

P B29 - Solution NMR structure of the α -conotoxin GI-HSV glycoprotein D chimera

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The α -conotoxins are small peptides, 12-25 residues long with either 2 or 3 disulphide bridges. They are found in the venom of marine cone snails and target neuromuscular and neuronal nicotinic acetylcholine receptors, binding to their acetylcholine sites and preventing channel opening. Two neuromuscular α -conotoxins, GI and SI both 13 residues long, have high sequence homology and two disulphides, between residues 2-7 and 3-13, but very different binding affinities. Their structures have been solved by NMR and crystallography [1-3]. Structural comparisons show that they share a common backbone fold, despite a significant residue substitution at position nine, namely a proline for arginine between SI and GI respectively. This backbone conformation is known as the α -conotoxin fold, and provides a rigidity to the overall conformation of these α -conotoxins. The region in these peptide structures which exhibits the highest similarity in backbone conformation is that which has the lowest sequence homology (residues 9-12). As this region is between the two disulphide bridges, it seems that the backbone conformation is being tightly constrained by the disulphides, despite significant differences in sequence.

In view of this rigidity of the α -conotoxin fold, it made it an ideal feature for switching the native residues 9-12 of GI, [RHYS], for the most critical residues of the epitope-binding region of the herpes simplex virus glycoprotein D (HSV gD), namely residues [NPVG] [4]. It was demonstrated that antibodies raised to this chimeric polypeptide successfully recognised the native HSV gD protein. Structural studies using solution NMR have been carried out to investigate whether the α -conotoxin fold is still retained in this chimeric peptide. Analysis of the NMR data strongly suggests that the chimeric molecule is far more flexible in the 9-12 region, namely adopting two distinct conformations, involving principally different disulphide conformations. It seems likely that the introduction of the highly flexible and sterically small glycine at position 12 in the chain has allowed the conotoxin fold to become more flexible in this region. Our results therefore suggest that while there is some retention of the classical conotoxin backbone fold within the chimera, due to the retained disulphide pattern, there has been an increase in the flexibility of the structure overall, resulting in two conformations of the C-terminal residues as a result.

References

- [1] Benie, A. J., Whitford, D., Hargittai, B., Barany, G. & Janes, R. W. (2000) *FEBS Lett.* 476, 287-295.
- [2] Gehrmann, J., Alewood, P.F. & Craik, D.J. (1998) *J. Mol. Biol.* 278, 401-415.
- [3] Guddat, L. W., Martin, J. A., Shan, L., Edmundson, A. B. & Gray, W. R. (1996) *Biochem.* 35, 11329-11335.
- [4] Mező, G., Drakopoulou, E., Paal, V., Ranjnavolgyi, E., Vita, C. & Hudecz, F. (2000) *J. Peptide Res.* 55, 7-17.

P B31 - Structural study of the HIV-1 gp160 cleavage site by native and modified sequences. 2. Role of the N-terminal secondary structure.

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The HIV envelope glycoprotein gp160 is intracellularly processed into the gp120 and gp41 glycoproteins by furin and other precursor convertases (PCs), newly discovered subtilisin-like serine-proteases. The cleavage of the gp160 is a prerequisite for the virus infectivity, as gp120 and gp41 play a key role in the fusion of viral and cellular lipid membranes. The proteolytic activation of the HIV-1 envelope glycoprotein gp160 selectively occurs at the carboxyl side of the sequence R508-E-K-R511 (site 1), in spite of the presence of the other consensus sequence: K500-A-K-R503 (site 2). These data indicate that other factors are required in addition to the consensus motif R/K-X-R/K-R for the molecular recognition. To investigate the possible role of specific secondary structure motifs, flanking the physiological cleavage site, a series of synthetic analogues were designed and characterized

	site 2	site 1
p498	PIKAKRRVQREKR	↓AVGVG
h-REKR	EHVNAIQEARRLLNREKR	↓AVGVG
r-REKR	DPKGVTIVVT	↓VIREKR↓AVGVG

p498 is 19-residues peptide, spanning the gp160 sequence P498-G516 around the processing sites 1 and 2, and properly digested by furin at the site 1. The conformational study showed that p498 is characterized by a C-terminal loop, exposing the physiological site 1, and a N-terminal helix, enclosing the site 2. [1] The 23-mer peptides h-REKR and r-REKR were then designed to exhibit the native, site 1 containing, gp160 sequence: R508-G516 at the C-terminal side (in black) and an helical [2] or random conformation, [3] respectively, at the N-terminal side (in grey). h-REKR was shown to be cleaved by furin with efficiency comparable to the full-native p498 sequence, unlike the random analogue r-REKR. Here the NMR conformational analysis of these two analogues is presented and related to the different exhibited activity.

References

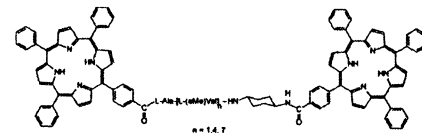
- [1] R. Oliva, M. Leone, L. Falcigno, G. D'Auria, M. Dettin, C. Scarinci, C. Di Bello and L. Paolillo, *Chem. Eur. J.* 2002, in press.
- [2] S. Fiori, S. Mammì, E. Peggion, P. Rovero, S. Pegoraro and R. P. Revoltella, *J. Pept. Sci.* 1997, 3, 336-346.
- [3] E. Krause, M. Beyermann, H. Fabian, M. Dathe, S. Rothermund, M. Bienert, *Int. J. Peptide Protein Res.* 1996, 48, 559-568.

P B30 - β -Turn and 3_{10} -helical peptide spacers for exciton coupled CD studies

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In exciton coupled CD studies acylating chromophores are introduced into the substrate, and the signs of split CD resulting from the coupling between the chromophores are used to determine the absolute sense of twist. This is a very versatile method but the extent of its potentiality has yet to be explored, particularly in conformational studies of large molecules. The extent of exciton coupling is inversely proportional to the square of the interchromophoric distance and proportional to the square of extinction coefficients of the coupled chromophores. Therefore, the intensity of chromophoric absorption is of prime importance in increasing the sensitivity over the large distance between interacting transition moments. 5-p-Carboxyphenyl-10,15,20-triphenylporphyrin (TPP), characterized by a sharp Soret band at 414 nm ($\epsilon=350,000$) has proven to be promising for the generation of new exciton coupled CD chromophores [1].



However, only preliminary investigations have been performed on the distance dependence of porphyrin CD exciton coupling [1]. For a CD study we decided to exploit the potentially 3_{10} -helical -L-Ala-[L-(α Me)Val]_n- oligopeptides as molecular rulers separating the two porphyrin chromophores. More specifically, we focused on the di-, penta- and octapeptides ($n = 1, 4, 7$) where the N- to C-terminus distance increases by one turn of the ternary helix (6.3 Å) from the lower to the immediately higher oligomer. We incorporated the achiral *trans*-1,4-diaminocyclohexane *semi*-rigid spacer at the C-terminus in order to provide the system with identical chromophores at either end.

