P.05.01.1

Acta Cryst. (2005). A**61**, C273

Crystallization of Cytochromes from *Thiocapsa roseopersicina*

Ivana Tomčová[a], Ivana Kutá Smatanová[a,b], [a]*Institute of Physical Biology, University of South Bohemia, Nové Hrady, Czech Republic.* [b]*Institute of Landscape Ecology, Academy of Sciences of the Czech Republic, Nové Hrady, Czech Republic.* E-mail: tomiva@centrum.sk

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.

This research was supported by grants MSM6007665808 and AVOZ60870520.

[1] Bagyinka C., *unpublished data*.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

P.05.01.2

Acta Cryst. (2005). A**61**, C273

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

Valeria Ferretti[a], Loretta Pretto[a], Mauro Marastoni[b], Anna Baldisserotto[b], Riccardo Gavioli[c], [a]*Chemistry Department andCcentre for Structural diffractometry.* [b]*Pharmaceutical Sciences Department.* [c]*Biochemistry and Molecular Biology Department, University of Ferrara, Italy*. E-mail: frt@unife.it

26S proteasome represents the multicatalytic proteinase of the ubiquitin/adenosine triphosphate-dependent proteolytic pathway. 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

P.05.01.3

Acta Cryst. (2005). A**61**, C273

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

Suryanarayanarao Ramakumar[a], Rudresh[a], U.A. Ramagopal[b], G. Madhvi[c], V.S. Chauhan[c], [a]*Department of Physics, Indian Institute of Science, Bangalore.* [b]*Department of Biochemistry, Albert Einstein College of Medicine, New York, USA.* [c]*ICGEB, New Delhi.* E-mail: ramak@physics.iisc.ernet.in

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

P5

P.05.01.4

Acta Cryst. (2005). A**61**, C273

Crystal Structures Puzzle of the DSDH Gramicidin Channel

Marek L. Glowka[a], A. Olczak[a], J. Bojarska[a], M. Szczesio[a], W.L. Duax[b], [a]*Institute of General and Ecological Chemistry, University of Technology, Lodz, Poland.* [b]*Hauptman-Woodward Medical Institute, Buffalo, N.Y., USA*. E-mail: marekglo@p.lodz.pl

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

P.05.01.5

Acta Cryst. (2005). A**61**, C273-C274

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

Yasuko In, Katsuhiko Minoura, Toshimasa Ishida, *Department of*