Copper homeostasis and trafficking in cells

Ivano Bertini

Magnetic Resonance Center - CERM, University of Florence, Via L. Sacconi, 6, 50019 Sesto Fiorentino, Italy

Copper is an essential element which has to be uptaken from outside the cell to inside and has to be accompanied to the target or to ATPases to cross internal membranes. Examples will be provided in bacteria, yeast and humans. Copper is also neeeded to assemble CuA in cytochrome c oxidase. The proteins involved are discussed and comparison is made between prokaryotes and eukaryotes. In both examples detailed and extensive biochemical pathways are presented.

A hub protein: many interaction partners

DYNLL: a multifunctional protein that binds to intrinsically disordered proteins

László Nyitray

Department of. Biochemistry, Eötvös Loránd University, Budapest, Hungary.

DYNLL1 and DYNLL2 are two mammalian paralogs of the conserved LC8 family of dynein light chains that were described as tail subunits of dynein and myosin Va motor proteins. Moreover, they were shown to interact with a wide variety of other polypeptides. They may serve as adaptors to bind cargos to the transport motors; however, they also have other functions in the cell unrelated to their role in motor complexes. We have localized the binding site of DYNLL2 on myosin Va tail within a disordered region, between two coiled-coil domains. DYNLL2 binds to this site with a K_d of 40 nM and stabilizes both flanking coiled-coils [1]. By studying DYNLL binding to their targets, we have found that the Ser88Glu mutant of DYNLL, mimicking phosphorylation by PAK1, an important regulator of cell motility, regulates activity of DYNLL, by dissociating the homodimer protein into two monomers that are able to bind only to dimeric partners. By analyzing the sequences of all known partners (>60), we have noticed that the only common feature of the DYNLL targets is that they are either intrinsically disordered proteins (e.g. the proaptototic proteins Bim and Bmf) or have their DLC binding sites within a disordered domain, moreover these site are often flanked by weak coiled coil sequences (e.g. myosin Va and the dynein intermediate chain). Thus, DYNLL isoforms are now recognized as chaperon-like "hub proteins" with well-defined structure that could promiscuously bind to diverse interactors that contain a short binding motif within a disordered protein or domain and contribute to their folding and/or regulation.

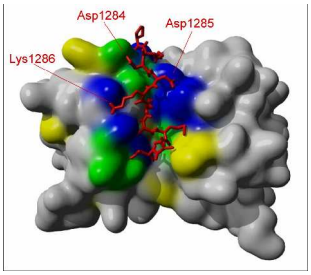


Fig.1. Structure of DYNLL2-myosin Va peptide complex

References

[1] Zs. Hódi, A. Németh, L. Radnai, Cs. Hetényi, K. Schlett, A. Bodor, A. Perczel, and L.Nyitray, "Alternatively spliced exon B of myosin Va is essential for binding of the tail light chain shared by dynein." Biochemisry, **45**, 12582-12595 (2006).

[2] Z. Hódi, P. Rapali, L. Radnai, T. Molnár, A. Szenes, J. Kardos, L. Buday, W.F. Stafford, and L.Nyitray. "The LC8 family of dynein light chains: multifunctional chaperon-like proteins." FEBS J., **274**: 106 (2007)

Combinatorial Phage-display Mutagenesis Reveals Complex Networks of Surface-Core Interactions in the Pacifastin Protease Inhibitor Family

Borbála Szenthe[1], András Patthy[2], Zoltán Gáspári[3], Adrienna Katalin Kékesi[4,5], László Gráf[1,2], Gábor Pál[1*]

[1]*Department of Biochemistry, ELTE, 1/C Pázmány P. street, Budapest, Hungary H-1117,*
[2]*Biotechnology Research Group of the HAS, ELTE, 1/C Pázmány P. street, Budapest, Hungary H-1117*
[3]*Institute of Chemistry, ELTE, 1/A Pázmány P. street, Budapest, Hungary H-1117*
[4]*Department of Physiology and Neurobiology, ELTE, 1/C Pázmány P. street, Budapest, Hungary H-1117*
[5]*Proteomics Group of the Biology Institute, ELTE, 1/C Pázmány P. street, Budapest, Hungary H-1117*

Pacifastin protease inhibitors are small cysteine-rich motifs of ~35 residues discovered in arthropods [1-5]. The family is divided into two related groups based on the composition of their minimalist inner core. In group I the core is governed by a Lys10-Trp26 interaction, while in group II it is organized around a Phe at position 10. Group I inhibitors exhibit intriguing taxon specificity: potent arthropod-trypsin inhibitors from this group are almost inactive against vertebrate enzymes. The group I member SGPI-1 and the group II member SGPI-2 are two extensively studied inhibitors [6-11]. SGPI-1 is taxon-selective, while SGPI-2 is not [6,7]. Individual mutations failed to explain the causes underlying for this difference. With comprehensive combinatorial mutagenesis and phage display we deciphered this phenomenon. We produced a complete chimeric SGPI-1 / SGPI-2 inhibitor-phage library, in which the two sequences were shuffled at the highest possible resolution of individual residues. The library was selected for binding to bovine and crayfish trypsin. Sequence analysis of the selectants revealed that taxon specificity is due to an intra-molecular functional coupling between a surface loop and the Lys10-Trp26 core. Five SGPI-2 surface residues transplanted into SGPI-1 resulted in a variant that retained the "taxon specific" core, but potently inhibited both vertebrate and arthropod enzymes. An additional rational point mutation resulted in a picomolar inhibitor of both trypsins. Our results challenge the generally accepted view that surface residues are the exclusive source of selectivity for canonical inhibitors. Moreover, we provide important insights into general principles underlying the structure-function properties of small disulfide-rich polypeptides, molecules that exist at the borderline between peptides and proteins.

References

[1] Hergenhahn, H. G., Aspan, A. & Soderhall, K. "Purification And Characterization Of A High-Mr Proteinase-Inhibitor Of Pro-Phenol Oxidase Activation From Crayfish Plasma." *Biochem. J.* **248**, 223-228. (1987).
[2] Nakakura, N., Hietter, H., Vandorsselaer, A. & Luu, B. "Isolation And Structural Determination Of 3 Peptides From The Insect *Locusta-Migratoria* - Identification Of A Deoxyhexose-Linked Peptide." *Eur. J. Biochem.* **204**, 147-153. (1992).
[3] Kellenberger, C., Boudier, C., Bermudez, I., Bieth, J. G., Luu, B., and Hietter, H. "Serine protease inhibition by insect peptide containing a cysteine-knot and a triple-stranded β-sheet." *J. Biol. Chem.* **270**, 25514-25519. (1995).
[4] Liang, Z., Sottrup-Jensen, L., Aspan, A., Hall, M. & Soderhall, K. "Pacifastin, a novel 155-kDa heterodimeric proteinase inhibitor containing a unique transferrin chain." *Proc. Natl. Acad. Sci. USA* **94**, 6682-6687. (1997).
[5] Hamdaoui, A., Wataleb, S., Devreese, B., Chiou, S. J., Vanden Broeck, J., Van Beeumen, J., De Loof, A. & Schoofs, L. "Purification and characterization of a group of five novel peptide serine protease inhibitors from ovaries of the desert locust, *Schistocerca gregaria*." *FEBS Letters* **422**, 74-78. (1998).
[6] Malik, Z., Amir, S., Pál, G., Buzás, Z., Várallyay, E., Antal, J., Szilágyi, Z., Vékey, K., Asbóth, B., Patthy, A. & Gráf, L. "Proteinase inhibitors from desert locust, *Schistocerca gregaria*: Engineering of both P1 and P1' residues converts a potent chymotrypsin inhibitor to a potent trypsin inhibitor." *Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology* **1434**, 143-150. (1999).
[7] Patthy, A., Amir, S., Malik, Z., Bódi, A., Kardos, J., Asbóth, B. & Gráf, L. "Remarkable phylum selectivity of a *Schistocerca gregaria* trypsin inhibitor: The possible role of enzyme-inhibitor flexibility." *Archives of Biochemistry and Biophysics* **398**, 179-187. (2002).
[8] Gáspári, Z., Ortutay, C. & Perczel, A. "A simple fold with variations: the pacifastin inhibitor family." *Bioinformatics* **20**, 448-451. (2004).
[9] Szenthe, B., Gáspári, Z., Nagy, A., Perczel, A. and Gráf, L. "Same fold with different mobility: backbone dynamics of small protease inhibitors from the desert locust, *Schistocerca gregaria*." *Biochemistry* **43**, 3376-3384. (2004).
[10] Fodor, K., Harmat, V., Hetényi, C., Kardos, J., Antal, J., Perczel, A., Patthy, A., Katona, G. & Gráf, L. "Extended intermolecular interactions in a serine protease-canonical inhibitor complex account for strong and highly specific inhibition." *J Mol Biol* **350**, 156-169. (2005).
[11] Gáspári, Z., Szenthe, B., Patthy, A., Westler, W. M., Gráf, L. & Perczel, A. "Local binding with globally distributed changes in a small protease inhibitor upon enzyme binding." *FEBS J* **273**, 1831-1842. (2006).

Analysis and evaluation of circular dichroism spectra with the updated CCA+ software

A fast and efficient quantitative tool for protein and peptide structure determination: the CCA+

Imre Jákli, András Perczel

Institute of Chemistry, Laboratory of Structural Chemistry and Biology, Eötvös Loránd University, Budapest, Hungary and Protein Modeling Group MTA–ELTE, Institute of Chemistry, Eötvös Loránd University, H–1538, Budapest, P.O.B. 32, Hungary

Advances in the structural biology and structural genomics needed high throughput determination of protein secondary structures. Electronic or UV Circular Dichroism (CD) spectroscopy was traditionally used in this field. In the past decades this aspect of the CD spectroscopy was effaced by X-ray crystallography and NMR spectroscopy since they give a more detailed description of the molecule. Although CD spectroscopy does not give atomic level resolution, – in some cases it is not needed – it has several advantages over the other methods used for structure elucidation. Unlike NMR, CD measurements can be performed at a lower concentration for proteins of any size. Measurement can be performed quickly, no lengthy data post processing is needed, to get a qualitative picture. It is highly sensitive: electronic and magnetic transitions of the amide bonds of polypeptide backbone are highly dependent on the conformation.

Most CD spectrum analyzing methods use the assumption that CD spectra can be calculated by summing the contribution of different conformers (spectral additivity). The goal of CD deconvolution is to determine **these base spectra** as well as the contribution to measured date. The Convex Constraint Analysis algorithm (CCA+) is unique in the sense of that it does not use any external database for the base spectra determination. These calculations give quantitative results on the spectral features, monitoring even small changes: ion, temperature, pH dependence etc. Because it has no external databases dependence, it can be used not only for proteins, but any other systems as well.

We present applications on how to use CCA+ both for smaller (peptides) and larger (protein) systems in different molecular environment (solvents, temperature, metal ions etc.):

- structure analysis of globular protein libraries
- temperature and pH dependence of miniproteins
- solvent dependence of linear and cyclic peptide models
- ion dependence (calcium and aluminum)

The CCA+ program can be downloaded free of charge from: http://www.chem.elte.hu/departments/jimre

New functions from the swapping of terminal arms: lessons from ribonucleases

3D domain swapping of proteins: the unusual features of Bovine Ribonucleases

Roberta Spadaccini[1], Carmine Ercole[2], Orlando Crescenzi[2], Teodorico Tancredi[3], Delia Picone[2]

[1]*Dipartimento di Scienze Biologiche ed Ambientali, Università del Sannio, 82100, Benevento, Italy,* [2]*Dipartimento di Chimica "Paolo Corradini", Università Federico II di Napoli, 80126, Napoli, Italy, and* [3]*ICB del CNR, 80078, Pozzuoli, Italy.*

There is an intimate connection between domain swapping and mammalian ribonucleases. The hypothesis that two (or more) polypeptide chains exchange their terminal extremities has been proposed initially for bovine pancreatic ribonuclease A (RNase A), to explain the recovery of biological activity upon mixing of two inactive derivatives, and was definitely proved after several years. More recently, the possibility that each polypeptide chain may dislocate more than one domain has again been observed for the first time in RNAse A. The family of mammalian ribonucleases includes also a dimeric protein, Bovine Seminal Ribonuclease (BS-RNase), made-up by two identical 124 residues subunits that are linked to each other through two disulfide bridges. The native dimeric protein exists as a ca. 2:1 equilibrium mixture of two isoforms with different tertiary structure, with (MxM) and without (M=M) swapping of the N-termini (1). In other words, due to the intersubunit linkages, the unswapped form of the protein is itself dimeric, and the exchange of N-terminal arms takes the form of a thermodynamic equilibrium. Besides the normal enzymatic activity, the protein displays some interesting biological functions, including cytotoxicity toward tumor cells, which can be traced back to its singular structural features. We have recently undertaken a systematic study of the structural bases of domain swapping in BS-RNase, based on site-directed mutagenesis, X-ray diffraction and NMR, with the long-term aim of designing proteins with improved anti-tumor activity. In contrast with all other cases of swapped proteins, substitutions at the hinge loop (i.e. the region that connects the dislocating arm to the main body of the protein) do not significantly affect the swapping propensity of BS-RNase, due to a balance between entropic and enthalpic effects (2). On the other hand, mutants obtained by substituting key residues either at located at the so-called open interface (*i.e.* the contact region between the two subunits), or involved in interactions with the hinge itself, show significant structural and functional differences (3,4). Here, some recent results about a structural and dynamical characterization of dimeric BS-RNase and its mutants in solution will be reported.

References

[1] Piccoli, R. et al. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**: 1870-1874.
[2] Picone, D. et al. (2005) *J. Biol. Chem.* **280**: 13771-13778.
[3] Ercole, C. et al. (2007) *Biochemistry* **46***:* 2227-2232.
[4] Picone, D. et al. (2008) *J. Mol. Biol.* **376**: 427-437.