The synthesis of these *bis*-TPP peptide conjugates was carried out by solution methods. An FT-IR absorption analysis showed that the peptides are folded in either the β -turn ($n = 1$ oligomer) or in the 3_{10} -helical ($n = 4$ and 7 oligomers) conformation in CDCl₃ solution. In CH₂Cl₂, CHCl₃ and toluene solutions well developed, exciton coupled CD curves are exhibited by the *bis*-TPP, ($n = 1$ and $n = 4$) peptides, but not by the *bis*-TPP peptide with the longest spacer ($n = 7$), thereby suggesting a remarkable distance effect. Surprisingly, the signs of the exciton couplet are inverted in TFE, HFIP, and DMSO solutions for both *bis*-TPP peptides. As for the shortest *bis*-TPP peptide ($n = 1$): (i) in the CDCl₃/TFE solvent mixture the CD transition takes place between 91% and 95% TFE, and (ii) heating of either the toluene or the DMSO solution to 60 °C does not produce inversion of the CD signals, but just a decrease of their intensities. In summary, the -L-Ala-[L-(α Me)Val]_n- ($n = 1-4$) β -turn and 3_{10} -helical peptides are excellent, tunable spacers in exciton coupled CD investigations. A significant porphyrin-porphyrin chromophoric interaction takes place at 17 Å ($n = 1$ spacer) and 23 Å ($n = 4$ spacer), but not at 29 Å ($n = 7$ spacer).

Reference

- [1] S. Matile, N. Berova, K. Nakanishi, J. Fleischhauer, and R. W. Woody, *J. Am. Chem. Soc.* 1996, 118, 5198-5

P B32 - Multifunctional antibacterial peptides

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Members of the proline-rich antibacterial peptide family, pyrrocoricin, drosocin and perhaps apidaecin appear to kill responsive bacterial species by binding to the multihelical lid region of the bacterial DnaK protein. Pyrrocoricin, the most potent among these peptides, is non-toxic to healthy mice, and can protect these animals from bacterial challenge. By observing a strict correlation between binding to the target protein and antibacterial activities of pyrrocoricin analogs we further verified that DnaK was the bacterial target macromolecule. A structure-antibacterial activity study of pyrrocoricin against *Escherichia coli* and *Agrobacterium tumefaciens* identified the N-terminal half, residues 2-10, the region responsible for inhibition of the ATPase activity, as the fragment that contains the active site. According to cell penetration assays, labeled versions of all three native peptides entered *E. coli* cells and mouse macrophages, and deletion of the C-terminal half of pyrrocoricin significantly reduced the peptide's ability to enter either types of cells. These findings highlighted pyrrocoricin's suitability in combating intracellular pathogens and the general utility of the peptide or its carboxy-terminal domain for delivering drug leads into mammalian cells. We provided further evidence for the domain structure of the proline-rich antibacterial peptides by using chimeras of the putative functional domains of pyrrocoricin and drosocin. Some of the mixed and matched sequences retained their ability to kill *E. coli*, *Salmonella typhimurium* and *A. tumefaciens*. While pyrrocoricin appeared to have a more universal pharmacophore, drosocin featured a more robust intracellular delivery unit. Interestingly, the N-terminal positive charges are also involved in the interaction with bacterial membranes. An Ala-scan on the D-E helix of *E. coli* DnaK identified key receptor residues for pyrrocoricin: Gln588, Glu589, Gln592, Gln595, Leu597, Met598, Ile600, Ala601, Gln602 and Gln604. They all sit nicely together on the X-ray structure-derived model and form a patch which appears to be an interaction site. On a flexible docking model, this surface intimately touches pyrrocoricin's Tyr6-Pro10 pharmacophore segment.

P B33 - PESCADOR: The peptides in solution conformation database: online resource.

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In recent years a large body of data has been obtained from Nuclear Magnetic Resonance (NMR) and Circular Dichroism (CD) experiments on the influence of the amino acid sequence and various other parameters on the conformational state of peptides in solution. Interpreting the experimental data in terms of the conformational populations of the peptides remains a key problem, for which current solutions leave appreciable room for improvement. Considering that making this body of data available for surveys and analysis should be instrumental in tackling the problem, we undertook the development of Pescador: The 'Peptides in Solution Conformation Database: Online Resource'. Here, we present the organization and data contents of Pescador: The 'Peptides in Solution Conformation Database: Online Resource', which stores available data from NMR and CD measurements for peptides in solution of up to 30 residues long as well as information on the structural parameters derived from these data. It also features specialized Web-based tools for data deposition, and means for readily accessing the stored information for analysis purposes. The database can be accessed on the WEB at the address: <http://www.ucmb.ulb.ac.be/Pescador/>. We illustrate how the data deposited in Pescador for alpha proton δ -values that have been measured for different peptides in different laboratories can be used to derive a new set of non-structured alpha proton δ -values. These values are shown to be similar to those obtained experimentally from series of glycine-based model peptides in water and their variation with increasing trifluoroethanol (TFE) concentration is similar to that reported for model peptides. We show, furthermore, that the chemical shift data in Pescador can be used to derive correction factors that take into account effects of neighboring residues, thus producing sequence-dependent non-structured alpha proton δ -values. These correction factors compare favorably with those recently derived from a series of model GGXGG peptides [1]. These encouraging results suggest that, as the quantity of NMR data on peptide deposited in Pescador increases, surveys of these data should be a valuable means of deriving key parameters for the analysis of peptide conformation.

Reference

- [1] S. Schwarzinger, G. J. Kroon, T. R. Foss, J. Chung, P. E. Wright, and H. J. Dyson: 2001, *J. Am. Chem. Soc.*, **123** (13), 2970-2978.

P B35 - Structure-function relationship studies on analogs of the 1-34 fragment of parathyroid hormone (PTH) containing β -alanine residues at positions 18 and 19.

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The parathyroid hormone (PTH) is an 84 amino acid residue peptide which plays a key physiological role in the regulation of calcium levels in animal serum. The N-terminal 1-34 sequence of PTH is equipotent and displays the full range of bone-relevant activities as in the intact PTH. On the basis of results from our previous studies on a series of active and inactive analogs of the PTH(1-34) [1], we suggested that the structural elements essential for biological activity are two N-terminal and C-terminal helical segments connected by hinges or flexible points around positions 12 and 19. To probe this hypothesis, in the present work we synthesized and characterized the following PTH(1-34) analogs containing β -Ala residues at positions 18 and 19:

[Nle⁸, β -Ala¹⁸, NaI²³, Tyr³⁴]hPTH(1-34)NH₂ (Analog I)

[Nle^{8,18}, β -Ala¹⁹, NaI²³, Tyr³⁴]hPTH(1-34)NH₂ (Analog II)

Biological activity and binding capacity of analog I are about one order of magnitude lower than those of the parent compound [Nle^{8,18}, NaI²³, Tyr³⁴]hPTH(1-34)NH₂. Biological potency and binding affinity are partially recovered in analog II, which is about two-fold more active than analog I. The conformational properties of the two analogs were studied in aqueous solution containing dodecylphosphocholine micelles by CD, 2D-NMR and molecular dynamics calculations. The structures of the two analogs are very similar and characterized by two N-terminal and C-terminal helical segments spanning the sequences 5-12 and 20-32, respectively. The NMR results indicate that stability of these helical sequences is practically identical in the two analogs. The only detectable difference between the two peptides is a less defined conformation in the central segment Leu⁵-Arg¹⁸ of analog I where helical NOEs are clearly missing. Insertion of β -Ala¹⁸ at position 18 eliminates the highly conserved hydrophobic side-chain (Leu, Val or Met) found in PTH(1-34) sequences of various species. Full activity is preserved in the Nle¹⁸ synthetic analogs. Our results suggest that the lower binding capacity of analog I compared to analog II is due to the absence of the hydrophobic side chain in position 18, which could be involved in a hydrophobic interaction with a complementary site in the receptor, and not to a conformational difference.

