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Crystallization of Cytochromes from *Thiocapsa roseopersicina*

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Cytochromes belong to colored proteins that play an important role in live cells. They incorporate prosthetic group - molecule of heme - that facilitates as a member in process of electron transport. Due to this important function, it is essential to study structural features of cytochromes with modern X-ray crystallographic methods.

Cytochrome *c* (cyt *c*) is a low-mass protein (26 kDa) transporting electrons among cytochrome b-c_1 complex and complex of cytochromoxidase. Cyt *c* from the purple photosynthetic bacterium *Thiocapsa roseopersicina* was isolated and purified according to Bagyinka [1].

Cyt *c* was crystallized using standard methods [2] based on vapor diffusion. Crystallization trials were performed in hanging and sitting drops [3] at room temperature. The most suitable concentration of protein (10mg/ml) and the precipitation agent (50% ammonium sulfate) were found. Ranging pH value higher than 7.5 the phase separation of protein appeared. First crystal growth was observed at pH 6.0.

Preliminary crystallization conditions are now being to be optimized in order to prepare monocrystals of cyt *c* suitable for X-ray diffraction measurement.

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[1] Bagyinka C., *unpublished data*. [2] Bergfors T. M., *Protein Crystallization. Techniques*, *Strategies and Tips*, International University Line, La Jolla, USA, 1999. [3] Ducruix A., Giegé R., *Crystallization of Nucleic Acids and Proteins*, Oxford University Press, Oxford, 1999.

Keywords: cytochrome c, crystallization, electron transport

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New Class of Proteasome 20S Inhibitors: a Crystallographic and Molecular Modelling Study

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26S proteasome represents the multicatalytic proteinase of the ubiquitin/adenosine triphosphate-dependent proteolytic pathaway. This large enzymatic complex is found in the cytosol and nucleus of eukaryotic cells, and plays a central role in the selective degradation of intracellular proteins. The 20S proteasome is a kind of proteolytic chamber formed by four stacked rings, where each of the two inner rings is made up of seven different β subunits. Proteasomes remove abnormal proteins and play a role in cell-cycle progression and apoptosis, representing thus a potential target for the development of therapeutic agents for the treatment of pathologies such as cancer, inflammation, immune diseases.

Very recently the synthesis and biological characterization of a new series of vinyl ester tripeptides acting as proteasome inhibitors have been reported [1]. In this communication we present the crystallographic structures of two of them, together with a conformational study of the molecules in the solid state, *in vacuum* and in a polar environment which is in turn the basis for a docking study of such inhibitors to the crystallographic structure of the 20S proteasome[2] in order to define the inhibitor-enzyme interaction subsite pockets.

[1] Marastoni M., et al., *J. Med. Chem*., 2005, *in press*. [2] Groll M., Koguchi Y., Huber R., Kohno J., *J. Mol. Biol*., 2001, **311**, 543.

Keywords: enzyme inhibitors, molecular conformations, docking

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Glycine Zipper Motif in the Association of Helices in a Designed Peptide

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The crystal structure of an apolar peptide Ace-Gly[1]-Ala[2]-ΔPhe[3]-Leu[4]-Gly[5]-ΔPhe[6]-Leu[7]-Gly[8]-ΔPhe[9]-Ala[10]-Gly[11]-NH2 is determined at 0.9Å resolution. The peptide was designed to mimic the interhelical interactions involving GxxxG like motifs seen in transmembrane helices. The peptide crystallizes as two conformers, one a right-handed and the other a left-handed 3_{10}-helix, displaying ambidextrous screw sense. It is interesting to note that despite the presence of L amino acids (Ala, Leu) in the sequence and more importantly bulky residues Leucine (Leu[4], Leu[7]) in the middle of the helix, one of the conformers is a left-handed helix. This is presumably to optimize helix - helix interactions, suggesting that global interactions can decide local conformation.