Charged single α-helix: a versatile protein structural motif

Dániel Süveges[1], Zoltán Gáspári[2], Gábor Tóth[3], László Nyitray[1]

[1]Department of Biochemistry and [2]Institute of Chemistry, Eötvös Loránd University, Pázmány Péter s. 1/C, H1117 Budapest, Hungary,
[3]Bioinformatics Group, Agricultural Biotechnology Center, Szent-Györgyi Albert u. 4, H2100 Gödöllő, Hungary

A few highly charged natural peptide sequences were recently suggested to form stable α-helical structures in water. In this paper we show that these sequences represent a novel structural motif called 'charged single α-helix' (CSAH). To obtain reliable candidate CSAH motifs, we developed two conceptually different computational methods capable of scanning large databases: SCAN4CSAH is based on sequence features characteristic for salt bridge stabilized single α-helices, while FT_CHARGE applies Fourier transformation to charges along sequences. Using the consensus of the two approaches, a remarkable number of proteins were found to contain putative CSAH domains. Recombinant fragments (50-60 residues) corresponding to selected hits obtained by both methods were produced and shown by circular dichroism spectroscopy to adopt largely α-helical structure in water. These segments differ substantially both from coiled coil and unstructured proteins, despite the fact that current detection methods recognize them as either or both. Analysis of the proteins containing the CSAH motif revealed possible functional roles of the corresponding segments. The suggested main functional features include the formation of relatively rigid spacer/connector segments between functional domains as in caldesmon, extension of the lever arm in myosin motors and mediation of transient interactions by promoting dimerization in a range of proteins (Fig 1.).

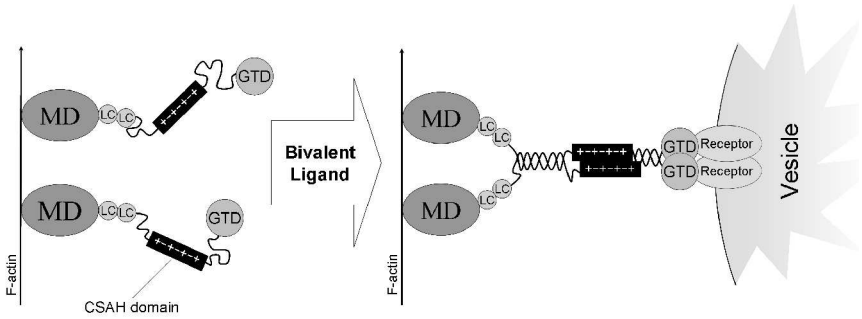


Fig 1. One possible role of CSAH sequences in proteins: the CSAH domain of myosin 6 could be responsible for dimerization upon binding to bivalent ligands.

Ambiguous disulfide bonds

Structure and dynamics of the antifungal protein PAF

Gyula Batta[1], Szabolcs Sándor[1], Zoltán Gáspári[2], Teréz Barna[1], Katalin E. Kövér[3], Éva Leiter[4], István Pócsi[4], Ulrike Binder[5], Bettina Sarg[5], Lydia Kaiserer[5], Herbert Lindner[5], Florentine Marx[5]

Departments of Biochemistry[1], Inorganic and Analytical Chemistry[3], Microbiology and Biotechnology4, Centre of Arts, Humanities and Sciences, University of Debrecen, Egyetem tér 1, H-4010 Debrecen, Hungary. [2]Institute of Chemistry, Eötvös Loránd University, Pázmány P. s. 1/A, H-1117, Budapest, Hungary. [5]Innsbruck Medical University, Biocenter, Division of Molecular Biology, Fritz-Pregl Strasse 3, A-6020 Innsbruck, Austria

The small molecular mass (55 aa, 6.2 kDa) cysteine-rich and basic antifungal protein of *Penicillium chrysogenum* (PAF) induces multifactorial detrimental effects in sensitive fungi. A deeper understanding of the mechanism of action of PAF is likely to lead to the development of novel antifungal drugs. Nearly 90% ^{1}H and full backbone assignment, $^{3}J_{NH,HA}$ couplings from ^{15}N-HSQC and ^{13}CA assignments were obtained at natural abundance. NOE assignments and structure calculation was performed using ATNOS/CANDID in combination with CYANA 2.0. We used combined NOE and S^2 order parameter restrained molecular dynamics simulation (MUMO)[1] for generating realistic ensemble structures. In spite of limited (47%) sequence identity of PAF and AFP, the two antifungal proteins exhibit striking similarity: the main PAF fold is composed of five β-strands forming two orthogonally packed β-sheets sharing a common interface and the six cysteines form three disulfide pairs. However, detailed NMR studies were not capable to unequivocally assign the SS bond patterns either in AFP or in PAF until now. ^{15}N relaxation experiments suggest 3 ns global correlation time and a compact monomeric structure. S^2 parameters from ^{15}N relaxation agreed quite well with chemical shift (RCI, Wishart) based predictions. ^{1}H and ^{15}N CSA/DD cross-correlated relaxation and NH-deuteration rate experiments also corroborate the proposed structure of PAF.

OTKA NK 68578 and EU-NMR Grant- Contract # RII3-026145 / CERM13 project Grant for access to 700 MHz NMR facilities in Florence are gratefully acknowledged.

References

(1.)Richter, B.; Gsponer, J.; Várnai, P.; Salvatella, X.; Vendruscolo, M. The MUMO (minimal under-restraining minimal over-restraining) method for the determination of native state ensembles of proteins. *J. Biomol. NMR* **2007** *37*, 117-135

(2.) Camposolivas, R.; Bruix, M.; Santoro, J.; Lacadena, J.; Delpozo, A. M. et al. Nmr Solution Structure of the Antifungal Protein from Aspergillus-Giganteus - Evidence for Cysteine Pairing Isomerism. *Biochemistry* **1995**, *34*, 3009-3021.

Structural and folding studies on a small miniprotein

A. Neumoin, J. Mares, S. Jurt, R. Walser, O. Zerbe

Institute of Organic Chemistry, University of Zurich, CH-8057 Zurich

The peptide YY (PYY) belongs to the NPY family of neurohormones. These are small, 36 amino acids comprising polypeptides, which are C-terminally amidated. PYY displays the so-called PP-fold in solution[1-2], which is characterized by an N-terminal polyproline helix that is folded against a C-terminal alpha-helix via a beta-turn. This motif is known as the PP fold and was originally observed for aPP by crystallography [3], and for bPP by NMR[4].

Herein, we have investigated molecular properties that result in the PP fold by a combination of NMR and mutagenesis[5]. We could show that through a single point mutation PYY can be converted into a molecule that posses the structural features of NPY. In particular, we have replaced each of the interface residues by Ala and observed a significant destabilization of tertiary structure in every case. From our data we outline a model for folding of this polypeptide in solution. Our mutagenesis data indicated that PYY folds cooperatively. Accordingly, we have conducted solvent- and heat-induced denaturation studies, and followed structural changes by monitoring NMR parameters such as the ^{13}C chemical shift of the C-alpha atoms, but also of sidechain moieties. Our data deliver a rather comprehensive picture of the folding process in this miniprotein.

Whereas PY is largely monomeric at NMR concentrations, the structurally highly related pancreatic polypeptide PP is dimeric. We have conducted similar experiments for unfolding using PP, and discuss differences that may be due to the dimeric nature of the peptide. We also report on attempts to convert monomeric PYY into a dimer.

References

1. Lerch, M., Mayrhofer, M. & Zerbe, O. (2004), J. Mol. Biol. 339, 1153-68.
2. Keire, D. A., Kobayashi, M., Solomon, T. E. & Reeve, J. R., Jr. (2000), *Biochemistry* **39**, 9935-42.
3. Blundell, T. L., Pitts, J. E., Tickle, I. J., Wood, S. P. & Wu, C.-W. (1981), *Proc. Natl. Acad. Sci. U S A* **78**, 4175-79.
4. Li, X. A., Sutcliffe, M. J., Schwartz, T. W. & Dobson, C. M. (1992), *Biochemistry* **31**, 1245-53.
5. Neumoin, A., Mares, J., Lerch-Bader, M., Bader, R., Zerbe, O., *J. Am. Chem. Soc.* **129** (2007), 8811-8817 .

DNA G-quadruplexes and cations

Interactions of nucleic acids and proteins

Janez Plavec

Slovenian NMR Center, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia

NMR is a major tool for structural analysis of bio-macromolecules. The advantages and disadvantages with respect to X-ray crystallography make it complementary and more flexible with respect to sample preparation and handling, where NMR has far superior flexibility in modifying conditions. Three-dimensional structure determination of bio-macromolecules by NMR is usually performed by computer simulations using structural constraints derived from the nuclear Overhauser effect, spin-spin coupling constants, residual dipolar couplings, etc. Recent progress in multidimensional NMR experiments and stable isotope-labeling techniques has simplified the procedure for obtaining these structural constraints, and thus accelerated the entire structural analysis process by NMR. It is therefore not surprising that NMR spectroscopy has contributed immensely to our basic understanding of nucleic acids and protein interactions with each other thus exhibiting various biological activities. Many bio-macromolecules undergo conformational changes as part of their function. RNA is unique in the magnitude of these structural transformations that can involve rearrangements at the secondary, tertiary, and quaternary structure level.

Guanine-rich DNA sequences can fold into four-stranded G-quadruplexes. Their structures are highly polymorphic. They are stabilized by cations.[1-4] We have used NMR in combination with 15N labeled ammonium ions to localize their preferred sites of binding and furthermore to follow their movement within the interior of 3D structural setup and with bulk solution.

References

1 P. Šket, M. Črnugelj, W. Koźmiński, J. Plavec *Org. Biomol. Chem.* 2003, **2,** 1970.
2 P. Šket, M. Črnugelj, J. Plavec *Nucleic Acids Res.* 2005, **33,** 3691.
3 P. Šket, J. Plavec *J. Am. Chem. Soc.* 2007, **129**, 8794.
4 P. Podbevšek, N.V. Hud, J. Plavec *Nucleic Acids Res.* 2007, **35**, 2554.

Theoretical analysis of peptide nucleic acid thermochemistry

Binding energy group contributions in PNA homoduplexes

Eufrozina A. Hoffmann[1], Tamás Körtvélyesi[1,2], Zoltán A. Fekete[2], Botond Penke[3]

[1]*Department of Physical Chemistry, University of Szeged, POB 105, H–6701 Szeged, Hungary*
[2]*HPC group, University of Szeged*
[3]*SZTE-MTA Szupramolekuláris és Nanoszerkezetű Anyagok Kutatócsoport, Szeged*

Peptide nucleic acids (PNAs) are mimics of natural nucleic acids, in which the entire sugar phosphate backbone is replaced by a polyamide backbone, with nucleobases are attached via a methylene carbonyl linker. They have improved biostability, as they are resistant to both nucleases and proteases. PNAs can hybridize with high affinity to complementary sequences of single-stranded RNA and DNA, forming Watson-Crick double helices, and can also invade double helices by formation of triplexes. They have become a general and versatile tool for DNA and RNA recognition.

PNAs also form homoduplexes and homotriplexes with themselves. In this study the stability of N-(2-aminoethyl)glycine (aeg) based PNA duplexes is investigated by theoretical methods. Group contributions are derived for the calculated binding energy of arbitrary aeg-PNA pair sequences. Starting from experimentally available stuctures from the Protein Database (accession codes 1PUP and 1RRU), full quantum chemical optimization was carried at the semiempirical PM3 and PM6 level. Separate calculations were also made with all possible dimer units at their relaxed conformations.

Regression analysis was facilitated with the „response equation based quantitative structure-property relationship“ (REQ-QSPR) method recently proposed by Fishtik *et al*. This approach rationalizes provides a simple concept for breaking down a dataset for heat of formation into linear combination of terms that are easy to interpret in the framework of chemical groups. Applied to our system, it is demonstrated that inter-residue junction conformation as well as base-pair constitution are important in determining stability of aeg-PNA homoduplexes.

Quantum chemical modeling and interpretation of nucleic acid vibrational spectra

Extracting more information about nucleic acid structure by quantum chemistry computations

Valery Andrushchenko and Petr Bour

Institute of Organic Chemistry and Biochemistry, Academy of Sciences,
Flemingovo nam. 2, Prague, 16610, Czech Republic

Nucleic acids exist in the natural environment in a variety of forms and conformations. Such a structural diversity is conditioned by a presence of different metal ions, proteins and other molecules. Significant structural modifications of nucleic acids can also occur upon interaction with various drugs. Among many other techniques vibrational spectroscopy (including infrared absorption, Raman, vibrational circular dichroism (VCD) and Raman optical activity (ROA)) plays an important role in investigation of the structure and conformational changes of nucleic acids due to rich structural information it provides, a wide availability, and relatively fast acquisition times. However, due to complexity of the nucleic acid vibrational spectra, large bandwidth, many overlapping vibrational bands and coupling of vibrations, clear and straightforward interpretation of the spectra with the respect to the nucleic acid structure and dynamics is an ambiguous and tedious task. Recent development of computer technologies and quantum chemistry tools allows to significantly simplify this task by computing vibrational spectra of large molecules with a high precision. Particularly, application of the tensor transfer techniques (1) of transferring molecular properties from smaller molecular fragments to larger systems allows one to compute vibrational spectra for relatively long oligomers of nucleic acids. This approach allows not only to assign most of the spectral features to vibrations of certain nucleic acid moieties, but also to investigate in detail the origin of the fine spectral changes and molecular energetics of the various conformers.

We present several examples documenting how the quantum chemical computations can help us in interpreting the vibrational spectra of nucleic acids in several conformations, as well as the influence of metal ion and drug binding on the nucleic acid structure.

Reference

[1]Bour, P., Sopkova, J., Bednarova, L., Malon, P., Keiderling, T. A. (1997): Transfer of molecular property tensors in Cartesian coordinates: A new algorithm for simulation of vibrational spectra. *J. Comput. Chem.* **18:** 646-659.