Reference

- [1] Peggion, E., Mammi, S., Schievano, E., Behar, V., Rosenblatt, M., Chorev, M., *Biopolymers* 50, 525 (1999) and references therein.

P B34 - Conformational and sequential requirements of interaction between the chemokine SDF-1 and the receptor CXCR4

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Chemokines constitute a superfamily of small (8-10kDa), inducible, pro-inflammatory cytokines that are involved in a variety of immune and inflammatory responses, acting primarily as chemoattractants and activators of specific types of leukocytes. About 50 human chemokines and nearly 20 receptors have been identified and characterized in little more than a decade since the discovery of interleukin-8 (IL-8), the first chemotactic cytokine [1].

Through the interaction with G-protein coupled receptor chemokines induce cytoskeletal rearrangement, firm adhesion to endothelial cells and directional migration. Latest studies report the involvement of chemokine receptor CXCR4 in human breast cancer metastasis[2]. Neutralizing the interaction of CXCR4 with its specific ligand Stromal Derived Factor-1 (SDF-1/CXCL12) significantly impairs tumor progression. Although the structure of SDF-1 and the basis for its functional activity like the separation of CXCR4 activation from binding [3] are known there is no structural knowledge of the chemokine receptor CXCR4.

In order to obtain synthetic molecules for novel diagnostic and therapeutic approaches for the treatment of breast cancer progression, currently, intense efforts are underway to identify the structural and sequential requirement of interaction between the chemokine SDF-1 and the receptor CXCR4 by conformational analysis of peptide analogs.

References

- [1] M. Baggiolini: *Journal of Internal Medicine* (2001); 250, 91-104
- [2] A. Muller et al.: *Nature* (2001); 410, 50-56
- [3] M.P. Crump et al.: *The EMBO Journal* (1997); 23, 6996-7002

P B36 - Which of the secondary structure elements of proteins may occur in vacuum?

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Protein architectures show strong similarities regardless of their amino acid composition: the backbone folds of the different secondary structural elements exhibit nearly identical geometries. To investigate the principles of folding properties, oligopeptide models {e.g. HCO-(NH-L-CHR-CO)_n-NH₂} were studied as suitable molecules. Previously, ab initio structure determinations have provided a handful information on the conformational building units of di- and tripeptides (e.g. HCO-L-Xxx-NH₂ and HCO-L-Xxx-L-Yyy-NH₂). With the exception of proline, for any natural α -amino acid residue a maximum of nine differently folded backbone types are available. All of these conformers have different relative energies. The present study compiles an ab initio database of optimized HCO-(L-Xxx)_n-NH₂ structures, where 1n8 and Xxx = Ala or Gly. All homoconformers (α -helix, β -sheet, collagen helix etc.) of the different backbone folds were optimized with additional β -turn type heteroconformer. The comprehensive analysis of more than 150 fully optimized polyalanine and polyglycine structures reveals the same energy preference profile of major secondary structures as found in globular proteins. The analysis of relative energies at 3 different levels of theory [RHF/3-21G, RHF/6-311++G(d,p),/RHF/3-21G and RHF/6-311++G(d,p)] for the above achiral and chiral molecular structures show how the above common secondary structure elements are gradually becoming more and more stable folds of these oligopeptides as the length of the polypeptide chain increases. This indicates that local energy preference seems to be a major driving force in peptide and protein folding. Furthermore, preferred conformers of the gas phase are rather similar as those observed in proteins crystallized from aqueous media. Indeed, relative energies for the computed different conformers show remarkable similarity to occurrence frequency of the same structures retrieved from a non-homologous X-ray database.

P B37 - Cyclic peptides from different flaxes

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Cyclic peptides from plants and other natural sources are recently objects of intensive investigations because of their biological activities [1-5], like suppression of the mammalian immune system [6,7] and antimalarial activity [8]. It is believed that cyclic peptides are components of the plant defense system [5], able to inhibit effectively pathogens and pest enzymes which makes these moderate size molecules attractive objects of investigations. This encouraged us to look for new structures in different strains of lin and in different part of these plants. An extensive study of chosen varieties of flax has shown the presence of different cyclic peptides. All investigated flaxes contain CLA, a cyclic peptide (PPFFLILV), while concentration of other peptides depends strongly on the strain of lin. The highest concentration of cyclic peptides was observed for seed and chaff material while leaves and stem do not contain such compounds. Such a distribution of cyclic peptides in plants strongly supports the view on their protective role in seeds. Almost all cyclic peptides from flaxes (except CLA and CLX) contain a methionine residue. This residue in cyclic peptides from flax is unusually sensitive to sulfur oxidation in aerobic conditions what increases the number of different chromatographically stable structures and diminishes yield of purifications. A very interesting property of pairs of cyclic peptides of identical sequences but with different HPLC and spectroscopical characteristics is reversibility of the temperature effects in NMR and CD spectra. The progress in studies on cyclic peptides from different flaxes will be presented.

References

- [1] Morita H., Shishido A., Matsumoto T., Itokawa H., Takeya K. *Tetrahedron*, 55 (1999) 967-976
- [2] Morita H., Yun S.Y., Takeya K., *Bioorg. Med. Chem.* 5 (1997) 2063-267
- [3] Morita H., Kayashita T., Takeya K., Itokawa H., Shiro M., *Tetrahedron*, 53 (1997) 1607-1616
- [4] Ding, Z., Zhou, J., Tan N., Teng, R., *Planta Med.*, 66 (2000) 386-388
- [5] Luckett Garcia S.R., Barker J.J., Konarev A.V., Shewry P.R., Clarke A.R., Brady R.L., *J. Mol. Biol.*, 290 (1999) 525-533
- [6] Fisher G., *Angew. Chem., Int. Ed. Engl.*, 33 (1994) 1415
- [7] Wieczorek Z., Bengtsson B., Trojnar J., Siemion I.Z., *Pept. Res.*, 4 (1991) 275
- [8] Bell A., McSteen P.M., Cebrat M., Picur B., Siemion I.Z., *Acta Pol. Pharm.*, 57 (2000) 134-136

P B39 - Peptoid residues and β -turn formation

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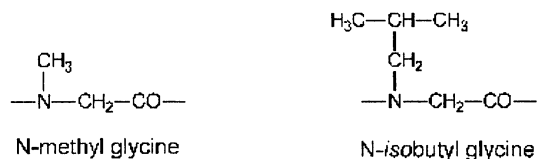
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Peptoid oligomers of N-alkylated glycines are a relatively new class of peptidomimetics that present interesting properties for the development of new drugs. They are more stable than peptides toward proteolytic enzymes.

The nature of their side chains is not restricted to those of protein amino acids, but can be based on the structure of known ligands.

The conformational space available to peptoids is larger than that of peptides because they lack the chiral α -carbon atom and the barrier of *cis/trans* isomerization about tertiary amide bonds is lower than that about secondary amide bonds.

As for peptides, the peptoid synthetic methods are amenable for automation.



In this work we investigated a variety of short peptoids, analogues of the bioactive tripeptide amide H-Pro-Gly-Leu-NH₂. By FT-IR absorption and 2D-NMR we characterized the conformations induced by the N-alkylated residues (N-methyl glycine and N-isobutyl glycine) in CDCl₃ solution. Special attention was devoted to determine the type of -turn adopted as a consequence of the *cis* or *trans* conformation of the tertiary amide bonds. Peak assignments were achieved by means of homo- and heteronuclear experiments and structural information was derived from the analysis of the ROESY spectra.