A remarkable feature is the occurrence of zipper like arrangement of main-chain to main-chain C^{α}-H···O hydrogen bonds consistently at three residue interval at Gly-Gly helix interface. The crystal structures of two other closely related peptides, where Gly at positions 5 and 8 have been replaced by Ala in one case and Val in the other have also been determined. Zipper like interaction motif involving Leucines is common to all the three peptide structures. A novel, aromatic side chain to main-chain C-H···O hydrogen bonded motif is observed in the last two peptides. The repertoire of weak interaction based motifs seen here, could be exploited for the de novo design of helical assemblies mimicking transmembrane helices.

Keywords: designed peptide, glycine zipper, transmembrane helix

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Crystal Structures Puzzle of the DSDH Gramicidin Channel

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The naturally occurring antibiotic gramicidin forms transmembrane channels specific for monovalent cations. In a solution several polymorphic gramicidin forms have been observed. All uncomplexed gramicidin crystal structures are reported to be left-handed antiparallel double-stranded double-helix (DSDH) dimers with 5.6 residues per turn. The same form was also observed by NMR in organic solvents. In contrast there are conflicting results concerning the crystal structures of complexed gramicidin. Despite the same space group and cell dimensions that agree to within 0.1%, two entirely different three-dimensional structures have been reported. Both structures contain DSDH dimers but they differ in h.b. patterns and the overall hand of the helices. The **right-handed** form agrees with NMR data in organic solvents, while no NMR data supporting the **left-handed** structure exists. Furthermore, the crystallographic, stereochemical, and chemical anomalies of the latter form suggest that the structure determination could be erroneous. Unfortunately, the reluctance of the authors to release their intensity data makes it impossible to unequivocally set to rest the question.

Therefore, we have made a thorough analysis of the two complexed gramicidin structures in question, including energy calculations and refinement of the LH form basing on RH diffraction data. (Research Project 3 T09A 047 26 from KBN).

Keywords: gramicidin polymorphs, double-stranded gramicidin channel, helices handness

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Conformational Comparison of μ-Selective Endomorphin-2 with Its C-Terminal Free Acid

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In order to make clear the structural function of C-terminal amide group of endomorphin-2(EM2:YPFF-NH_2) the conformations of EM2 and its C-terminal free acid (EM2OH:YPFF-OH) were analyzed by ^{1}H-NMR spectroscopy and X-ray crystal analysis.

The NMR spectra in trifluoroethanol(TFE) and water solvents indicated that both peptides were in equilibrium between the *cis*- and *trans*-rotamer around Tyr-Pro peptide bond, respectively. However they take almost trans rotmer in dodecylphosphocholine(DodPCho), micells, except for the EM2OH in water solvent at pH5.2. With the use of the proton-proton distance derived from ROESY cross peaks, possible fifty 3D structures are generated by dynamical simulated annealing method and were classified in four groups of two open and two fold conformers according to the folding of backbone structure.

On the other hand, two independent conformational isomers per asymmetric unit and seven water molecules were existed in the crystal structure of EM2OH. Both conformers were crystallized as neutral zwitterionic forms and took a folded-form with *cis*-configuration in around Tyr-Pro peptide bond.

Based on the conformational features of EM2 and EM2OH in solution and solid state, we would like to discuss the possible function of C-terminal amide group.

Keywords: NMR, X-ray conformation analysis, molecular conformation

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New Monocyclic and Acyclic hNK-2 Antagonists Retaining the β-turn Feature

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The human tachykinin NK-2 receptor is a promising target for important pathologies at respiratory, gastrointestinal and genitourinary level, where this receptor is mainly localized. Several peptidic and non-peptidic antagonists to this receptor are known, and a few of them are undergoing clinical studies. The bicyclic peptide MEN10627 [1] is one of the most potent antagonists for the neurokinin NK-2 receptor. However its low bioavailability prevents it to be used as a drug. We have already shown how, by selecting a proper part of its structure, i.e. that featuring the β-turn, it is possible to obtain simpler peptides still retaining their activity. The monocyclic series which originated was designed on the basis of theoretical assumptions with the support of modeling [2]. In the present contribution we show how subsequently that rationale has been experimentally validated through X-ray structure determination of a novel monocyclic hNK-2 antagonist (MEN13365). Moreover the same structural features have been retained in MEN15596, which belongs to a new non cyclic series of hNK-2 antagonists developed to circumvent the low oral bioavailability. Antagonists from this last series are presently undergoing preclinical development.