Effect of Structural Cell Proteins on the Peptide and Peptide Nucleic Acid (PNA) Binding to HSP90

Effect of Structural Cell Proteins on the Peptide and Peptide Nucleic Acid (PNA) Binding to Heat Shock Protein 90

Tamás Körtvélyesi, [1] Botond Penke, [2] Antal Orosz [3]

[1]Department of Physical Chemistry, University of Szeged, Hungary, [2]Institute of Medicinal Chemistry, University of Szeged, Hungary, [3]Tumor Cell Biology Lab., Anticancer Drug Research Foundation, Budapest, Hungary

Almost all heat shock protein 90 (HSP 90) inhibitors have toxic side effects and problems with solubility. Novel short peptide derivatives were synthetized and studied as HSP 90 inhibitors. Some of them has non-toxic antiproliferative effect [1] and the ability to reverse of multi drug resistance (MDR) [2]. The binding mode of the effective short peptides in the pocket was studied at flexible ligand and flexible ligand-flexible side chain of the protein by docking procedures. The motion of the side chains and the possible movement of the backbone were also simulated by molecular dynamics methods [3]. Some experimental results support that ligands can bind with more effectiveness in the presence of cell structural proteins. In order to support this phenomenon, the binding mode of the short peptides and some known drugs (Geldanamycin) were studied by theoretical methods. α,β-tubuline and β-actine was used as the proteins interact with HSP 90. After protein-protein docking and molecular dynamics calculations, we found that these molecules bound near to the mouth of the pocket and deform it with the effect of changing slightly the binding site. Peptide nucleic acids binding to HSP 90 was also studied and compared with the peptides which are effective.

This work was supported by the Hungarian Research Fund (OTKA K61577).

References

[1] Orosz, A.., Szabó, A.., Szemán, G., Janáky, T., Somlai, C., Penke, B., Bodor, A., Perczel, A., Int. J. Biochem. & Cell Biol., 38(8), 1352-62(2006).
[2] Molnár, J., Engi, H., Mandi, Y., Somlai, C., Penke, B., Szabó, H., Orosz, A., In Vivo 21(2) 429-433(2007).
[3] Körtvélyesi, T., Penke, B., Orosz, A., Prepared for publication.

DNA- and metal-binding of RTN1-C_{CT} peptide: towards the function of a reticulon protein

Ridvan Nepravishta[1], Alessia Bellomaria[1], Francesca Polizio[2], Paci Maurizio[1] and Sonia Melino[1]

[1]Department of Sciences and Chemical Technologies, University of Rome "Tor Vergata", Italy; [2]Department of Biological Sciences, University of Rome "Tor Vergata", Italy

RTN1-C protein is a membrane protein localized in ER and expressed in the nervous system and its biological role is still not clarified, although interactions of the N-terminal region of RTN1-C with proteins involved in the vesicle tracking have been observed. In order to investigate on the role of the cytosolic C-terminal region of RTN1-C we synthesized RTN1-C_{CT} peptide corresponding to the region from 186 to 208 residue of RTN1-C and performed structural and interaction studies on this peptide. We identified in the C-terminal region of RTN1-C a unique consensus sequence characteristic of H4 histone protein and demonstrated a high ability of RTN1-C_{CT} peptide to bind and condensate the nucleic acids using electrophoretic and spectroscopic techniques. Moreover, we identified the presence in the peptide of a N-terminal metal-binding site and studied the ability of the peptide to bind the copper and nickel ions and to cleave DNA. The results allowed us to propose that RTN-1C could be implied in the nucleic acid interaction and that Cu/Ni-RTN1-C_{CT} complexes might be used as small nucleases and therapeutic agents.

The thermodynamic evaluation of G-quadruplex stability

G- quadruplex motifs in the genome

Viktor Víglaský, Luboš Bauer, Martin Walko, Katarína Tlučková

P.J.Šafarik University, Faculty of Sciences, Institute of Chemistry, Department of Biochemistry, Moyzesova 11, 04154 Košice, Slovakia

The sequences repeated in tandem, with three or four adjacent guanines, have been known to form polymorphic quadruplexes containing G-quartets stabilized by cyclic Hoogsteen hydrogen bondings. Quadruplex structures are highly stable DNA or RNA structures formed in G-rich sequences. Naturally occurring G-rich DNA sequences that are able to form parallel/antiparallel G-quadruplex structures appear as potential targets for anti-cancer chemotherapy, and therefore play an important role in cellular processes, such as cell aging, death and carcinogenesis. The telomeric regions of DNA and nuclease hypersensitive elements of human c-myc, VEGF and PDGF-A promoters represent a very appealing target for many drugs and may interfere with normal DNA function. For example platinum complexes bind covalently to nucleobases and especially to the N7 atom of guanines and the four guanines of a G-quartet have their N7 atoms involved in hydrogen bonding. Therefore, within a G-quadruplex structure, only the guanines out of the stack of G-quartets should react with electrophilic species such as platinum (II) complexes. Platinum complexes significantly moderate the formation of G-quadruplexes. Results obtained by CD spectroscopy, PCR and Temperature gradient-gel electrophoresis (TGGE) (1) clearly demonstrate that DNA platination significantly affect folding of G-quadruplex for telomeric sequences; the abundance of unfolded DNAs to G-quadruples is proportional to platinum concentration.

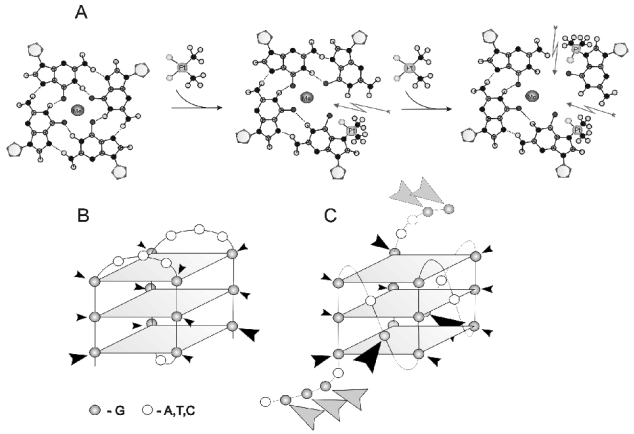


Fig.1. Proposed influence of DNA platination on G-tetrad (A) and cisplatin occupancy of guanines in telomeric and cMYC sequences (B and C).

This study is supported by grants from the Slovak Grant Agency (1/1274/04 and 1/3254/06) and the Science and Technology Assistance Agency (APVT-20-006604).

Reference

[1] Viglasky, V., Danko P .(2007): Intercalators: contra cruciform extrusion in DNA. Anal. Biochem. **360:** 7-13.

Metal binding chimeric peptides with nuclease activity

Artificial metallonucleases for gene therapy

Béla Gyurcsik[1], Emőke Endreffy[2], Kyosuke Nagata[3]

[1]*Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary,*
[2]*Department of Pediatrics, Albert Szent-Györgyi Medical Center, University of Szeged, Szeged, Hungary,*
[3]*Department of Infection Biology, Graduate School of Comprehensive Human Sciences and Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Japan*

Duchenne Muscular Dystrophy is the most common and severe form of the muscular distrophies, where the patients mostly lose the ability to walk by age 12, and generally don't survive beyond their 20s. The cause of the disease is in most cases a deletion of a part of the DMD gene, the largest gene known in human genome. As a consequence, disfunctional protein called dystrophin is expressed, thereby causing progressive muscle weakness, as individual muscle cells dye [1].

Although there have been a lot of efforts, up to now there is no treatment for this disease. One option could be the regeneration of the genetic material. Recently it has been shown that the specifically targeted chromosomal cleavage by zinc-finger nucleases even in the human genome increased the frequency of homologous recombination events []. In addition, zinc-finger nucleases could be designed to recognize practically any nucleotide sequence choosen from a genomic DNA.

Based on this approach, first we have determined the breakpoint within the ~ 2.2 million bases-long gene of a DMD patient. The breakpoint exerts a unique base sequence which can be targeted by zinc finger nucleases to initiate the insertion of the supplied missing DNA sequence by homologous recombination, by use of the cellular DNA repair machinery. Next, rationally optimized phage display selection of a three zinc finger peptide was performed.

The therapeutic disadvantage of the currently known zinc-finger nucleases fused to FokI nuclease domain is their minor citotoxicity, due to the cleavage at nonspecific target sites. The unvanted site recognition is mainly due to the homodimerization instead of the heterodimer formation. In order to avoid this type of side reaction, we have introduced modified nuclease domains based on peptide molecules promoting hydrolysis of nucleic acids in the presence of zinc(II) ions. The DNA binding and cleavage activities of the new chimeric peptides were tested. The recent results will be presented.

References

[1] Nowak, K.J., Davies, K.E. (2004): Duchenne muscular dystrophy and dystrophin: pathogenesis and opportunities for treatment. *EMBO Rep.* **5**: 872-876.

[2] Moehle, E.A., Rock, J.M., Lee, Y.-L., Jouvenot, Y., DeKelver, R.C., Gregory, Ph.D., Urnov, F.D., Holmes, M.C. (2007) Targeted gene addition into a specified location in the human genome using designed zinc finger nucleases. *Proc. Natl. Acad. Sci. USA* **104**: 3055-3060.

Proteomic Studies on "Off Target" Effects in Nucleic Acid Therapy – An Experimental Approach towards Pharmaceutical Systems Biology

Christian R. Noe, Martina Stessl

Departement of Medicinal Chemistry, University of Vienna

In biological studies, the "antisense effect" of oligonucleotides is one of the most important tools to regulate gene expression. Surprisingly, after more than 20 years of research, therapeutic application of this approach is still far away from reaching its tremendous perspectives. Obviously, in the first "heroic" phase of gene therapy, the perfect target specificity of oligonucleotides has drawn the attention of the researchers too far away from important criteria of drug development, such as toxicity and pharmacokinetic ADME parameters.

Focused on bcl-2 down regulation in a melanoma cell line, effected either by antisense oligonucleotides (AONs) or by siRNA, the lecture will deal with the detection of "off target" effects by means of 2D-gel electrophoresis. Although the concept is straightforward in principle, technical details - such as validation of methods or staining techniques for quantitative analysis - are prerequisites to come up with relevant interpretations. In general - at the same level of down regulation - AONs induce clearly more significant differences in the expression pattern of the proteins than siRNA does. A set of proteins has been identified, whose level had changed along with the down regulation of bcl-2. This set opens up the opportunity to study physiological effects associated with the down regulation of the target protein – both related to wanted effects and to unwanted side effects. In a more general perspective, this method provides an efficient experimental approach towards pharmaceutical systems biology.

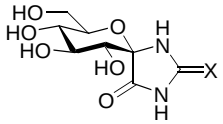
Design, Synthesis, and Structure–Activity Relationships of Glycogen Phosphorylase Inhibitors

Glucose Derivatives against Type 2 Diabetes Mellitus

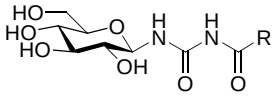
L. Somsák[1], V. Nagy[1], N. Felföldi[1], B. Kónya[1], K. Telepó[1], T. Docsa[2], P. Gergely[2], E. D. Chrysina[3], K.-M. Alexacou[3], S. E. Zographos[3], D. D. Leonidas[3], N. G. Oikonomakos[3]

[1]Department of Organic Chemistry, [2]Institute of Medical Chemitsry, University of Debrecen, Debrecen, Hungary
[3]Institute of Organic and Pharmaceutical Chemistry, The National Hellenic Reserch Foundation, Athens, Greece

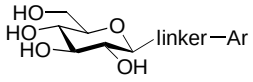
The regulation of glycogen metabolism is a major therapeutic strategy for blood glucose control in type 2 diabetes. Because glycogen phosphorylase (GP) catalyzes the first step in the phosphorolysis of glycogen, it has become a potential key target for controlling hyperglycemia in this disease (1-3). D-Glucose is a physiological regulator of GP and several glucose derivatives have proved to be potent inhibitors of this enzyme being thus potential antihyperglycemics. The first micromolar inhibitors of GP were spiro-hydantoins **1** and **2**. The most efficient glucose analog inhibitors to date are *N*-acyl-*N'*-β-D-glucopyranosyl urea derivatives (e. g. **3** and **4**).



1 X = O K_i = 3.1 μM
2 X = S K_i = 4.2 μM



3 R = Ph K_i = 4.6 μM
4 R = 2-naphthyl K_i = 0.35 μM



5

The presentation will focus on the design of analogous inhibitors **5** based on X-ray crystallographic structural elucidation of enzyme-inhibitor complexes as well as on synthetic efforts to establish structure–activity relationships as to the composition and length of the linker and the size and orientation of the aromatic acyl part. Further synthetic modifications, kinetic and crystallographic results will be presented on parallel posters.

Cell Membrane Active Cyclodextrin Derivatives

Cyclodextrin Derivatives for Drug Delivery

Lajos Szente[1], Éva Fenyvesi[1], Róbert Iványi[1], Ferenc Fenyvesi[2,3], Gábor Szabó[2], Miklós Vecsernyés[3], Dóra Mehn[4], György Báthory[4], Tamás Janáky[5], András Váradi[6]

[1] *CycloLab Cyclodextrin R&D Laboratory Ltd., Budapest, Hungary*
[2] *Department of Biophysics and Cell Biology, Univ. Debrecen, Debrecen, Hungary*
[3] *Department of Pharmaceutical Technology, Univ. Debrecen, Debrecen, Hungary*
[4] *Solvo Biotechnology Inc., Szeged, Hungary*
[5] *Department of Medicinal Chemistry Univ. Szeged, Hungary*
[6] *Institute of Enzymology of the Hungarian Academy of Sciences, Budapest, Hungary*

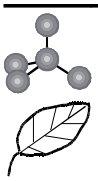
The relationship between the structure of cyclodextrins and their membrane modulating effect has been studied. The influence on the membrane function measured in various cellular and vesicular assays changed in accordance with the interaction of cyclodextrins with cholesterol. The highest affinity toward cholesterol is shown by the methylated beta-cyclodextrin derivatives having membrane-damaging effect. By chemical tuning of 2,6-dimethyl and random methylated beta-cyclodextrin new, "second generation" cyclodextrin derivatives having both ionic and methyl substituents were designed and synthesized aiming at selecting the derivatives, which can reduce the ABC transporter activity in the cell membrane without hemolytic and other cytotoxic effects. Their cholesterol- and drug-solubilizing potential were compared and correlated to the effects on the cell membrane characterized by various tests.