Our results support the view that: (i) An N-alkylated glycine induces a remarkable increase in the extent of β -turn formation in a peptoid sequence compared to a peptide based exclusively on protein amino acids. This effect is only slightly lower than that brought about by a prolyl residue. (ii) An N-alkylated glycine in position *i*+2 tends to favour the onset of type-II and type-VI β -turns. These latter findings are related to the lack of chirality of the *i*+2 residue and the facile isomerization of the Xxx-N-alkylated glycine peptide bond, respectively.

P B38 - Analogues of aggregating peptide models with fluorescent probe

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One of the most researched devastating degenerative disorders of the brain that affects human are Alzheimer and prion diseases also called "conformational diseases". The main postulated factor responsible for the nerve cells degeneration is formation of the protein aggregates. Mechanism and kinetics studies of peptide and protein amyloidogenesis are important and necessary to understand the basis of the illness and to plan further therapy. The published kinetic study of aggregate formation has been performed in much higher concentrations than possible physiological conditions. To simulate the low concentration conditions the fluorescent, dansyl marker has been attached to model fragments of peptides. We synthesized few fragments of β -amyloid (22-35, 25-35, 29-42) and prion protein (113-126, 109-126). In the communication, the determination of the dependence of aggregation rate in different conditions (pH, concentration, solvents) using various analytical methods will be presented.

P B40 - Polylysine characterization using mass spectrometry

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Polylysine and polylysine based branched polypeptides have been used in drug discovery for a long time, and they are applied in many fields of research. Polylysine based bioconjugates are effective synthetic antigens for immunization and monoclonal antibody production. Polylysine conjugates are also used for target specific drug delivery and applied in cancer research. In the last years a new application of this compound got into the focus of the interest. It has been described that this polycationic polyelectrolyte form noncovalent complexes with DNA and they can be utilized as non-viral gene delivery systems. The application of polylysines is strongly restricted by the fact, that their structure can not be characterized in sufficient detail using currently available analytical procedures.

Molecular mass measurement and mass distribution are indispensable for characterization of biopolymers. These experiments are most accurately performed by mass spectrometry. However, in the case of polymeric polylysines, mass spectrometric studies up to now were not successful for obtaining molecular mass information. It is likely due to a broad molecular mass distribution combined with a high degree of protonation. These difficulties are further complicated by solubility problems. For these reasons polylysines are most often characterized only by gel filtration and by sedimentation equilibrium measurements. While these yield useful information, their accuracy is not always sufficient. Due to the increasing demand to obtain more specific information on the structure of polylysines, we have investigated the feasibility to obtain molecular mass information using mass spectrometry.

In our work, we used matrix assisted laser desorption-ionisation and electrospray ionization mass spectrometry in combination with gas phase charge reduction to examine oligo- and polylysine mixtures and polylysine polymers. Our results showed that the charge state of oligo- and polylysine is much higher than that of proteins with a similar molecular mass. For this reason the newly developed gas phase charge reduction technique proved essential. With this technique the molecular mass (distribution) of relatively low molecular weight polylysines were successfully determined. With increasing the degree of polymerization and with increasing microheterogeneity analysis becomes progressively more demanding.

P B41 - Influence of the substrate peptide sequence shortening on papain activityM. Szabelski⁽¹⁾, K. Guzow⁽¹⁾, W. Wiczek⁽¹⁾

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The activity of proteolytic enzymes is usually measured by means of synthetic chromogenic or fluorogenic peptide substrates [1]. Resonance fluorescence energy transfer, a phenomenon where excitation energy is transferred from the excited fluorescent donor chromophore (D) to a quenching acceptor chromophore (A), has been used to develop spectrofluorimetric assays for various proteases. Cleavage of the peptide bond in fluorogenic substrates produces an increase in fluorescence because a donor and an acceptor are separated. We synthesized three fluorogenic substrates possessing a donor-acceptor pair designed to find the shortest sequence which is efficiently hydrolyzed by papain.

BOX-Lys-Phe-Gly-Gly-Ala-Ala-Tyr(NO₂)BOX- Phe-Gly-Gly-Ala-Ala-Tyr(NO₂)BOX- Gly-Gly-Ala-Ala-Tyr(NO₂)

They containing new highly fluorescent amino acid derivative, 3-[2-(2-benzofuranyl)benzoxazol-5-yl]-alanine (BOX) [2], as an energy donor and 3-nitro-L-tyrosine (Tyr(NO₂)) as an acceptor. The peptide chain of these substrates is derived from the sequence proposed by Garcia-Echeverria and Rich [3], displayed high specificity for papain. The high fluorescence quantum yield and photostability of 3-[2-(2-benzofuranyl)benzoxazol-5-yl]-alanine give the possibility to use very low concentrations of the substrate without detection sensitivity loss of hydrolysis reaction.

The kinetic of cleavage followed the Michaelis-Menten model. Kinetic parameters, k_{cat} and K_m , were evaluated. These short fluorogenic substrates containing D-A pair (BOX - Tyr(NO₂)) seem to be a very useful and very promising instrument for enzymology and medicine because of easy and inexpensive method of synthesis and, what is more, their high affinity to the papain.

References

- [1] Gershkovich, A. A.; Kholodovych, V. V., *J. Biochem. Biophys. Methods*, 33, 1996, p. 135-162.
- [2] Guzow, K.; Szabelski, M.; Malicka, J.; Wiczek, W., *Helv. Chim. Acta*, 84, 2001, p. 1086-1092.
- [3] Garcia-Echeverria, C.; Rich, D. H., *FEBS Lett.*, 297, 1992, p. 100-102.

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P B43 - Hemoglobin degradation pathways in human erythrocytesO. N. Yatskin⁽¹⁾, A. A. Karelin⁽¹⁾, M. M. Philippova⁽¹⁾, V. T. Ivanov⁽¹⁾

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Hemoglobin fragments are widely presented in extracts of mammalian tissues and many of them are biologically active [1]. In this work human erythrocytes were examined as a possible source of such peptides. Previously more than 30 hemoglobin fragments have been isolated and sequenced from the erythrocyte lysate under acidic extraction conditions. Comparison of their structures with those of hemoglobin fragments isolated from erythrocyte primary culture supernatant and from tissue extracts allowed to develop a model of step-by-step hemoglobin degradation in the organism [2]. Here we examine further the hemoglobin-degrading potential of erythrocytes. Starting from the in vivo intraerythrocyte pattern (erythrocyte lysate peptide fraction isolated under conditions excluding proteolysis) we followed the degradation process in the erythrocyte lysate under various conditions. The starting material contains over 50 endogenous intraerythrocyte peptides. The majority of them are N- and C-terminal fragments of hemoglobin α - and β -chains of various length. We assume that these peptides result from the following two step mechanism: (i) primary endopeptidase cleavage in the middle part (segments 95-110 for α -chain and 70-118 for β -chain), the resultant "halves" stay as a non-covalent complex with a native-like 3D structure; (ii) exopeptidase degradation of primary fragments (N-terminal fragments - from the C-terminus and vice versa). Addition at neutral pH of 2 M GuaHCl triggers intensive proteolysis by a (phenylmethylsulfonyl fluoride-sensitive) serine or thiol protease(s), presumably due to partial unfolding of hemoglobin. Under acidic pH another protease(s), inhibited by pepstatin, is activated in the erythrocyte lysate, giving rise to earlier described peptides [1,2]. The basic properties of the process (pH optimum, K_d, saturation time, etc.) were investigated. A remarkable feature of this complex is the sensitivity to organic solvents: the proteolysis was completely blocked by 20% acetonitrile. The most intensive cleavage sites correspond to cathepsin E-like specificity, however with some significant exceptions. For example, neokytorphin formation (cleavage of Leu136-Thr137 bond in α -chain) does not correspond the cathepsin E specificity. Thus, there are at least 4 distinct hemoglobin degradation pathways in the erythrocytes. The in vivo functional significance of these pathways will be discussed.

