations in determining the substrate reactivity is instead less investigated. On the ground of the available structural and modeling data, here the effect of the local conformation on the cleavability of the scissile peptide bond is investigated by ab initio calculations. Oligopeptide analogues, representing the minimal recognition sequence in the basic cleavage sites, are employed to investigate the influence of local conformational effects, as well as the role played by the inductive effect of basic residues, in modulating the peptide bond reactivity.

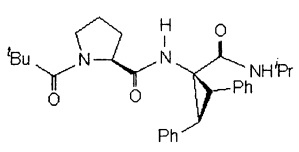
References
[1] Devi L., *FEBS Letters* 1991, 25, 189-94.
[2] Brakch N., Rholam M. Simonetti M., Cohen P., *Eur. J. Biochem.* 2000, 267, 1626-33.

P D27 - Structural analysis of model dipeptides incorporating a highly constrained cyclopropane analogue of phenylalanine

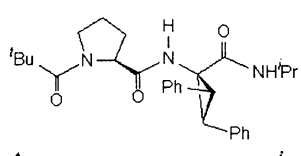
A. I. Jimènez[1], S. Royo[1], C. Cativiela[1]

1. Dept. of Organic Chemistry, Institute of Materials of Aragon, University of Zaragoza-CSIC, 50009 Zaragoza - Spain

In order to evaluate the possible influence of the side chain orientation on the backbone conformation we have synthesized the model dipeptides ^{t}BuCO-L-Pro-c$_3$diPhe-NH^{i}Pr, where c$_3$diPhe represents (2*S*,3*S*)- and (2*R*,3*R*)-1-amino-2,3-diphenylcyclopropanecarboxylic acid, two cyclopropane analogues of phenylalanine. Their structural behaviour has been studied in solution by FT-IR and ^{1}H-NMR experiments, and in the solid state by X-ray diffraction.
Racemic Boc-c$_3$diPhe-OH was prepared by 1,3-dipolar cycloaddition of phenyldiazomethane to (*Z*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone followed by transformation of the oxazolone ring. Peptide synthesis was carried out using the mixed anhydride method with isobutyl chloroformate as the coupling agent. The diastereomeric dipeptides ^{t}BuCO-L-Pro-(2*S*,3*S*) c$_3$diPhe- NH^{i}Pr and ^{t}BuCO-L-Pro-(2*R*,3*R*) c$_3$diPhe-NH^{i}Pr were obtained in good yield and easily separated by column chromatography on silica gel. The absolute configuration of the c$_3$diPhe residue was deduced from the crystal molecular structure of the dipeptides.
In the solid state, the (2*S*,3*S*)c$_3$diPhe-containing compound adopts a classical βII-turn disposition, with a ^{i}Pr*NH* to ^{t}Bu*CO* hydrogen bond. In contrast, the dipeptide incorporating the (2*R*,3*R*) enantiomer accommodates an *open* βII-turn structure (lacking the usual *i*+3 to *i* hydrogen bond), with a γ-turn centred at the c$_3$diPhe residue and stabilized by a ^{i}Pr*NH* to Pro-*CO* hydrogen bond. This result is highly remarkable, since the γ-turn (C7 structure) has only rarely been observed in crystallised small linear peptides. The IR and NMR data indicate that, in solution, the two dipeptide sequences accommodate the type I and II β-turns in different proportions, depending on the solvent and the c$_3$diPhe chirality. Thus, in chlorinated solvents, (2*S*,3*S*) c$_3$diPhe mainly induces a type I β-turn, whereas the type II β-turn is largely favoured by the (2*R*,3*R*) residue.
These peptides constitute an excellent example of the correlation between side chain orientation and peptide backbone conformation.



^{t}BuCO-L-Pro-(2*S*,3*S*)c$_3$diPhe-NH^{i}Pr



^{t}BuCO-L-Pro-(2*R*,3*R*)c$_3$diPhe-NH^{i}Pr

P D28 - Conformation characterization of flexible oligopeptides

A. Kalászi[1], G. Mező[2], F. Hudecz[2], Ö. Farkas[1]

1. Eötvös Loránd University, Dept. of Organic Chemistry - Hungary
2. Research Group of Peptide Chemistry, Hungarian Acad. Sci. - Hungary

We propose a method to explore and characterize typical conformations, conformational families and to obtain more detailed insight into the conformational behavior of large flexible molecules, e.g. oligopeptides. Our new tool is also intended to help interpretations of experimental and biological results.
Determining the crystal structure of such molecule is usually not feasible or does not contain sufficient information on all conformers existing in solution. On the other hand, often only few distance constraints can be derived from NMR studies, which may lead to uncertain structures.
The present method is based on high energy molecular dynamics studies, in vacuum, incorporating geometry optimization algorithms to explore the full conformational space. The resulting structures are used as an input for multiple dynamics simulations in a solvent environment which finally provides the data for conformational characterization and classification of the conformational families. The applications were carried out using the GROMACS force field.
Apart from the conformational characterization of peptides, additional properties were also studied. For example, in the case of oligotuftsin (TKPKG)$_4$, a new peptide-based drug carrier [1], accessibilities to specific reaction sites were determined. In another example, selected members of a TXTXT peptide library, corresponding to a mucin-2 glycoprotein epitope motif [2], the conformational basis of antibody binding was investigated. The structural information obtained form the CD spectra of the above examples were also compared to the computed conformers.

References
[1] Mező et al., Peptides 2000, *ed.: Jean Martinez, Jean-Alain Fehrentz, EDK, Paris, France 2001*, p. 253-254
[2] Uray et al., Peptides 1998, *ed.: S. Bajusz, F. Hudecz, Akadémiai Kiadó, Budapest*, p. 638-639