The following derivatives were prepared: carboxymethyl heptakis(2,6-di-O-methyl)-β-cyclodextrin (**CM-DIMEB),** heptakis(2,6-di-O-metil-3-sulfato)-β-cyclodextrin (**S-DIMEB),** sulfopropyl heptakis(2,6-di-O-metil)-β-cyclodextrin (**SP-DIMEB),** succinyl random methylated β-cyclodextrin (**Su-RAMEB),** 6-monoamino-random methylated β-cyclodextrin **MA-RAMEB.** The order in *in vitro* cholesterol solubilizing capacity:

RAMEB, DIMEB, TRIMEB> Su-RAMEB > MA-RAMEB >> SP-DIMEB ~ CM-DIMEB

was similar to the order obtained for the hemolytic effect, cell viability, membrane permeability, detergent resistance of the lipid rafts, showing that cholesterol complexation plays an important role in these cell functions. The effect of these cyclodextrin derivatives on the drug transport using model drugs was also studied.

The work and the presentation were supported by the Hung. R. &. D. Fund (NKFP-1A-041/2004)



Non-covalent Cyclodextrin/Protein Interactions

Biopharmaceutical Uses of Cyclodextrins

István Puskás, Lajos Szente

CycloLab Cyclodextrin R&D Laboratory Ltd., Budapest, Hungary

Over the past decade, the number of papers and patents dealing with the non-covalent interactions between peptides/proteins and cyclodextrins (CD) has been dramatically increasing. Out of presently known 34,400 publications on cyclodextrins, more than 2500 are dedicated to the CD/Protein interactions.

This presentation intends to survey the presently known main directions of research and development dedicated to the pharmaceutical utilization of peptide/CD interactions. The structural aspects of protein/peptide CD interaction will be surveyed with emphasis on the type and strength of association and on the stoichiometry aspects of peptide/CD inclusion phenomena.

The CD-assisted prevention of oligopeptide and protein aggregation will be discussed in the light of literature data and on the example of selected oligopeptides from CycloLab's in-house experience. The stabilization of proteins in chemical sense will be illustrated and evaluated with regard to shelf-life data and to the cyclodextrin-enabled protection of proteins against enzymatic degradation *in vivo* and *in vitro*. The practical importance of cyclodextrin-facilitated peptide and protein delivery across absorptive mucosae will be shown on a number of oral, nasal, transdermal, transpulmonar and ocular delivery systems.

CD-based biodegradable polymeric scaffolds for sustained protein and peptide delivery will be discussed on the example of such biopolymer implants and injectable, long-lasting depot formulations. Examples for the pharmacological relevance of protein/cyclodextrin interactions will be shown by discussing the usefulness of tailor-made CD-derivatives that specifically affect membrane pores and membrane-anchored proteins.

Aldoximinopyridium Cyclodextrin Derivatives

Cyclodextrin Cholineseterase Regenerators

László Jicsinszky[1], Marianna Petró[1,2], Győző Horváth[2], József Szejtli[1], James L. Way[3], Ilona Petrikovics[3]

[1]*CycloLab Cyclodextrin R&D Laboratory Ltd., Budapest, Hungary*
[2] *F. J. Curie Inst. Of Radiation Biology, Budapest, Hungary*
[3] *Texas A&M University, Texas, US*

Cyclodextrin-drug conjugates are such cyclodextrin derivatives where the drug substance is covalently bound to the cyclodextrin. The resulted new chemical entities should require numerous and expensive toxicological studies, therefore the amount of drug loaded to cyclodextrin should be high, the preparation of the conjugate must be easy and the drug substance itself should be very effective due to a possible decreased activity of the conjugate. An additional requirement is the accessibility of the active site of the conjugate.

Aldoximinopyridium iodides (PAMs) are used as agents which antagonize anticholinesterase alkylphosphate intoxications. Owing to the rapid urinary excretion of large amounts of unchanged PAM and only small amounts of metabolites suggest that residence time in the bloodstream should be enhanced to reach an improved efficiency in regeneration of the targeted enzyme. Cyclodextrin conjugates of the two smallest PAMs, 2-PAM and 4-PAM, were prepared to modify the excretion properties of the active molecular moiety. The molecular weight ratio of cyclodextrin derivatives and PAMs forms a feasibility limit of appropriate cyclodextrin derivatives.

In our studies the number of bound PAMs and hydrophilicity of the cyclodextrins were varied. It was found that the more effective 4-PAM could be introduced most difficultly with low yield. The convergent synthesis of the conjugates consists of 2-7 steps starting form the naked cyclodextrin and the appropriate intermediary product of PAM, depending on the targeted cyclodextrin derivative.

In vivo experiments on white male mice confirmed the theory: the prepared cyclodextrin-PAM conjugates were excreted at considerably smaller rate than the parent PAMs. The full substitution at the primary rim of cyclodextrins resulted in almost the half of the efficiency of the identical amount of PAM, but their mean residence time were more than 100 times higher than the unconjugated PAMs. Amphiphilic cyclodextrins obtained by exhaustive methylation on the secondary hydroxyl side resulted in toxic molecules. The promising preliminary results give a real example for using cyclodextrin drug conjugates to improve the bioavailability of fast excreted small molecules.

Synthesis of oligosaccharide fragments of poly-β-(1→6)-*N*-acetyl-glucosamine

Anikó Fekete[1], Anikó Borbás[1], Vince Pozsgay[2], András Lipták[1,3] and Sándor Antus[1,4]

[1]*Research Group for Carbohydrates of the Hungarian Academy of Sciences, H-4010, Debrecen, PO Box 94, Hungary*
[2]*National Institute of Child Health and Human Development, National Institutes of Health, 31 Center Dr. MSC 2423 Bethesda, MD, 20892-2423 United States*
[3]*Institute of Biochemistry, University of Debrecen, H-4010, Debrecen, PO Box 55, Hungary*
[4]*Department of Organic Chemistry, University of Debrecen, H-4010, Debrecen, PO Box 20, Hungary*

In the last few decades staphylococci have become the most prominent organisms that are responsible for infections associated with implanted biomaterials [1]. Because of their resistance to most antibiotics [2], prevention may be alternative strategy against infections.

One of the surface polysaccharides of *Staphylococcus aureus* and *S. epidermidis* is a poly-*N*-acetyl glucosamine (PNAG) antigen. It has been established that purified PNAG can elicit protective immunity against both coagulase-negative staphylococci and *S. aureus*, suggesting that PNAG is a potencial vaccine for staphylococci [3].

Here we present the synthesis of di-, tetra-, hexa- and octasaccharide of poly-β-(1→6)-N-acetylglucosamine with 7-(1,3-dioxan-2-yl) heptyl aglycone (Figure 1).

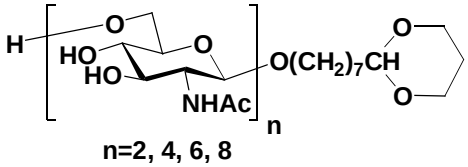


Figure 1.

Our long-term goal is to prepare the protein conjugates of synthetic oligosaccharides for evaluation of their immunogenicity.

References

[1] Huebner, J.; Goldmann, D. A.; *Annu. Rev. Med.* **1999**, *50*, 223-236.
[2] Lowy, F. D. *J. Clin. Invest.* **2003**, *111*, 1265-1273.
[3] Maira-Litran, T.; Kropec A.; Goldmann, D. A.; Gier, G. B. *Vaccine* **2004**, *22*, 872-879.

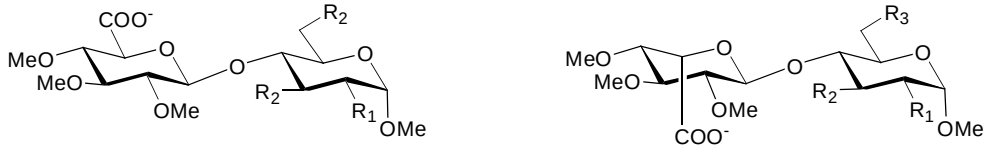
Synthesis of sulfonic acid analogues of heparinoid oligosaccharides

A. Borbás,[1] L. Lázár,[1] M. Herczeg,[1] S. Antus,[2] A. Lipták[1]

[1]*Research Group for Carbohydrates of the Hungarian Academy of Sciences, H-4010, Debrecen, PO Box 94, Hungary*
[2]*Department of Organic Chemistry, University of Debrecen, H-4010, Debrecen, PO Box 20, Hungary*

Heparin is a well-known member of glycosaminoglycans that play a crucial role in maintaining the haemostatic state of blood. Heparin interacts with antithrombin III (AT-III), a serine protease inhibitor that blocks thrombin and factor Xa in the coagulation cascade. The active pentasaccharide fragment (1) of heparin and many simplified analogues (2) were prepared.

The aim of the present work is to prepare sulfonic acid analogues of heparinoid oligosaccharides, in which sulfate esters are partially replaced by methylenesulfonic acid moieties. Sugar-*O*-sulfates and sugar methylene sulfonates are bioisosters, therefore these type of analogues can show antithrombin activity. These negatively charged sulfonic acid analogues can be resistant towards esterases, accordingly the desired biological effect in the organism can be sustained for a longer period of time.



1 $R_1 = CH_2\text{-}SO_3^-$, $R_2 = R_3 = OSO_3^-$

2 $R_1 = R_3 = OSO_3^-$, $R_2 = CH_2\text{-}SO_3^-$

3 $R_1 = R_2 = OSO_3^-$, $R_3 = CH_2\text{-}SO_3^-$

4 $R_1 = CH_2\text{-}SO_3^-$, $R_2 = R_3 = OSO_3^-$

5 $R_1 = R_3 = OSO_3^-$, $R_2 = CH_2\text{-}SO_3^-$

6 $R_1 = R_2 = OSO_3^-$, $R_3 = CH_2\text{-}SO_3^-$

Figure 1. Targeted heparinoid disaccharide sulfonic acids

References

[1] K. A. Bauer, D. W. Hawkins, P. C. Peters, M. Petitou, J.-M. Herbert, C. A. A. van Boeckel, D. G. Meuleman (2002); *Cardiovasc. Drug Rev.* **20**: 37-52.

[2] (a) C. A. A. van Boeckel, M. Petitou (1993); *Angew. Chem. Int. Ed. Engl.* **32**: 1671-1690.
(b) Christian Noti and Peter H. Seeberger (2005); *Chemistry and Biology* **12**: 731-756.
(c) P. Westerduin, C. A. A. van Boeckel, J. E. M. Basten, M. A. Broekhoven, H. Lucas, A. Rood, H. van der Heijden, R. G. M. van Amsterdam, T. G. van Dinther, D. G. Meuleman, A. Visser, G. M. T. Vogel, J. B. L. Damm, G. T. Overklift (1994); *Bioorg. Med. Chem.* **2**: 1267-1280.

Evaluation of Modified Cyclodextrins (CDs) as Inhibitors of Anthrax Toxins

Cationic Cyclic Oligosaccharides as Inhibitors of Anthrax Toxins

Konstantina Yannakopoulou,[1] Vladimir Karginov,[2] Nikolaos Mourtzis,[1] Chrysie Aggelidou,[1] Irene M. Mavridis[1]

[1]*Institute of Physical Chemistry, NCSR "Demokritos" Aghia Paraskevi,*
[2]*Innovative Biologics, Inc 13455 Sunrise Valley Dr. Suite 200 Herndon, VA 20171, USA*

Anthrax infections are caused by *Bacillus anthracis* that secretes three polypeptides, PA (protective antigen), LF (lethal factor) and EF (edema factor). PA forms a heptameric pore [1] to facilitate transport of LF and EF into the cyctosol resulting in death of the infected subject. Cyclodextrins, cyclic oligomers of glucose, consisting of six (α-CD), seven (β-CD) or eight (γ-CD) units and have a 6- 7- or 8-fold molecular symmetry, respectively. Positively charged cyclodextrin (CD) derivatives have been shown recently [1-2] to act as promising inhibitors of the Anthrax lethal toxin by blocking the transmembrane PA pore, in potencies related to their charge and structure [2]. Aminoalkyamino and guanidinalkylamino CDs, shown recently to penetrate effectively cell walls and interact with DNA [3] are also effective inhibitors of Anthrax toxin, however the only β-CD and the γ-CD derivatives, while α-CD derivatives were ineffective. Testing of the compounds for their ability to block ion conductance through the PA channels incorporated into the bilayer lipid membranes is in progress.

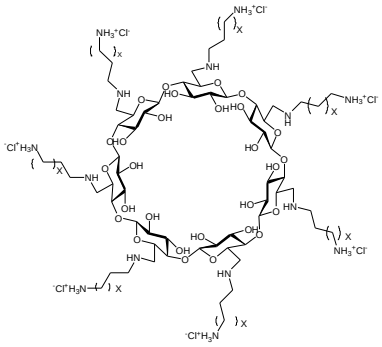


Fig.1. A β-CD derivative functionalised with aminoalkylamino groups, positively charged at pH 7.