References

- [1] Ivanov V.T., et al. *Biopolymers. Peptide Science* (1997) 43: 171
- [2] Karelin A.A., et al. *Russian Journal of Bioorganic Chemistry* (1998) 24: 241

P B42 - Structure-activity relationship of melanin-concentrating hormone.R. M. Vitale⁽¹⁾, C. Isernia⁽¹⁾, P. Amodeo⁽²⁾, R. Fattorusso⁽¹⁾, L. Zaccaro⁽³⁾, G. Perretta⁽³⁾, B. Di Blasio⁽¹⁾, M. Saviano⁽²⁾, C. Pedone⁽²⁾

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Melanin-concentrating hormone (MCH), a hypothalamic cyclic peptide, was identified initially in teleost fish as a regulator of pigmentary changes in background adaptation. MCH was later also found in mammals to have a particular role in the regulation of food-intake behaviour and associated pathologies such as obesity. The conformational analysis of human MCH, a heterodetic cyclic nonadecapeptide, is object of considerable interest with the aim of gaining insight into a possible bioactive conformation. Previous studies on MCH and its analogs demonstrated the importance of conformational features in determining the biological activity. In this view, we aimed at structure characterizing of MCH in solution, and at exploring MCH conformational stability toward changes of environment. In fact, the influence of the environment onto bioactive conformation of neuropeptides that bind transmembrane receptors is up to date an open problem. With this goal in mind, we have determined the peptide structure both in acetonitrile-water mixture and in water by ¹H-NMR and molecular dynamics simulations in solution. Here we report the comparative analysis of the two sets of structures, as obtained in these two solvent systems, and of the conformational preferences adopted by the residues responsible of peptide biological activity. These structural informations will be used to build a model of MCH/MCHR complex and to design analogs with high receptor affinity.

P B44 - A study of energetics of formation of molecular and ionic heterocomplexes modelling acid-base interactions in polipeptides by using potentiometric and *ab initio* methodsL. K. Chmurzyński⁽¹⁾, M. Makowski⁽¹⁾, A. Kozak⁽¹⁾, M. Czaja⁽¹⁾

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Acid base and hydrogen bonding equilibria play an important role in living systems, e.g. the formation of hydrogen-bonded salt bridges between ammonium and carboxylate groups contributes to the stability of protein structure and surface loop structure formation. To examine such interactions, the anionic homoconjugating acetic acid - acetate, cationic homoconjugating n-butylammonium cation - n-butylamine, imidazole cation - imidazole, as well as heteroconjugating acetic acid - n-butylamine, acetic acid - imidazole systems were studied in two polar protophobic aprotic non-aqueous solvents acetone and nitromethane using the potentiometric-titration method. The systems under considerations were designed to model acid-base and hydrogen-bonding phenomena that involve acid and basic amino-acid side chains in proteins. To perform main goal of this study in both solvents studied the pK_a values, cationic and anionic homoconjugation, as well as heteroconjugation (in molecular and ionic experimental systems) constant values were determined. On the basis of the obtained constant values the influence of such solvents properties as polarity and basicity on acid-base equilibria has been discussed.

Experimental studies were supplemented by theoretical *ab initio* studies at the RHF (Restricted Hartree Fock) and MP2 (Møller-Plesset) levels on energetic parameters of the protonation, homo- and heteroconjugation reactions studied. To estimate solvation effects self-consistent reaction field (SCRF) and polarizable continuum (PCM) models were applied.

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P B45 - Computational design and combinatorial assembly of synthetic proteins

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The aim of protein design is to create novel proteins with tailored structural and functional properties. Extensive research efforts have provided a wide variability in biomimetic molecules. But the lack in understanding the folding of proteins and the difficulty in screening physical parameters has limited these studies to rather small numbers of protein variants. The assembly of synthetic proteins on a cyclic template (TASP) is an efficient strategy for the synthesis of proteins with partially pre-determined structures. A library of metallo proteins has been synthesized from small libraries of peptide building blocks which were attached in a combinatorial approach to templates that were bound to the cellulose membranes. A cleavable linker has been used to attach the cyclic decapeptide template to the cellulose membrane which allows to control the synthesis steps. In addition, spectroscopic screening directly on the solid support has been established to monitor the properties of the individual proteins (Rau et al. (2000) *Angew. Chem.* 39, 250). This strategy has been used to synthesize several hundred four-helix bundle proteins with heme groups and to characterize their redox potential. It was also successfully applied to the synthesis of mono-His ligated heme proteins with enzymatic activity of heme oxygenase. Proteins with desired properties have been synthesized also in solution for detailed physical characterization. This approach has been extended to an iterative optimization of copper proteins by using a combination of three different peptide libraries. The best hit of the first hundred proteins (Schneppf et al. (2001) *J. Am. Chem. Soc.* 123, 2186) has been used as starting structure for a second approach which led to copper proteins with properties of type I, type II, and di-copper centres with remarkable stability. These proteins have an α -helical structure which is not found in natural copper proteins.

To further improve the strategy for novel proteins we have used a computational design of proteins. An assembly of a hydrophobic core from a rotamer library of amino acids was extended to the packing of a complete β -sandwich protein from eight anti-parallel β -strands. The final protein was energy minimised with a mobile backbone structure. This protein designed *in silico* was synthesized and assembled from four hairpin structures on a cyclic decapeptide template. Its folding is confirmed by a fraction of 80 % of β -structure as estimated from FTIR-measurements in H₂O. Synthesis and solubility of the protein was improved in a second variant with two additional Asp residues. We believe that the combination of the two strategies of computational protein design and combinatorial assembly will help to improve the synthesis of novel proteins with tailored functions.

P B47 - Toward rationally designed glycopeptide antigens recognizing autoantibodies in multiple sclerosis patients

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The glycopeptide CSF114, containing a β -D-glucopyranosyl residue linked to an Asn residue, is an important synthetic antigen able to detect specific antibodies (Abs) by ELISA on sera of patients affected by Multiple Sclerosis (MS), the most known demyelinating autoimmune disease of the central nervous system. Interestingly, the Ab titre correlates with disease activity. Further studies demonstrated that auto-Abs can be detected only using glycosylated antigens, whose specific epitope is restricted to the Asn(Glc) moiety. Conformational studies, based on mono and bi-dimensional NMR experiments, indicated that the peptide structure, characterised by a β -hairpin, contributes to the presentation of this epitope on the solid-phase of the immunoenzymatic assay (ELISA).