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Keywords: molecular scaffold, β-turn, tachykinin

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Absolute Configuration of the κ-Agonist Salvinicin A

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Salvinorin A and B are potent κ selective opioid receptor agonists from *Salvia divinorum*. An infusion prepared from fresh or dried leaves is used by the Mazatec Indians to stop diarrhea, relieve headache and rheumatism, and is also used in traditional spiritual practices to produce "mystical" or hallucinogenic experiences.[1] Young adults and adolescents have begun to smoke the leaves and leaf extracts of the plants to induce powerful hallucinations.[2] The stereochemistry of Salvinorin has not previously been determined. In an effort to determine the sterochemistry of this opioid agonist a 3,4-dichlorobenzoyl derivative was prepared. Single crystal x-ray diffraction was able to unambiguously determine the absolute configuration of this dichloro derivative and by extension that of Salvinorin A.

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Keywords: κ-opioid receptor, structure, stereochemistry

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Crystal Structures of Cholesterol Derivatives

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We have undertaken a series of crystal structures of the esters, carbonates and ethers of cholesterol. These are cholesteryl formate, pentanoate, hexanoate, heptanoate, crotonate, isobutyrate, aniline, 2,4-dichlorobenzoate and hemisuccinate, cholesteryl phenyl acetate, methyl carbonate, ethyl carbonate, propyl carbonate, butyl carbonate, isobutyl carbonate, isopropyl carbonate, pentyl carbonate, hexyl carbonate, crotyl carbonate, cholesteryl ethyl ether, isopropyl ether and methyl ether.

Among these structures, (1) cholesteryl ethyl carbonate, propyl carbonate, crotyl carbonate, crotonate are isostructure each other, (2) cholesteryl pentyl carbonate, hexyl carbonate, hexanoate, heptanoate are also isostructural,

These structures are remarkable in forming layer structures in which the central region of the layers, composed largely of semi-rigid cholesteryl groups is closely packed and the packing of the flexible fatty acid or carbonate chains and the isoprenoid tail of the cholesterol form the interface region between layers. Some of the crystals show the liquid crystalline states. Typical packing modes will be discussed.

Keywords: cholesteryl ester, cholesteryl carbonate, cholesteryl ether

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Structure and Tautomerism of Mercapto-1,2,4-triazole Derivatives in the Solid State

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Molecular and crystal structures and tautomerism of new mercapto-1,2,4-triazole derivatives, which are structurally labile compounds capable to exist in different tautomeric forms, are discussed. X-Ray single crystal diffraction experiments show the existence of only 1*H*-triazole tautomer in crystal. As a result of our investigations it can be concluded, that for 3,5-substituted 1,2,4-triazoles usually crystallizes the tautomer, where hydrogen atom is bonded with the nitrogen (one of two neighbouring) situated near the electronodonor group, that is 3-R_A-5-R_D-1,2,4-(1*H*)-triazole. For 3-phenyl-5-mercap-to-1,2,4-triazole two thion-thiolic tautomers were found in one crystal: two molecules of four symmetrically independent ones are 3-phenyl-4,5-dihydro-(1*H*)-1,2,4-triazole-5-tion tautomers, and the rest are 3-phenyl-5-mercapto-(1*H*)-1,2,4-triazole. The asymmetric part of the unit cell of 3(5)-(2-hydroxyethyl)thio-1,2,4-triazolinium oxalate consists of two cation-anion pairs. The two cations are the endocyclic tautomers: one of them is 3-(2-hydroxyethyl)thio-(1*H*),(4*H*)-1,2,4-triazolinium cation and the other is 5-(2-hydroxyethyl)thio-(1*H*),(4*H*)-1,2,4-triazolinium cation. The