References

[1] (a) Karginov, V. A., Nestorovich, E. M., Moayeri, M., Leppla, S. H., Berzukov, S. M. (2005): Blocking Anthrax Lethal Toxin at the Protective Antigen Channel by Using Structure-Isnspired Drug Design. *Proc. Natl. Acad. Sci.* USA **102:** 15075-15080. (b) Karginov, V. A., Nestorovich, E. M., Yohannes, A., Robinson, T. M., Eddine Fahmi, N., Schmidtmann, F., Hecht, S. M., Berzukov, S. M. (2006): Search for Cyclodextrin-Based Inhibitors of Anthrax Toxins: Synthesis, Structural features and Relative Activities. *Antimicrob. Agent. Chemother.* **50(11):** 37403753-15080.

[3] Mourtzis, N., Paravatou, M., Mavridis, I. M., Roberts, M. L., Yannakopoulou, K, (2008): Synthesis, Characterization, and Remarkable Biological Properties of Cyclodextrins Bearing Guanidinoalkylamino and Aminoalkylamino Groups on Their Primary Side. *Chem. Eur. J.* **14:** 4188-4200.

Synthesis of Glycoclusters by Click Chemistry : Binding Studies with Lectins

Glycoconjugates as Probes for Studying Lectin-Carbohydrate Interactions

Samy Cecioni[1], Ruth Lalor[2], Susan E. Matthews[2], Anja Mueller[2], Anne Imberty[3], Jean-Pierre Praly[1], Sébastien Vidal[1]

1ICBMS, Laboratoire de Chimie Organique 2 – Glycochimie, UMR CNRS 5246, Université Lyon 1, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne, France
[2]School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, UK
[3]CERMAV, CNRS, BP 53, F-38041 Grenoble cedex 9, France

Lectin-carbohydrate interactions (1) play a role in biological recognition events involving cells and proteins.(2) Although this interaction is highly specific, the affinity is usually weak (K_d ~ mM) for monovalent interactions. Therefore, Nature uses the so-called "glycoside cluster effect" (3,4) to overcome this weak interaction by presenting several saccharides ligands interacting simultaneously with one or more of their receptor(s). We developed a flexible synthetic approach for the preparation of a large number of glycoclusters (5,6) using microwaves assisted "click chemistry" methodology for the conjugation of carbohydrates to *geometrically well-defined scaffolds* such as calix[4]arenes. Binding studies will determine both the kinetic and thermodynamic aspects of the interactions with lectins. *We want to evaluate the influence of the three-dimensional arrangement of a multivalent glycoconjugate on its binding to a lectin with the eventual appearance of selectivity of some glycoclusters for a specific lectin.*

References

[1] H. Lis, N. Sharon, *Chem. Rev.*, **1998**, 98, 637-674.
[2] R.A. Dwek, *Chem. Rev.*, **1996**, 96, 683-720.
[3] J.J. Lundquist, E.J. Toone, *Chem. Rev.*, **2002**, 102, 555-578.
[4] M. Mammen, S.-K. Choi, G.M. Whitesides, *Angew. Chem. Int. Ed.*, **1998**, 37, 2754-2794.
[5] C. Bouillon, *et al.*, *J. Org. Chem.*, **2006**, 71, 4700-4702.
[6] F. Morvan, *et al.*, *Bioconjugate Chem.*, **2007**, 18, 1637-1643.

Synthesis strategy for the generation of a heparin oligosaccharide library

Towards understanding the specificity of heparin-protein interactions

Péter Fügedi

Chemical Research Center, Hungarian Academy of Sciences, H-1525 Budapest, P.O.Box 17, Hungary

Heparin and heparin sulfate are sulfated polysaccharides belonging to the glycosaminoglycans. Both heparin and heparan sulfate show a great deal of structural heterogeneity due to structural variations in their carbohydrate backbone and mainly as a consequence of their highly varied sulfation pattern. Heparin, well-known as a blood anticoagulant, expresses a series of additional biological activities, such as antitumor, antiviral, antiinflammatory, and antiasthmatic effects. The diverse biological effects of heparin are the results of its interactions with various proteins; heparin-protein interactions modulate important physiological processes. More than a hundred heparin binding proteins have been identified so far.

Understanding heparin-protein interactions at the molecular level is of critical importance both for the understanding of the specificity of carbohydrate-protein interactions and for the potential pharmaceutical implications. In most cases, relatively small oligosaccharide units, and not the whole polysaccharide, are accountable for heparin-protein interactions. It is commonly assumed that individual proteins recognize specific oligosaccharide epitopes within the heparin chain in specific interactions. The identifications of oligosaccharide ligands of heparin-binding proteins is subject to intense research. Testing for the carbohydrate ligands of heparin-binding proteins requires a library of homogeneous oligosaccharide fragments which are, however, not available by degradation of the natural polysaccharide. Current chemical syntheses of heparin oligosaccharides are lengthy, laborious, multi-step processes which provide only one target oligosaccharide from each synthesis and therefore are not suitable for library generation.

We have developed a new synthesis strategy, based on orthogonal protection, which is capable of generating a multitude of target compounds from a small number of common intermediates. Ready access to these homogeneous, structurally well-defined oligosaccharides should greatly facilitate the understanding of the specific interactions of heparin with its receptors.

Multidimesional QSAR

Modeling ligand-protein interactions

Jaroslaw Polanski

Institute of Chemistry, University of Silesia, Katowice, Poland

Quantitative Structure Activity Relationship (QSAR) is an approach mapping chemical structure to properties that should convert molecular data to drugs by property prediction and design. A significant development can be observed along two last decades in this method. From a traditional Hansch analysis based on the logP and Hammett constant towards a growing importance of 3D structure, conformational dynamics and finally receptor data and solvation effects. However modeling interactions of chemical molecules in biological systems still provides highly noisy data, which makes activity predictions a roulette risk. (1). This can be classified as the data, superimposition, molecular similarity, conformational and molecular recognition noise. We will discuss robust methods that improve the performance of multidimensional (m-)QSAR (2).

Molecular recognition uncertainty in traditional receptor independent m-QSAR cannot be eliminated but by the inclusion of the receptor data. Modeling ligand-receptor interactions is a complex computational problem. This limited the development of the receptor dependent (RD) m-QSAR. However, a steady increase of computational power, has also improved modeling ability in chemoinformatics, and novel RD QSAR methods appeared, e.g., RD-4D-QSAR, 5 and 6 D QSAR (3).The strategy for these methods will be briefly discussed. Does this change the place of QSAR in a molecular design landscape?

Finally, we will present Drug Design Toolbox® (DDT®) for MATLAB, the software designed to assist modeling m-QSAR that can be obtained from our laboratory as a free download (4). We will also discuss modeling and visualization tools for the RD-m-QSAR in this software.

References

[1] Daweyko, A. (2004): 3D-QSAR illusions. *J. Comput.-Aided Mol. Des*. **18:** 587-596.

[2] Polanski, J., Gieleciak, R., Bak, A., Magdziarz, T. (2006): Modeling Robust QSAR. *J. Chem. Inf. Model.* **46:** 2310-2318.

[3] Santos-Filho, O.A., Hopfinger, A.J. (2006): Structure-Based QSAR Analysis: An Application of the Receptor-Dependent (RD) 4D-QSAR Formalism, *J. Chem. Inf. Model.,* **46:** 345 -354; Vedani A., Dobler M., Lill M.A. (2005): Combining protein modeling and 6D-QSAR. Simulating the binding of structurally diverse ligands to the estrogen receptor. *J Med Chem.* **48:**3700-3.

[4] T. Magdziarz, J. Polanski, DDT®, http://uranos.cto.us.edu.pl/~zchorg/.

Identification of Kinase Inhibitors: Comparative Evaluation of Theoretical and Experimental Approaches

Virtual and Experimental High Throughput Screening Against Kinase Targets

György M. Keserű

Discovery Chemistry, Gedeon Richter Plc

Virtual screening (VS) is usually considered as “in silico” analogue of in vitro HTS. Both technologies require the selection of the target, the development of the assay/protocol and the definition of the screening library in a suitable physical/virtual format. Hits are typically identified in a highly automated process generating huge amounts of data that should be stored, treated and analyzed. HTS and VS have also a common conceptual framework having the limited accuracy that is compensated by the number of compounds investigated. In addition to true actives and inactives both high throughput technologies surely miss some active compounds (false negatives) but pick up inactives (false positives).

Recent efforts on the integration of these technologies involve (i) sequential, (ii) iterative, (iii) fully integrated, (iv) parallel, and (v) independent screening strategies. There is only limited experience with the first three strategy. In most application HTS and VS are performed independently using distinct physical and virtual compound collections. Since missed and false hits have a serious impact on the applicability domain of these technologies we investigate them comparatively using the strictly parallel strategy using the very same set of compounds for both experimental and virtual screening.

Our goal was to analyze the screening results of several libraries obtained both in silico and in vitro on three different kinase targets. Similar enrichment observed for HTS and VS suggests that virtual screening protocols could identify active ligands from large libraries even in real screening situations. We found that the hit rates in VS are typically higher than that obtained by HTS. Direct comparison of complete hit lists and clusters of hits revealed that the overlap between the results of the two approaches is limited but acceptable. The large number of false positives and false negatives in virtual screening, however, suggests VS to be a complementary rather than competitive approach to HTS.

Affinity chromatography method in off target identification and chemistry driven drug development

Zoltán Varga[1], Zoltán Greff[1], Henrik Daub[2], László Őrfi[1,3,5] Axel.Ullrich[2] György Kéri[1,4,5]

[1]Vichem Chemie Research Ltd. Herman Ottó u. 15., H-1022, Budapest, Hungary,
[2]Max Planck Institute of Biochemistry, Am Klopferspitz 18., D-82152, Martinsried, Germany,
[3]Semmelweis University, Department of Pharmaceutical Chemistry, Hőgyes Endre u. 9., H-1092 Budapest, Hungary,
[4]Pathobiochemical Research Group of Hungarian Academy of Sciences in Semmelweis University, POB 260, 1444. Budapest 8, Hungary,
[5]Rational Drug Design Laboratory CRC, Semmelweis University, POB 131, 1367, Budapest 5, Hungary

Targeted inhibition of protein kinases by small molecule drugs has evolved into a viable approach for anticancer therapy. These inhibitors are widely used in signal transduction therapy, although the true selectivity of them has remained unclear.

Together with scientists at the Max Planck Institute for Biochemistry in Martinsried, GPC Biotech and Kinaxo Biotechnologies we have developed a sophisticated chemical derivatization based affinity chromatography technology for selectivity profiling and off-target identification of kinase inhibitors. The strategic relevance of the KinaTor™ technology in lead optimization is twofold. Firstly, it provides the selectivity panel for a lead of any ongoing optimization program on distinct kinase targets, and secondly, it identifies targets for an investigated compound series templated on a privileged structure.

The KinaTor™ technology relies on the coupling of small molecule kinase inhibitors via a linker to a matrix with the aim to generate an affinity chromatography material, which exploits the strong binding affinities between kinase inhibitors and their molecular targets. A key element of the technology is the proper chemical modification of the kinase inhibitor. Once immobilized ligands are processed in an affinity chromatography approach to identify all the relevant targets and off-targets from a crude extract, which bind to this matrix. MS analysis of the identified protein spots and a parallel LC-MS/MS analysis permit detection of all the bound proteins.

In my presentation I will descibe this target indentification method and I will present two examples to prove its usefulness in drug discovery and development.

Glycopeptides – a synthetic challenge Part I

Gábor K. Tóth[1], Kinga Kádár[1], Orsolya Hegyi[1], Orsolya Csikós[1], László Kalmár[2], János Kerékgyártó[2]

[1]*University of Szeged, Department of Medical Chemistry, Hungary,*
[2]*University of Debrecen, Department of Biochemistry, Hungary*

The number of possible combinations of the twenty proteinogenic amino acids is enormous even for smaller oligopeptides. However, Nature was not satisfied with this variability. Great amount of new compounds, hardly similar to the mother compound, can be formed by partly post-translational modifications following the ribosomal protein synthesis, partly modifications of amino acids by mostly plants, microorganisms and fungi. Beside glycosylated, phosphorylated, etc. peptides and proteins, such compounds are amino acid- and peptide-based heterocyclic compounds (alkaloids and some antibiotics, etc.) widespread in the plant kingdom and used as medicines. These post-translational modifications have fundamental importance in biological recognition processes. One of the most challenging task among of them the rational preparation of the glycosylated peptides especially having oligosaccharide moieties. There are two main strategies for the synthesis of glycopeptides: the synthon and global (convergent) method. Both of them can be implemented in liquid or solid-phase. Since the glycosylation could appear on O and N atoms of the amino acid side-chain, due to the different reactivity of the glycosidic linkage different chemical strategies will necessitate. In this presentation we compare several chemical strategies for the preparation of two model peptides (Leu-Lys-Asn*-Gly-Gly-Pro, Gly-Val-Glu-Asp-Ile-Ser*-Gly-Leu-Pro-Ser-Gly,*site of glycosylation). As glyco-part several mono, di and trisaccharide (see part II.) were used and several of the applied strategies led to successful preparation of these glycoconjugates.

(We are grateful to OTKA-71753 for financial support.)