It is evident the importance to optimise the synthetic autoantigens to be used in ELISA, not only for the diagnosis but also for the follow-up of this important autoimmune disease. To this aim, we undertook a structure-activity relationship study. We synthesized glycopeptides shortened at both termini to find out the minimal glycopeptide sequence able to detect the autoantibody response. Moreover, the role of each amino acid of the sequence was investigated by an alanine-scan. Finally, a structure based design was carried out to select β -hairpin mimetic peptide sequences, able to optimally expose the Asn(Glc) epitope.

The optimized peptides were tested on MS patients sera, in the context of the "Regional Coordination Centre for Plasma Filtration and Immunoabsorption", at the Hospital Unit Careggi (Firenze, Italy), with the aim to validate a diagnostic kit.

P B46 - Convergent molecular modelling of α -helix- and β -sheet-inducers towards artificial transcription factors of the GCN4 and Met-repressor classes

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Stabilization of short peptides in their α -helical conformation is achieved by combination of three stereochemical principles: first the inherent narrowing of available conformational ϕ, ψ -space through incorporation of α -branched unnatural amino acids into peptide chains, secondly by integration of this branch as a 2-aminotetraline-2-carboxylic acid [2] derived moiety at the n+3 position, which is thirdly cyclized retaining helical stereocompatibility. These peptidomimetic constructs are suitable to enhance helicity and do reflect the DNA-binding specificity of GCN4 transcription factor mimetics. On the other hand, construction of β -sheet transcription factor mimetics such as derived from the met-repressor protein is approached by heterochiral β -turn type II' spanned cyclic octadecapeptides. Thereby a characteristic gramicidin-S like L - β -sheet twist ('GrS like') is crucial for binding to the DNA major groove [1]. These features are consistently reflected by force-field minimized molecular models applying a recently introduced software suite [3]. The MAB force-field contained therein as a core component has been calibrated for a large number of small molecule pharmacophores, proves however to equally well reflect conformational and molecular properties of small peptides such as the investigated mimetics compared with their parent large regulatory protein factors. These approaches illustrate the potential of convergent molecular modelling for design in biostructural studies involving both proteins and nucleic acids.

References

- [1] Lehmann, C. In Lebl, M. and Houghten, R.A. (Eds.) *Peptides: Wave of the Future* (Proceedings of the 17th American Peptide Symposium), American Peptide Society 2001, in press, and ref. cited therein.
- [2] Obrecht, D., Altorf, M., Bohdal, U., Daly, J., Huber, W., Labhardt, A., Lehmann, C., Müller, K., Ruffieux, R., Schönholzer, P., Spiegler, C. and Zumbund, C., *Biopolymers* 1997, 42, 575-626.
- [3] P. R. Gerber: MOLOC - A Molecular Design Software Suite: <http://www.moloc.ch>

P B48 - Probing AT-1 receptors with "branched" cyclopentapeptides

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3D models of the "receptor-bound" conformation of angiotensin II (Asp¹-Arg²-Val³-Tyr⁴-Val⁵-Ile⁶-His⁶-Pro⁷-Phe⁸, AII) and its complex with transmembrane (TM) region of the AT-1 receptor [2] have been used for design of several types of "branched" cyclopentapeptides (CPPs), namely:

cyclo(Xxx-Val-His-Asp(D-Phe)-Yyy), Xxx = Gly, D-Nle (Type I)
cyclo(Xxx-Val-His-D-Asp(D-Phe)-Yyy), Xxx = Gly, Nle (Type II)
cyclo(Dap(Xxx)-Val-His-D-Asp(D-Phe)-Yyy), Xxx = Val, Arg-Val (Type III)
(in all compounds Yyy = Tyr, D-Tyr)

In compounds I and II, the β -carboxyl group of the Asp/D-Asp residue was used to attach the Phe residue that is one of the three residues indispensable to manifest agonistic activity of AII (Tyr⁴, His⁶ and Phe⁸). In compounds III, additionally, the β -amino group of the Dap residue (2,3-diaminopropionic acid) was used to attach peptide fragments to model the N-terminal part of AII. According to preliminary molecular modeling studies, some of the above compounds may form a complex with TM region of AT-1 receptors similar to that with the parent AII [2], which was not the case for a series of CPPs studied by us earlier [3]. This presentation will describe molecular modeling, syntheses, NMR studies and bioassays for the above compounds

References

- [1] Nikiforovich, G.V., Marshall, G.R. *Biochem. Biophys. Res. Commun.* 195,222 (1993)
- [2] Nikiforovich, G.V., Marshall, G.R. *Biochem. Biophys. Res. Commun.* 286,1204 (2001)
- [3] Nikiforovich, G.V.; Lindenberg, G. Kövér K. E.; Ye, Y.; Frandberg, P.-A. et al. *Peptides: The Wave of the Future*. Proceedings of the Seventeenth American Peptide Symposium; American Peptide Society, 2001; pp 917-918

P B49 - *Ab initio* conformational analysis of the *cis-trans* isomerization of prolyl-proline peptideA. Perczel⁽¹⁾, I. Hudáky⁽¹⁾

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Consecutive proline residues connected by a *cis* peptide linkage are reported to be found in several naturally occurring cyclic peptides with immunosuppressive activity, such as antamanide [1], cyclolinopeptide A [2], cycloleonorinin [3] and cycloleonorinipeptide D [4]. Non-globular protein collagen contain many repeats of the prolyl-hydroxyproline sequence unit and one of the rate-limiting steps in its folding is demonstrated to be the *cis-trans* isomerisation of the peptide linkage between proline and hydroxyproline [5]. By *ab initio* method, *cis-trans* isomerization reaction path was studied on N-acetylproline methylamide, a simple model of proline residue in peptides and proteins [6].

In the present study, *ab initio* investigation of *cis-trans* isomerization of the peptide bond between the two proline residues of For-L-Pro-L-Pro-NH₂ was carried out first at RHF/3-21G then at RHF/6-31+G(d) levels of theory. At the RHF/6-31+G(d) level of theory the global minimum is $\tau_{E_L}+\tau_{L^+}$ with all *trans* peptide bonds, ϵ_L and γ_L backbone conformations, and both prolines having a positive χ_1 value [7]. $\tau_{E_L}+\alpha_{L^+}$ has a *cis* peptide bond between the two prolines, and at 300 K this conformer ($\Delta E=0.01$ kcal/mol) is expected to have almost the same population as the global minimum. Transition structures with ω torsional angles around +90° as well as around -90° were searched for. Transition structures are quantitatively characterized through energetic parameters, backbone torsional angles and pseudorotational coordinates of proline residues [8], nitrogen pyramidality as well as stabilizing hydrogen bonds.