Glycopeptides – a synthetic challenge Part II

János Kerékgyártó[1], László Kalmár[1], Zoltán Szurmai[1], János Harangi[1], Orsolya Hegyi[2], Gábor K. Tóth[2]

[1]*University of Debrecen, Department of Biochemistry, Hungary,*
[2]*University of Szeged, Department of Medical Chemistry, Hungary*

N-Glycoproteins play central roles in biological processes, such as recognition on cell membranes, intracellular communication, cell growth and they may also represent tumor-associated antigens. The common core-region of the N-glycosylated glycoproteins is the following trisaccharide: β-D-Manp-(1→4)-β-D-GlcNAc-(1→4)-D-GlcNAc attached to the amide of an Asn-unit. In contrast to peptides glycopeptides, the characteristic partial structures of glycoproteins, are not easily available by means of gene technology. Therefore, chemical synthesis provides a valuable possibility to produce these compounds allowing to study structure-function relationship. In order to investigate the coupling reaction between glycosylamines and selectively protected peptides, with respect to the size of each component and choice of protection for the carbohydrate hydroxyl functions, the preparation of the fully *O*-benzylated glycosyl azides GlcNAc(β1-N_3), GlcNAc(β1-4)GlcNAc(β1-N_3) and Man(β1-4)GlcNAc(β1-4)GlcNAc(β1-N_3) representing the reducing terminal of the core structure of N-glycans were prepared. Chemoselective reduction of the azido function of glycosyl azides resulted in fully *O*-benzylated glycosylamines. The coupling reactions by *in situ* trapping of the amines with selectively protected activated aspartic acid and Leu-Lys-Asn*-Gly-Gly-Pro hexapeptide resulted in our target carbohydrate-aspartic acid and carbohydrate-hexapeptide derivatives, respectively. For the preparation of carbohydrate-hexapeptide conjugates by post-synthetic glycosylation of the suitable protected hexapeptide appropriate reaction conditions had to be elaborated in order to minimize undesired side reactions. Both the ^{1}H- and ^{13}C-n.m.r. spectra revealed that the anomerization of the amines could be avoided under the applied reaction conditions. The glycosylated aspartic acid derivatives after catalytic hydrogenation are suitable building blocks for solid phase peptide synthesis (see part I.).

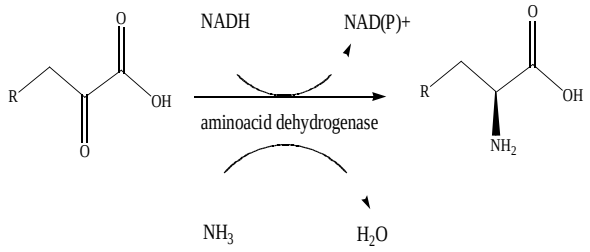
(We are grateful to OTKA-71753 for financial support.)

Novel approach to the synthesis of aliphatic and aromatic keto acids and their application in biotransformations

Daniele Balducci, Francesca Paradisi

UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4 Ireland

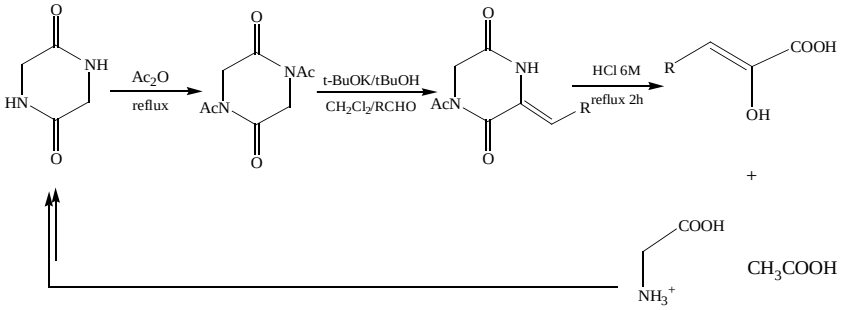
Keto acids are very useful substrates in the synthesis of chiral amino acids as suitable dehydrogenases enzymes can be utilised as biocatalysts (Scheme 1). [1-2].



Scheme 1

In the literature different methods have been reported for the synthesis of either aromatic or aliphatic keto acids, with yields that vary from very good to very poor.[3]

Here, an efficient synthesis of keto-acids is achieved by employing a N-acetyl diketopiperazin scaffold. The synthesis encompasses both aromatic and aliphatic substrates proving to be versatile and innovative with excellent carbon economy and recycling of the glycine by-product (Scheme 2).



Scheme 2

References

[1] P. Busca, F. Paradisi, E. Moynihan, A. R. Maguire, P. C. Engel "*Enantioselective Synthesis of Non-natural Amino Acids using Phenylalanine Dehydrogenases Modified by Site-directed Mutagenesis" Organic and Biomolecular Chemistry* **2004***, 2*, 2684 - 2691

[2] F. Paradisi, S. Collins, A. R. Maguire, P. C. Engel "*The use of phenylalanine dehydrogenase mutants as efficient biocatalysts for the synthesis of non-natural phenylalanine derivatives" J. Biotechnol.*, **2007,***128*, 408-411**.**

[3] A. J.L. Cooper, J.Z. Ginos, A. Meister *"Synthesis and Properties of the α-Keto Acids" Chem. Rev.* **1983**, 83, 321-358

Transformation of sanguinarine to dihydrosanguinarine in vivo

Sanguinarine: Pharmacokinetics and metabolism

Pavel Kosina, Vilím Šimánek, Jitka Ulrichová

Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic

Medicinal plants containing the quaternary benzo[c]phenanthridine alkaloid (QBA) sanguinarine (SG) *Sanguinaria canadensis*, *Chelidonium majus* and *Macleaya cordata* (Papaveraceae) were in the past utilized in traditional medicine. SG was the subject of sustained practical and research interests owing to its widespread biological activities. Mechanistic studies in normal and/or cancer cell types evidenced effects of SG on apoptosis, angiogenesis, proliferation, differentiation and transformation. In animal experiments SG displayed mainly antimicrobial and anti-inflammatory effects. SG is the active component in SANGROVIT®, a weight gain stimulant for farm animals. The QBA fraction from *M. cordata* has been used in oral hygienic preparations. On the other hand, a well-known toxic effect attributed to SG and its dihydroderivative (DHSG) is the epidemic dropsy syndrome caused by consumption of edible oils contaminated by the oil from *Argemone mexicana* seeds. A case report study has reported that the long-term use of oral products containing SG appears to be associated with an increased prevalence of oral leukoplakia. The Achilles heel of the assessment of safety/toxicity SG and/or DHSG was until recently the full absence of data about their pharmacokinetics, biodistribution and metabolism, i.e. topics presented in this lecture. The SG pharmacokinetics was studied in the rat after a single oral dose (10 $mg.kg^{-1}$ body weight). Alkaloid determination in the plasma and liver was carried out by validated HPLC/ESI-MS method. The pharmacokinetic parameters (t_{max}, c_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$) were determined for SG. The major metabolite detected in the plasma was DHSG. Neither SG nor DHSG were detected in the urine. The formation of the less toxic DHSG (the reduction of the iminium bond of SG) might be the first step of SG detoxification in the organism and its subsequent elimination in phase II reactions. Both compounds were completely eliminated from the plasma and liver after 24 h. After a single oral dose of ^{3}H-sanguinarine, radioactivity was detected in all organs and tissues, over 42% of the ingested radioactivity being present in the gastrointestinal tract. Benz[*c*]acridine, the only sanguinarine metabolite referred to in the literature, was not detected in the plasma, liver or urine.

After SG, we focused on DHSG and evaluated its toxicity in rats at concentrations of 100 and 500 ppm DHSG in feed for 90 days. No significant alterations in any studied parameters (biochemical, hematological, histological examinations, etc). Thus, repeated dosing of DHSG for 90 days at up to 500 ppm in the diet (i.e. approximately 58 mg/kg/day) showed no evidence of toxicity, in contrast to results published in the literature. In parallel, DHSG pharmacokinetics was studied in rat after oral doses 9.1 or 91 mg/kg body weight. The results showed that DHSG undergoes enterohepatic cycling with maximum concentration in plasma at the first or second hour following the application. DHSG was cleared from the body after 12 or 18 h, respectively. The investigation of further metabolic pathway of SG (DHSG) is the area of our ongoing research, which should lead to the better understanding of behavior of sanguinarine in living systems.

Acknowledgements : This work was supported by the Grant Agency of the MSMT (grant No. MSM 6198959216) and by the Grant Agency of the Czech Republic (GA CR 525/07/0871).

Halogenated alkaloids as potent effectors of myosin function

Allosteric inhibition of myosin motor activity

Roman Fedorov[1], Markus Böhl[2], Georgios Tsiavaliaris[3], Falk K. Hartmann[3]; Petra Baruch[1], Bernhard Brenner[4], René Martin[5], Hans-Joachim Knölker[5], Herwig O. Gutzeit[2], Dietmar J. Manstein[1,3]

[1]Research Centre for Structure Analysis, OE8830, Hannover Medical School, 30623 Hannover, Germany;
[2]Department of Zoology, TU Dresden, Zellescher Weg 20b, 01217 Dresden, Germany;
[3]Institute for Biophysical Chemistry, OE4350, Hannover Medical School, 30623 Hannover, Germany;
[4]Institute for Molecular and Cellular Physiology, OE4210, Hannover Medical School, 30623 Hannover, Germany;
[5]Department of Chemistry, TU Dresden, Bergstr. 66, 01069 Dresden, Germany

We identified the marine natural product pentabromopseudilin (PBP) as potent inhibitor of myosin motor activity. IC_{50} values of 1.2, 23, and 50 μM were obtained for the actin-activated ATPase and motile activities of chicken myosin-5a, skeletal muscle myosin-2, and Dictyostelium myosin-1E. PBP interferes with ATP-binding, ATP-hydrolysis, and the communication between the actin and nucleotide binding sites. PBP does not block the formation of strong binding states but its presence leads to a marked increase in the time that the motor domain spends per catalytic cycle in weak binding states. To identify the binding mode of PBP, we crystallized the Dictyostelium myosin-2 motor domain Mg^{2+}ATP-metavanadate complex in the presence of PBP. The structure of the ternary complex was refined to 2.8 Å resolution. The electron density for PBP is unambiguous and shows the inhibitor to bind to a novel allosteric site involving residues in the 50 kDa upper domain of myosin, loop-2, and the strut loop.

The effects of related halogenated alkaloids on the interaction of myosin with F-actin will be described and myosin isoform-specific differences will be discussed.

An allosteric myosin inhibitor alters ligand-induced conformational changes

Utility of a myosin inhibitor in deciphering the structural mechanism of force generation

Balázs Takács, Máté Gyimesi, Bálint Kintses, András Málnási-Csizmadia, Mihály Kovács

Dept. of Biochemistry, Eötvös University, H-1117 Budapest, Pázmány P. sétány 1/C, Hungary

Blebbistatin is a recently discovered inhibitor of class 2 myosins, a group which includes molecular motors working in muscle and during cell division of all cell types. Previously we have shown that blebbistatin is an allosteric inhibitor: it binds at the bottom of myosin's actin binding cleft and stabilizes a complex of myosin with ATP hydrolysis products. In this study we assessed the effect of blebbistatin on the nucleotide-induced structural changes that occur during the working cycle of myosin. We used single tryptophan *Dictyostelium* myosin 2 catalytic domain constructs containing site-specific tryptophan sensors at key locations (in the ATP binding site, and at the base of the lever arm). Our results show that, during the ATP hydrolysis cycle, blebbistatin stabilizes a weak actin binding intermediate that forms before the powerstroke of the motor. Furthermore we find that, under certain conditions, myosin's complex with blebbistatin resembles an essential but unexplored intermediate of the force generation pathway that has been inaccessible due to its low abundance and short lifetime in the absence of the inhibitor.

L(+)-tartrate and vanadate: nonlinear competitive inhibitors of prostatic phosphatase catalytic activity

Inhibitors of allosteric human prostatic acid phosphatase

Magdalena Górny, Natalia Hutyra, Ewa Luchter-Wasylewska

Department of Medical Biochemistry, Jagiellonian University, Kraków, Poland

Human prostatic acid phosphatase (PAP) is built of two identical subunits; each is composed of two domains of various size. The active center, with his^{12}, is located in a large open cleft between domains.

PAP catalyses hydrolysis of many phosphoesters (phosphosugars, nucleotides, phospholipids and phosphoproteins on phosphoserine, phosphothreonine, and phosphotyrosine residues). Additionally PAP displays phosphotransferase and peptidase catalytic activities. To the physiological substrates dephosphorylated by PAP belongs receptor cErbB2 with protein tyrosine kinase activity as well as seminal fluid LPA and semenogelin.

Positive cooperativity in substrate binding, exhibited by PAP, was stated by us [1]. The substrate saturation curves, described by Hill rate equation, are sigmoidal. Thus, the hydrolysis substrates are positive homotropic effectors (activators) of PAP and PAP belongs to regulatory enzymes. The extent of cooperativity grows when enzyme concentration is increased.

Studies on inhibition of phenyl phosphate (FP) hydrolysis, catalysed by PAP, are continued by us. Two inhibitors: vanadate (substrate analogue) and L(+)-tartrate (transient-state analogue), were used at selected enzyme concentrations (10, 25 and 50 nM). The experimental data were fitted to the Hill rate equation in order to determine the values of catalytic constant k_{cat}, of half saturation constant $K_{0,5}$, and of Hill cooperation coefficient h.

L(+)-tartrate and sodium vanadate were found to be non-linear competitive inhibitors of PAP-catalysed hydrolysis of FP. When inhibitor concentration grows, the values of half saturation constants ($K_{0,5}$) are increased but the values of turnover numbers (k_{cat}) remain constant. When L(+)-tartrate concentration grows the Hill cooperation coefficient (h) is decreased. L(+)-tartrate thus diminishes the cooperative character of PAP. Sodium vanadate, at growing concentration, does not change the cooperative character of substrate binding to PAP.

Reference

[1] Luchter-Wasylewska, E. (2001) Cooperative kinetics of human prostatic acid phosphatase. *Biochim. Biophys. Acta* **1548**, 257-264.

Small bioactive molecule interactions in crystalline environment

Inhibitor – enzyme interactions inferred from crystal structures: a comparative study

Barbara J. Oleksyn[1], Ewa Żesławska[2], Wojciech Nitek[1], Jan Śliwiński[1]

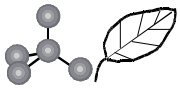
[1]Faculty of Chemistry, Jagiellonian Unversity, [2]Pedagogical University; Kraków, Poland,

The crystal structures of ligand – protein complexes are important sources of information about the interactions between small bioactive molecules and the macromolecules. The knowledge of such interactions helps to understand the mechanism of action of the studied molecules and to elucidate the biochemical processes, which run with their participation. Interesting examples are the enzyme-inhibitor complexes, especially those in which the role of inhibitors is played by drugs or potential drugs, while the enzyme is engaged in some pathological phenomena in living organisms. The well known examples are anti- Inhibitor – enzyme interactions inferred from crystal structures anti-inflammatory, anti-HIV and anticancer drugs. The X-ray structure analysis of the enzyme – inhibitor complex reveals such intermolecular interactions as covalent bonds, co-ordination bonds, ionic bonds, hydrogen bonds, π - π interactions and some other short contacts.

In our studies of the crystalline complexes of urokinase type plasminogen activator (uPA) inhibitors with this enzyme (I-E) and with small picrate anion (I-P) we noticed geometrical similarity between the intermolecular interactions formed by the inhibitors in I-E crystals and in I-P crystals [1,2]. This observation prompted us to check occurrence of such tendency in other systems found in PDB (for inhibitor-enzyme complexes) and CSD (for inhibitors in the environment of small molecules). In this contribution we describe the results of comparison between the intermolecular interactions formed by each of the inhibitors with two different types of molecules (enzyme and small molecules).

Reference

[1] E. Zeslawska, U. Jacob, J. Sturzebacher, B.J.Oleksyn (2006), Bioorg. Med. Chem. Lett. **16,** 228 – 234.



Cyanobacterial indole-3-acetic acid: production and impact on plant growth

Mehboob Ahmed, Shahida Hasnain

Department of Microbiology and Molecular Genetics, Quaid-e-Azam Campus, University of the Punjab,Lahore-54590, PAKISTAN

Selective biotransformations in continuos flow reactors

Csaba Csajági[1], Gábor Szatzker[2], Mariann Szigeti[2], Anna Tomin[2], Enikő R. Tőke[2], Sarolta Pilbák[2], László Ürge[1], Ferenc Darvas[1], László Poppe[2]

[1]*ThalesNano Inc., Graphisoft Park, H-1031 Budapest, Záhony u. 7., Hungary,*
[2]*Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, H-1111 Budapest, Műegyetem rkp. 3, Hungary*

Continuous-flow mode enantiomer selective acetylations of racemic secondary alcohols (*rac*-**1a-c**, *Fig.1.*) were performed in small lipase-filled stainless steel packed-bed reactors. Highly enantiomer selective (E > 100) kinetic resolutions were achieved with several lipases. Comparison of the continuous-flow and batch mode biotransformations indicated similar enantiomer selectivities (E) but higher productivities (specific reaction rate: r) in the corresponding continuous-flow reaction. The effect of temperature (0-60 °C) and pressure (1-120 bar) on the continuous-flow acetylation of *rac*-**1c** was investigated in a reactor filled with *Candida antarctica* lipase B (CaLB). Whereas pressure had no significant effect, an increase in pruductivity (r) was observed within this temperature range. Surprisingly, the enantiomer selectivity had a maximum (E ~25, at 20 °C) and a minimum (E ~7, at 50 °C).

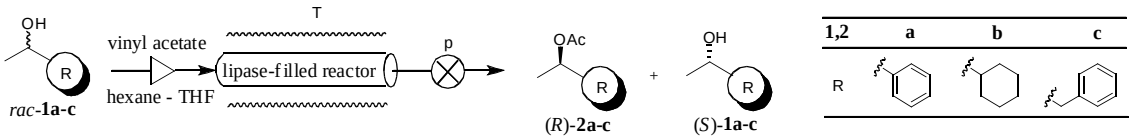


Fig.1. Enzymatic kinetic resolutions in continuous flow reactor

Stereoselective biotransformations of further racemic alcohols with coupled continuous mode extractions and asymmetric acylation of prochiral diols were also investigated in continuous flow systems.

Reference

[1] Csajági,Cs.; Szatzker, G.; Tőke, E. R.; Ürge, L.; Darvas, F.; Poppe, L. (2008): Enantiomer selective acylation of racemic alcohols by lipases in continuous-flow bioreactors. *Tetrahedron:Asymmetry*, **19:** 237-246.

Allyl thiosulfate, a natural apoptotic inducer affects the cell detoxification system

Renato Sabelli[1], Sonia Melino[1], Giuditta Viticchiè[1], Egidio Iorio[2], Franca Podo[2], Maurizio Paci[1]

[1]*Department of Sciences and Chemical Technologies, University of Rome "Tor Vergata", Italy;*
[2]*Department of Cellular Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy.*

Recently, the induction of the programmed cell death by sulfane sulfur/seleno compounds open relevant questions about their metabolism and on the role in the cancerogenesis processes of the of the enzymes involved in it. Sodium 2-propenyl-thiosulfate (2-PTS) a water-soluble organo-sulfane sulfur compound (OSC), isolated from garlic induces apoptosis in a number of cancer cells. The molecular mechanism of action of 2-PTS is not clarified. In this work we investigated, *in vivo* and *in vitro,* the effects of this sulfane sulfur compound on the activity of enzymes involved in the detoxification system and in the "managing" of the redox state of the cell. Our data suggest that the rhodanese, thioredoxin reductase and GST may be target enzymes of this garlic OSC and that GSH might be involved in the antiproliferative effect of 2-PTS.

Molecular simulation of hyaluronates

Metal binding to hyaluronates

Elizabeta Tratar Pirc[1], Jernej Zidar[2]

[1]*University of Ljubljana, Faculty of Chemistry and Chem. Technol., Ljubljana, Slovenia*
[2]*National Institute of Chemistry, Ljubljana, Slovenia*

The glycosaminoglycan hyaluronan (hyaluronic acid, HA) is becoming increasingly important as its many biological functions and biomedical applications are discovered. Chemically, HA is a hetero-polysaccharide composed of repeating disaccharide units (-GlcNAc-β1,4-GlcA-β1,3-)$_n$ where GlcA is D-glucuronic acid and GlcNAc is N-acetyl-D-glucosamine, and n is approximately 10.000. Because of the repeating anionic carboxylic sites HA is a polyanion under physiological conditions, its interaction with cations is an important factor determining the supermolecular structure and function. The coordination geometry around the cation has a strong influence on the conformation of the macromolecule and its biological activity. The new properties can then be further exploited in pharmaceutical practice or in materials science. Solid metal hyaluronates have not been extensively studied. The present study is focused on the interaction of HA molecule with Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and Ag^{1+} ions, in particular on differences in ion coordination which influences the behavior of the whole macromolecule. Molecular modeling was used to complement experimental data. In the first step the monomers, D-glucuronic acid and N-acetyl-D-glucosamine were constructed and optimized with quantum mechanical calculations. Coordination, symmetry data and counterion (Ca^{2+}) for hyaluronic acid were used to build a bundle of hyaluronic acid fibers. To investigate the binding of heavy metal ions to hyaluronic acid at atomic level a combination of quantum modeling (QM) and molecular modeling (MM) was used. Within this approach the simulated system is simplified by dividing it into a smaller QM part, calculated using quantum mechanics and a larger MM part, computed using molecular mechanics.

We assumed the overall conformation of the hyaluronan moiety would not change significantly during simulations of hyaluronate chains with other heavy metal ions. This allowed us to replace Ca^{2+} ions with metal cations, adjust the parameters to reflect the change of ion and redo the simulation. The results of simulations are in good agreement with experimental data. The results of our QM/MM simulations also confirm that the overall conformation of the hyaluronan moiety does not change during simulations of hyaluronate chains with different heavy metal ions. The observed change of coordination geometry is probably caused by the change of the number of water molecules (i.e. hydration) around the heavy metal ion.

References

[1] Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., Karplus, M. (1983): CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. In: *J. Comput. Chem*, **4:** 187–217.
[2] Tratar Pirc, E., Zidar, J., Bukovec, P., Hodoscek, M., (2005): Molecular modeling of cobalt(II) hyaluronate. In: *Carbohydr. Res.,* **340**: 2064–2069.

Flexible peptides as interaction hubs

Focal adhesion assembly

Iain D Campbell

Department of Biochemistry, University of Oxford, South Parks Rd, Oxford OX1 3QU

Important biological processes such as cell migration depend on the formation of transient self-assembling complexes called focal adhesions. These have many protein components made from modular proteins with flexible linkers. Components include extracellular matrix proteins, such as fibronectin [1], membrane-spanning receptors, such as integrins and CD44 [2], and numerous intracellular proteins, including talin [3] and filamin [4] that link short cytoplasmic integrin tails to actin filaments. I will review structural biology studies of some of the dynamic complexes formed by these proteins. Examples will be given from our recently published [1-4] and unpublished work. Emphasis will be placed on complexes formed between folded protein domains and intrinsically unfolded peptides.

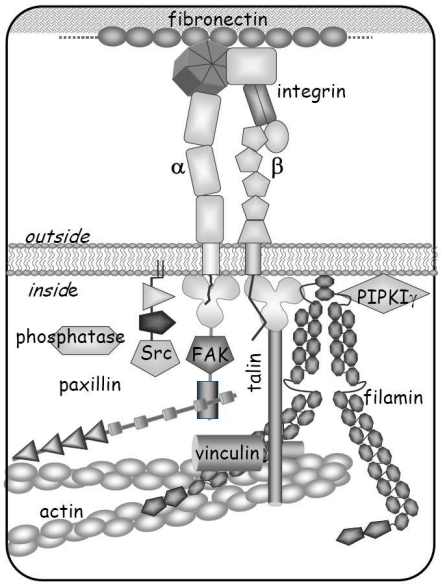


Fig.1. A schematic view of a focal adhesion complex. The proteins are drawn so as to indicate their modular nature and some of the flexible linker regions joining them. The extracellular matrix (ECM) at the top of the diagram is made up of molecules like fibronectin. Integrins, membrane spanning $\alpha\beta$ heterodimers, bind to ECM components using their extracellular regions. Short flexible intracellular integrin tails are linked to the actin cytoskeleton by talin, which has a cloverleaf-like FERM head domain and a rod-like actin binding domain, and filamin-A, a large two-armed dimer, each arm of which has an actin binding domain and 24 Ig-like domains. Important other associated molecules include kinases (e.g. Src, PIPKIγ and FAK) and phosphatases. Paxillin and vinculin facilitate the formation of the dynamic complexes by cross-linking several different proteins

References

[1] Vakonakis I. *et al.* Intra-domain association in fibronectin: insight into cryptic sites and fibrillogenesis (2007) *EMBO J* **26** 2575-83.
[2] Banerji S *et al.* Structures of the CD44–hyaluronan complex provide insight into a fundamental carbohydrate protein interaction (2007) *Nat Struct and Mol Biol* **14**, 234-9.
[3] Wegener KL *et al.* Structural basis of talin activation of integrins (2007) *Cell* **128**, 171-182.
[4] Kiema T et al. Structural details of integrin-filamin interactions reveal competition with filamin (2006) Molecular Cell **21**, 337-47.

Revealing and characterizing interactions between proteins, metal ions, partner proteins and organic ligands

Claudio Luchinat

Magnetic Resonance Center - CERM, University of Florence, Via L. Sacconi, 6, 50019 Sesto Fiorentino, Italy

The activity of the structural biology laboratory at CERM spans from the production and the structural determination of proteins and metalloproteins [1], to the investigation of their dynamic properties [2], to the study of the multiple interactions between biological components of cells or organelles [3], to the interactions with candidate drugs [4]. NMR is playing an important role in all these activities. When dealing with metalloproteins, the presence of the metal offers even more opportunities to exploit the potential of NMR. If the metal is paramagnetic, or can be substituted with a paramagnetic one, the NMR parameters are altered in many ways, and these alterations contain additional information on both structure and dynamics [5]. Examples will be reported of how the combination of X-ray and NMR information provides a complete picture, for example to obtain hints on the metal uptake and release of metal storage proteins [6], or to address the problem of multidomain proteins that require, or may require, interdomain conformational freedom for their function [7]. By this combination of tools we address problems such as the mechanisms of intracellular calcium signalling [7-10], of extracellular hydrolytic activities carried out by matrix metalloproteinases [11], and of candidate drug binding to phosphatases [12].

References

[1] Ab, E., Atkinson, A. R., Banci, L., Bertini, I., Ciofi-Baffoni, S., Brunner, K., Diercks, T., Doetsch, V., Engelke, F., Folkers, G., Griesinger, C., Gronwald, W., Gunther, H., Habeck, M., de Jong, R, Kalbitzer, H. R., Kieffer, B., Leeflang, B. R., Loss, S., Luchinat, C., Marquardsen, T., Moskau, D., Neidig, K. P., Nilges, M., Piccioli, M., Pierattelli, R., Rieping, W., Schippmann, T., Schwalbe, H., Trave, G, Trenner, J. M., Wohnert, J., Zweckstetter, M., and Kaptein, R., *NMR in structural proteomics*, **Acta Crystallogr. D Biol. Crystallogr.** 62: 1161-1183, 2006.

[2] Fragai, M., Luchinat, C. and Parigi, G., *"Four-dimensional" protein structures: examples from metalloproteins*, **Acc. Chem. Res.**, 39: 909-917 (2006).

[3] Banci, L, Bertini, I, Ciofi-Baffoni, S, Kandias, NG, Spyroulias, GA, Su, XC, Robinson, NJ and Vanarotti, M. *The delivery of copper for thylakoid import observed by NMR*. **Proc. Natl. Acad. Sci. USA** 103: 8325, 2006; Banci, L, Bertini, I, Cantini, F, Felli, IC, Gonnelli, L, Hadjiliadis, N, Pierattelli, R, Rosato, A and Voulgaris, P. *The Atx1-Ccc2 complex is a metal-mediated protein-protein interaction*. **Nat. Chem. Biol.** 2: 367-368, 2006.