References

- [1] Karle, *J. Am. Chem. Soc.*, 1974, 96, 4000
- [2] Blasio et al, *J. Am. Chem. Soc.*, 1989, 111, 9089
- [3] Morita et al, *Tetrahedron*, 1997, 53, 7469
- [4] Morita et al, *Tetrahedron*, 1997, 53, 1617
- [5] Buevich et al, *Biochemistry*, 2000, 39, 4299
- [6] Fischer et al, *J. Am. Chem. Soc.*, 1994, 116, 11931
- [7] Hudáky et al, *Peptides 2000*, 417
- [8] Hudáky et al, *J. Mol. Struct. (THEOCHEM)*, in press

P B51 - A helical peptide receptor for [60]fullereneA. Bianco⁽¹⁾, D. M. Guldi⁽²⁾, M. Crisma⁽¹⁾, E. Sartori⁽³⁾, C. Corvaja⁽³⁾, M. Maggini⁽¹⁾, C. Toniolo⁽¹⁾

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A detailed understanding of the electronic properties between non-covalently assembled donor-acceptor partners is heavily based on the design of molecular structures with a well defined geometry [1]. In this context, we have conceived and prepared by solution methods a helical, Aib-rich nonapeptide, potentially able to encapsulate a [60]fullerene moiety [2]. This peptide is constituted by six Aib residues, a Gly spacer and two ferrocenyl side-chain substituted L-Tyr residues in positions 2 and 8. FT-IR absorption and 2D-NMR analyses indicate that a ₃₁₀-helical conformation is adopted by this rigid peptide in structure-supporting solvents. As five amino acid units separate the two substituted L-Tyr residues in the peptide sequence, the two side chains will face each other in solution after two complete turns of the ternary helix. By carrying out a detailed photophysical analysis, we have demonstrated that the electron-rich, hydrophobic and wide cavity generated by the nonapeptide template with the two ferrocenoyloxybenzyl walls is able to host N-methylfulleropyrrolidine (MFP). Upon photoexcitation, the singlet excited state of MFP is therefore prompted for a rapid intra-complex deactivation by the ferrocenoyl groups. Conversely, in the CHCl₃/HFIP (1,1,1,3,3,3-hexafluoropropan-2-ol) solvent mixture that disfavors the formation of a host-guest complex, no evidence for intra-complex processes has been obtained. Instead, MFP reacts in its triplet excited state with ferrocene predominantly *via* diffusion-controlled dynamics. Further evidence for this superstructure has been provided by a mass spectrometric investigation in the gas phase. We have also synthesized a control nonapeptide in which the two electron-rich ferrocenoyloxybenzyl side chains are replaced by two methyloxybenzyl side chains. An X-ray diffraction analysis of this peptide unambiguously proved the high tendency of the -C(=O)-Aib-L-Tyr(Me)-(Aib)₂-Gly-(Aib)₂-L-Tyr(Me)-Aib- backbone to adopt a regular, ₃₁₀-helical conformation. To our knowledge the ferrocenoyl derivatized nonapeptide is the first peptide-based (1:1) mini-receptor reported for [60]fullerene.

References

- [1] Voyer, N., Lamothe, *J. Tetrahedron* 1995, 51, 9241.
- [2] Bianco, A., Corvaja, C., Crisma, M., Guldi, D. M., Maggini, M., Sartori, E., Toniolo, C. *Chem. Eur. J.*, in press.

P B50 - Modelling of bioactive peptides with the inclusion of synthetic amino acidsM. A. Preto⁽¹⁾, A. S. Melo⁽¹⁾, S. P. Costa⁽²⁾, H. L. Maia⁽²⁾, M. J. Ramos⁽¹⁾

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Angiotensin II (ATII) is an octapeptide which is involved in the blood pressure control mechanism [1]. Blood hypertension is related to excess production of this natural peptide. In this case, the over stimulation of the type 1 receptor (AT-1) induces vasoconstriction, renal tubular sodium reabsorption, aldosterone release, vascular smooth muscle remodelling and stimulation of the central and peripheral sympathetic activity, thus leading to increases in blood volume and blood pressure [2]. Antagonists of ATII for its AT-1 receptor are well tolerated and are effective in decreasing blood pressure.

However, the peptide antagonists have large problems of bioavailability, because they are decomposed by the digestive enzymes [3].

In this work, new ATII peptide antagonists have been designed by inclusion of novel synthetic amino acids [4]. These peptides are expected not to be well recognized by the digestive enzymes, being suitable for oral administration as drugs of high commercial value.

Molecular modelling, a powerful tool to build new active biomolecules [5], has been used to design these biomimetic peptides of ATII to the AT-1 receptor.

References

- [1] Dina, R.; Jafari, M.; *Am. J. Health-Syst. Pharm.* 2000; 57: 1231-1238;
- [2] Bernstein, K. E.; Berk, B. C.; *Am. J. Kidney Dis.* 1993; 22: 745-54;
- [3] Frishman, W. H.; Fozailoff, A.; Bin, C. et al. *J. Clin. Pharmacol.* 1994; 34: 837-880;
- [4] Toniolo, C.; Lelj, F.; Fraternali, F.; Langran-Goldsmith, A.; Maia, H. L. S.; *J. Chem. Soc. Perkin Trans. 2* 1990: 1481-1487;
- [5] Ramos, M. J.; Melo, A.; Henriques, E. S.; *Theoretical and Computational Chemistry*, vol 9, chapter 13: 539-595; 2001 Elsevier Science B. V.

P B52 - Identification of the physiological binding partners of SH3-domains in a whole proteome by combining phage display and SPOT-technologyC. Landgraf⁽¹⁾, L. Castagnoli⁽²⁾, S. Panni⁽²⁾, J. Schneider-Mergener⁽¹⁾, G. Cesareni⁽²⁾, R. Volkmer-Engert⁽¹⁾

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The formation of macromolecular complexes and pathways that coordinate specific biochemical functions in the cell are often mediated by small protein recognition modules which were found repeatedly in several protein structures. Each domain family (e. g. SH3, SH2, PDZ, WW) binds ligands displaying a core structural motif and, within each domain family, specificity is modulated by key residues flanking the core binding motif. The identification of the physiologically relevant binding partners of a given protein domain family in a whole proteome is of general interest. Recently, a combined experimental and computational strategy to define a protein interaction network for the different members of the SH3 domain family in a whole proteome was described [1]. In this communication a strategy for the identification of physiological SH3-domain ligands in the whole *Saccharomyces cerevisiae* proteome is described using the combination of phage display and SPOT-synthesis technique [2,3]. The SH3 protein domains Abp1, Rvs167 and Boil were used as model systems to verify this strategy. The approach involves the following steps: (i) Determine a rough domain recognition consensus pattern by phage display; (ii) synthesize peptide arrays which include all the peptides that conform to this pattern in the *Saccharomyces cerevisiae* proteome using the SPOT-synthesis technique; (iii) incubate the peptide arrays with the different SH3-protein domains in order to derive the potential physiological binding partners; (iv) pin down the physiological ligands using semi-quantitative spot-intensity evaluation and BIAcore studies of the high affinity peptides which were found in the screening experiment. Here, 12 peptides among the 729 class 1 consensus sequences and 27 peptides among the 735 class 2 consensus peptides were identified as ligands of the SH3 domain of Rvs167. All the proteins that were known, by classical experiments, to interact with the protein Rvs167 via the SH3 domain contain at least one peptide that is included in this list. This result supports the power of the proposed approach in the identification of physiological partners.

References

- [1] Tong AH, Drees B, Nardelli G, Bader GD, Brannetti B, Castagnoli L, Evangelista M, Ferracuti S, Nelson B, Paoluzi S, Quondam M, Zucconi A, Hogue CW, Fields S, Boone C, Cesareni G., *Science*, 295 (2002), 321-4.
- [2] Frank R., *Tetrahedron*, 48 (1992), 9217-32.
- [3] Reineke, U., Volkmer-Engert, R., Schneider-Mergener, J., *Curr. Op. Biotech.* 12 (2001), 59-64.

P B53 - New design of synthetic peptide nanotubesK. Rosenthal-Aizman⁽¹⁾, A. Uden⁽¹⁾

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Peptides containing an even number of regularly alternating D- and L- α -amino acids possess unique structural features. These type of peptides was previously demonstrated to adopt flat ring conformations and sample into β -sheet like hydrogen-bounded large tubular aggregates. [1] However there are limited data available about effects of amino acid composition of peptides on association.

In this study our goal was to investigate the influence of factors like side chain hydrophobic effects and steric interactions and explore further the effect of amino acid substitution on self assembly process.

We found that if appropriately designed the self associating properties of D/L peptides can be drastically changed and lead to the novel features. For example, introduction of sterically hindered amino acids may provide control over degree of aggregation. Solid phase synthesis and association behaviour of structurally modified model hexa and octapeptides will be presented.

Reference

[1] Ghadiri, M. R., Granja, J. R., Milligan, R. A., McRee, D. E., Khasanovich N. *Nature* 366, 324-327 (1993).

P B54 - Evolutionary combinatorial chemistry, a novel tool for SAR studies on peptide transport across the blood-brain barrier.M. Teixidó⁽¹⁾, I. Belda⁽²⁾, S. González⁽³⁾, M. Fabre⁽³⁾, S. Vilaró⁽³⁾, J. M. Garrell⁽²⁾, X. Roselló⁽⁴⁾, F. Albericio⁽¹⁾, E. Giralt⁽¹⁾

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The ability to predict the pharmacokinetic behaviour of a drug is a crucial element in drug design. Successful drug development requires not only optimization of specific and potent pharmacological activity at the target site, but also efficient delivery to that site. Drugs directed towards targets in the CNS must overcome the blood-brain barrier. Many peptides with therapeutic potential for treatment of CNS disorders have recently been identified. In this work we use the Evolutionary Combinatorial Chemistry to study transport of peptides across the blood-brain barrier. This novel tool combines the selection and synthesis of compounds with a Genetic Algorithm (GA). In our work peptides are treated as individuals whose physico-chemical properties are encoded by genomes composed of the following genes: length, aromatic residues, methylated residues, C-Terminal, N-Terminal, secondary structure, hydrogen-bonding, pI, polycation, amphiphilic and LogP. Each gene can exhibit different values (e.g. α -helix, β -sheet, random coil for the secondary structure gene).

The GA starts with a set of different individuals ranked according to their measured fitness (i.e. their experimental permeability in an *in vitro* model of blood-brain barrier).

New genomes are generated by the following GA functions:

Crossover: Mixing of two genomes to yield a new genome

Mutation: Change in the value of a gene in the parent genome

Replication: Regeneration of an equivalent genome

Once a new generation of genomes (children) has been generated it is translated into a new population of peptides which are synthesized and evaluated in a *in vitro* model. This artificial life cycle is repeated until satisfactory properties of the individuals (peptides) are obtained.

This approach should provide us with insight into what characteristics enable a peptide to cross the blood-brain barrier. In this communication, this approach, which can be used to accelerate the drug discovery process, will be broadly discussed.

P B55 - Relationship between structure and cell permeability of oligopeptides and related compoundsM. Akamatsu⁽¹⁾, R. Ano⁽¹⁾, Y. Kimura⁽¹⁾, M. Shima⁽¹⁾, R. Matsuno⁽¹⁾

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Clinical development of orally bioavailable peptide-based drugs has been restricted not only by their low molecular hydrophobicity, which limits their intestinal mucosal permeation, but also by their lack of stability against enzymatic degradation. Peptidomimetics are therefore designed to enhance their oral absorption and depress the enzymatic degradation. Most drugs and peptides are transported across the intestinal epithelium by a passive diffusion process and/or active carrier-mediated routes. Passive absorption can occur via either the transcellular or paracellular route.

It is often generalized that rapidly transported hydrophobic compounds are absorbed across the membrane by the transcellular route, whereas slowly transported hydrophilic compounds are absorbed through the tight junctions via the paracellular route. P-glycoprotein, a membrane glycoprotein expressed in intestinal epithelial cells, is also involved in intestinal absorption. This is a system that mediates drug transport in a secretory direction. However, as yet, it is unclear what structural features of compounds are favorable for their intestinal permeation. The successful development of orally active peptidomimetics will be aided by an understanding of the structural factors which influence epithelial permeation of peptides. In this study, Caco-2 cell permeability of oligopeptides containing Trp and related compounds was measured as an *in vitro* absorption model in human intestinal epithelial cells. The artificial lipid membrane permeability was also measured to evaluate absorption of the compounds across the membrane by the transcellular route. Firstly, we quantitatively analyzed the permeability across the artificial lipid membranes using the physicochemical parameters of the compounds. Hydrophobicity, pK_a, and hydrogen-bonding potential were significant in governing variations in the permeability. Then correlation between the Caco-2 cell and artificial lipid membrane permeability was investigated.

P B56 - Isolation of casein-derived antibacterial peptides from rabbit milkM. Baranyi⁽¹⁾, U. Thomas⁽²⁾, A. Pellegrini⁽²⁾

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It is well established that proteolytic digestion of bovine casein yields several bioactive peptide fragments. To investigate whether antimicrobial peptides can be generated during the proteolytic digestion of rabbit casein, the acid-precipitated fraction of rabbit milk, the "whole casein" was enzymatically digested by trypsin. The peptide fragments were separated by reversed-phase chromatography. The collected fractions were pooled and their antimicrobial properties investigated against *E. coli*, *B. subtilis* and *S. lentus*. Three pools derived from the trypsin digestion were found to strongly inhibit the growth of *B. subtilis*, whereas two pools inhibited this bacterial strain moderately. Two pools also inhibited the multiplication of *S. lentus* moderately. Three bactericidal peptide fragments present in two pools were isolated and identified. Their sequences were HVEQLLR (residues 50-56 of β -casein), ILPFIQSLFPFAER (residues 64-77 of β -casein), and FHLGHLK (residues 19-25 of α -S1-casein). Following characterization these three peptides were synthesized purified and their bactericidal activity was investigated up to a concentration of 6.0 mM against Gram-positive and Gram-negative bacteria. The peptides were active against the Gram-positive bacteria only. Our results suggest a possible microbicidal function of rabbit caseins. Although rabbit 'whole casein' has no effect against all the bacterial strains proofed, antibiotic peptides can be released from casein during the digestion of milk proteins. It is conceivable that bactericidal peptides can be generated by endopeptidases of the mammalian gastro-intestinal possibly providing protection for newborn rabbits against aggression of microorganisms.

P B57 - SP8ca, a peptide enhancing transport of model compounds across Caco-2 cell monolayers

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Many potential therapeutic agents, including small molecules, peptides and proteins, show poor permeability through physiological barriers, including intestinal and blood-brain barrier. This permeability is limiting the application of drugs. Improvement of their transport could be achieved by application of carriers that are capable of crossing the epithelium using specific transcellular mechanisms.

SP8ca is a 16-mer linear peptide. It was shown to enhance permeability of a bacterial phage expressing the peptide across caco-2 cell monolayers, as well as human brain endothelium cell (HCEC) monolayers. The peptide conjugated to peroxidase also increased the transcellular transport of the enzyme through caco-2 cell monolayers. The present communication describes the capability of the peptide to carry a model small organic molecule. The peptide has been conjugated to a fluorescent tag, tetramethylrhodamine (TAMRA). The kinetics of the transport of the tag across caco-2 monolayers was determined, and compared to that of TAMRA conjugated to a non-relevant peptide. The permeability of TAMRA-SP8ca was doubled, as compared to the reference.

The communication will also describe the work on other conjugates of SP8ca as potential drug delivery systems.