[4] Pellecchia, M, Bertini, I., Cowburn,D, Dalvit, C, Giralt, E, Jahnke, W., James, T. L, Homans, S. W., Kessler, H, Luchinat, C., Meyer, B, Oschkinat, H., Peng, J, Schwalbe, H, Siegal, G., *Perspectives on NMR in Drug Discovery: A Technique Comes of Age*, **Nat. Drug Disc.**, in press.

[5] Bertini, I., Luchinat, C., Parigi, G. and Pierattelli, R. *NMR of paramagnetic metalloproteins*, **ChemBioChem**, 6: 1536-1549 (2005).

[6] Calderone, V., Del Bianco, C., Dolderer, B., Echner, H., Hartmann, H. J., Luchinat, C., Mangani, S. and Weser, U. *The crystal structure of yeast copper thionein: The solution of a long-lasting enigma*, **Proc. Natl. Acad. Sci. USA**, 102: 51-56 (2005).

[7] Bertini, I., Del Bianco, C., Gelis, I., Katsaros, N., Luchinat, C., Parigi, G., Peana, M., Provenzani, A. and Zoroddu, M. A. *Experimentally exploring the conformational space sampled by domain reorientation in calmodulin.* **Proc. Natl. Acad. Sci. USA** 101:6841-6846 (2004).

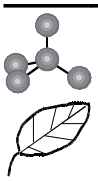
[8] Bertini, I., Gupta, Y. K., Luchinat, C., Parigi, G., Peana, M., Sgheri, L. and Yuan, J., *Paramagnetism-Based NMR Restraints Provide Maximum Allowed Probabilities for the Different Conformations of Partially Independent Protein Domains*, **J. Am. Chem. Soc.**, 129, 12786-12794 (2007).

[9] Bertini, I., Kursula, P., Luchinat, C., Parigi, G., Vahokoski, J., Wilmanns, M. and Yuan, J., *A combined X-ray and NMR structural investigation of calmodulin with DAPk and DRP1 peptides: detection of rearrangement in solution*, submitted.

[10] Arendt, Y., Bhaumik, A., Del Conte, R., Luchinat, C., Mori, M. and Porcu, M., *Fragment docking to S100 proteins reveals a wide diversity of weak interaction sites,* **ChemMedChem**, 2, 1648-1654 (2007).

[11] Bertini, I., Calderone, V., Cosenza, M., Fragai, M., Lee, Y.-M., Luchinat, C., Mangani, S., Terni, B. and Turano, P. *Conformational variability of MMPs: beyond a single 3D structure*, **Proc.Natl.Acad.Sci.USA**, 102: 5334-5339 (2005).; Bertini, I., Calderone, V., Fragai, M., Luchinat, C., Maletta, M. and Yeo, K. J., *Snapshots of the Reaction Mechanism of Matrix Metalloproteinases*, **Angew.Chem.Int.Ed** , 45: 7952-7955 (2006); Bertini, I., Calderone, V., Fragai, M., Jaiswal, R., Luchinat, C., Melikian, M., Mylonas, E. and Svergun, D. I., *Evidence of reciprocal reorientation of the catalytic and hemopexin-like domains of full-length MMP-12*, **J.Am.Chem.Soc.**, 130, 7011-7021 (2008).

[12] Bertini, I., Fragai, M., Luchinat, C., and Talluri, E., Water-based ligand screening for paramagnetic metalloproteins, **Angew.Chem.Int.Ed.**, 47, 4533-4537, (2008)



Transition metal complexes of small multihistidine peptides

Models of the active centres of some metalloenzymes

Sarolta Timári, Csilla Kállay, Imre Sóvágó, Katalin Várnagy

Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010 Debrecen, Hungary

It is well known that the essential metal ions play important roles in the synthesis and transport of biomolecules or the catalysis of acid-base and redox processes of biological systems. Therefore, the very low concentration of other metal ions are dangerous and toxic for all organisms. It is also obvious, that among the organic compounds the proteins through their side chain donor atoms play the most important role in the metal ion binding.

Histidine imidazole nitrogens are the most frequent binding side chain donor groups, which is well demonstrated by different metalloenzymes (superoxide-dismutase (SOD), carbonic anhydrase, carboxypeptidase etc) and other proteins including prions, amyloid-β-peptides, histones and SPARC. The peptides containing two or more histidinyl residues exhibit a very high structural variety in the complex formation reactions with metal ions, because in addition to the common binding modes of peptides, the formation of macrochelates or other bent structures and/or polynuclear complexes is also possible. Moreover, the multihistidine peptides are often considered as promising models to mimic the structures and catalytic activities of various metalloenzymes including Cu,Zn-SOD and copper containing oxidases.

For this reason nickel(II)-, zinc(II)- and cobalt(II) complexes of several multihistidine and/or terminally protected tri-, tetra- and penta-peptides including Ac-HGH-OH, Ac-HGH-NHMe, Ac-HHGH-OH, Ac-HAHVH-NH_2, Ac-HVHGH-NH_2 and Ac-HGHVH-NH_2 were studied by potentiometric, UV-Vis, CD and ^{1}H NMR spectroscopic methods. In all cases, the two imidazole N coordinated MLH (ML for tripeptides) and three imidazole N coordinated ML complexes are formed in the acidic pH range. The increase in the number of histidyl residues results in enhanced stability of ML complexes and these complexes are present predominantly under slightly acidic pH conditions (pH 5-7). With nickel(II) ion the cooperative deprotonation of amide nitrogens can be observed at high pH and $NiLH_{-2}$ complexes are formed.

Acknowledgements: This work was supported by the Hungarian Scientific Research Fund (T048352 and T072956).

Imidazole versus hydroxamate preference in Cu(II) and Ni(II) complexes of histidine containing peptidehydroxamic acids

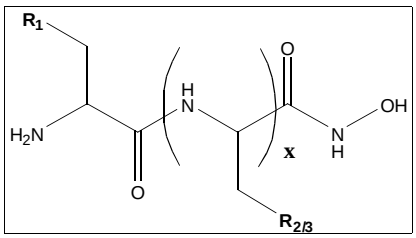
Peptidehydroxamic acids as potential metalloenzyme inhibitors

Edit Csapó[1], Etelka Farkas[1], Chiara A. Damante[2], Giuseppe Di Natale[2]

[1]*University of Debrecen, H-4010 Debrecen, Hungary*
[2]*Dipartimento di Scienze Chimiche, Università di Catania, V.le A. Doria 6, 95125 Catania, Italy*

In a large number of metalloenzymes, metal ions like Cu(II), Ni(II) or Zn(II), are quite often coordinated by imidazole residues. On the other hand, a hydroxamic group (its inhibitory effect, towards *e.g.* metalloproteinases (MMP), or histone-deacetylases, is well-known[1]) is also an effective metal binding function. Since this latter moiety is often intended for metal binding group (MBG) in newly synthesized inhibitor molecules (in many cases peptide derivatives), any effect, which modifies the coordination of MBG, highly affects also the metalloenzyme inhibitory behaviour of the molecule.

In the present work new di- and tripeptide derivatives (structures see below), all involving both of the above mentioned effective functions in a single molecule, have been synthesized and studied in solution by using pH-potentiometry, UV-Vis, CD and ESI-MS methods. The obtained results clearly show that the amino-N and peptide function(s) are play significant role in the metal binding, but there is an interesting, metal and pH-dependent competition between the imidazole-N and the hydroxamate-N donors. On the conference, the results for the Cu(II) and Ni(II) complexes are planned to present, demonstrating that Cu(II) ion "chooses" imidazole instead of hydroxamate-N in many cases, but the hydroxamte-N is unambiguously preferred by the Ni(II) ion.



	R_1	R_2	R_3
L-Ala-*L*-HisNHOH (x=1)	-H	-Im	-
L-Ala-*L*-Ala-*L*-HisNHOH (x=2)	-H	-H	-Im
L-His-*L*-AlaNHOH (x=1)	-Im	-H	-

(-Im: imidazole group)

Acknowledgement: Thanks for the financial support for OTKA T049612 National Hungarian Fund

References

[1] E. M. F. Muri, M. J. Nieto, R. D. Sindelar, J. S. Williamson, Current Med. Chem. **9** (2002) 1631-1653

Role of serum proteins in transformation of antidiabetic zinc(II)-complexes

Interaction between antidiabetic zinc(II)-complexes and serum components

Éva Anna Enyedy, László Horváth, Tamás Kiss

University of Szeged, Department of Inorganic and Analytical Chemistry, Szeged, Hungary

Certain simple zinc(II)-complexes show high antidiabetic effect, *e.g.* they can enhance the glucose uptake into the glucose-metabolising cells. However, the exact mechanism of their action has still not been completely revealed in the literature. The potential complexes can be considered as prodrugs and most probably the original forms are different from the actual ones. Besides their classical aqueous solution characterisation, the knowledge of their interaction with relevant bioligands such as the serum components is crucial for the understanding of the mechanism of the drug candidates. Interaction of these complexes with cysteine is the most considerable among the low molecular mass serum components, while out of the serum proteins, albumin has predominant role in the complete or partial displacement of the carrier ligand of the complexes (1). The possibility of formation of serum protein-ligand adducts was not taken into consideration before, but it can have important role in the metabolism of the antidiabetic zinc(II)-complexes. The binding strength and the most probable binding sites were characterised by ultrafiltration, ICP-AES, UV-Vis, CD and spectrofluorimetric measurements.

Complexation of zinc(II)-ion with the carrier ligands leads to the formation of neutral, *bis* complexes enhancing their lipophilic character and the scale of absorption from the digestive tract. Since the biological effects of the complexes are always closely correlated to their octanol-water distribution coefficients (2) which can have effect on the binding to serum proteins too, the hydro-lipophilicity of the zinc(II)-complexes and the carrier ligands were reinvestigated in the present work as well.

References

[1] É. A. Enyedy, L. Horváth, K. Gajda-Schrantz, G. Galbács, T. Kiss (2006): *J. Inorg. Biochem.* **100**: 1936-1945.
[2] H. Sakurai, A. Katoh, Y. Yoshikawa (2006): *Bull. Chem. Soc. Jpn*. **79:** 1645-1664.

Preparation and study of metal complexes mimicking the SOD enzyme

Biological importance of the metal complexes mimicking the SOD enzyme

Imre Labádi, Kata Markó, Mária Benkő

University of Szeged, Department of Inorganic and Analytical Chemistry

The SOD enzyme (superoxide dismutase) is one of the enzymes which active centre is known. In most of the SOD enzymes, in the active centre there are a copper(II)- and a zinc(II)ion bridged by an imidazolate anion, and surrounded by nitrogen atoms (from the imidazole part of the histidine) and one carboxylic oxygen atom(in side of the zinc(II)ion). Mimicking the structure of this active centre, an mixed ligand, imidazolate bridging, diethylenetriamine(in copper side) and tris(aminoethyl)amine (in zinc side) metal complexes were prepared, characterized by IR spectroscopy, EPR spectroscopy, mass spectrometry and their SOD activity was tested [1-5].

Following these studies, other nitrogen and oxygen atom-containing ligands (dipyridine, terpyridine, aminoalcohols) were used as a ligands on the copper (II) and zinc(II) sites, while the imidazolate ion bridge between the copper(II) and zinc(II)ions did not change.

References

[1] I. Szilágyi, I. Labádi, K. Hernádi, I Pálinkó,T. Kiss: J. Mol. Str. 744-747 (2005) 495
[2] I. Szilágyi, I. Labádi, K. Hernádi, I Pálinkó, I. Fekete, L. Korecz, A. Rockenbauer, T. Kiss: New J. of Chemistry 29(2005) 740
[3] I. Szilágyi, I. Labádi, K. Hernádi, T. Kiss, I Pálinkó: Stud. In Surface Science and Catalysis 158 (2005) 1011
[4] I. Szilágyi, Z. Kele, I. Labádi, K. Hernádi, I Pálinkó,T. Kiss: Rapid Comm. In Mass Spectrometry 19 (2005) 2878
[5] I. Szilágyi, L. Horváth, I. Labádi, K. Hernádi, I Pálinkó,T. Kiss: Central Eur. J, of Chemistry 4 (2006) 118

Comparative studies on the biospeciation of antidiabetic vanadium and zinc compounds

What are the actual forms of antidiabetic metal compounds in the blood serum?

Tamás Kiss, Éva Anna Enyedy, Tamás Jakusch

Biocoordination Chemistry Research Group of the Hungarian Academy of Sciences, Department of Inorganic and Analytical Chemistry, University of Szeged, H-6701 Szeged, Hungary

Intensive studies have been carried out during the last two decades on the antidiabetic (AnD) effects of several metal ions among others chromium, vanadium, tungsten and zinc. Vanadium compounds seem to be the most effective, however their non-essential character is a great disadvantage and their occurrence and potential accumulation in the organism may produce strong aversion to their practical application. For this reason, the essential Zn compounds may receive a more positive acceptance.

Due to the presence of numerous endogenous and exogenous metal ion binders the original AnD complex may undergo transformations in the biological fluids and tissues and thus the real biological/physiological activity may be bound to an entirely different chemical entity.

In order to say something about the actual solution state(s) of these metal ions during their transport in the blood stream we studied their interactions with the relevant low molecular mass (lmm) ligands, such as citrate, lactate, oxalate, phosphate, histidine and cysteine and high molecular mass (hmm) protein components, such as albumin, transferrin and α-macroglobulin of blood serum. In this work we give an overview of the work. Solution speciation and spectral (EPR, UV-Vis) studies revealed that citrate for vanadium, while histidine and cysteine for zinc(II) are the most important lmm binders, while transferrin for vanadium and α-macroglobulin and albumin for zinc(II) are the most important hmm binders of the serum. Ultrafiltration studies helped to separate and determine the two fractions bound metal content of the serum.

In vitro chromatographic separations made on human plasma confirmed our results obtained by modeling speciation calculations.

Acknowledgements. This work was carried out in the frame of a COST D21 collaboration and supported among others by the Hungarian Science Research Fund (OTKA T49417, NI61786 and PD 050011).