PS04.01.56 CRYSTAL STRUCTURE OF FIV dUTP PYROPHOSPHATASE. G. Sridhar Prasad, E. A. Stura, D. E. McRee, C. Hasselkus-Light, G. S. Laco, J. H. Elder, C. D. Stout, Department of Molecular Biology, The Scripps Research Institute, La Jolla, 92037

The crystal structure of the dUTP pyrophosphatase (dUTPase) from feline immunodeficiency virus (FIV) has a similar fold to the *E. col i* enzyme (1) in which the C-terminal strand of an antiparallel ß-sandwich participates in the ß-sheet of an adjacent subunit to form an interdigitated, biologically functional trimer. dUTPase hydrolyzes dUTP to dUMP and pyrophosphate. By maintaining low cellular concentrations of dUTP the enzyme prevents incorporation of uracil into DNA, which if unchecked leads to numerous strand breaks due to extensive excision repair by uracil-DNA-glycosylase. As an essential enzyme of nucleotide metabolism, dUTPase is a potential target for drug design. The objective of this study is to understand the structural basis for the substrate specificity, Mg^{2+} ion dependence and chemical mechanism of dUTPase. The active site of the *E. Coli* enzyme has not yet been defined (1).

Three crystal forms of FIV dUTPase have been obtained using recombinant protein expressed in both *E. Coli* and baculovirus (monomer MW 14,350) (2). The structure has been solved by MIR methods using a $P6_3$ crystal form containing two monomers per asymmetric (one trimer on the 6_3 screw axis and one trimer on the 3fold axis). For two Hg derivatives the figure of merit is 0.67 to 2.7Å resolution (phasing power 2.90 and 2.97). Complete native data to 1.8Å resolution has been collected at 90°K. Refinement of the structure is in progress. The $P6_3$ crystal form requires Mg^{2+} and also binds Sm^{3+}, Gd^{3+} and Yb^{3+} at a site on the 3-fold axis. However, in soaking experiments this form does not accommodate substrates. A second crystal form, which is orthorhombic and contains one trimer per asymmetric unit is grown in the presence of dUDP, an inhibitor of the enzyme. A third crystal form, in space group $P6_1$, contains 4 trimers per asymmetric unit. Complete data have been collected to 3.0Å resolution for this crystal form. Progress in the study of dUTPase Mg^{2+}, substrate and inhibitor complexes in these crystal forms will be reported.

1. E. S. Cedergren-Zeppezauer, *et al.*, Nature **355**, 740 (1992).
2. P. C. Wagaman, *et al.*, Virology **196**, 451 (1993); and J. H. Elder, *et al.*, in preparation.

PS04.01.57 CRYSTAL STRUCTURE OF RNase-FORM I (COMPLEXED WITH NICKEL): Rama Balakrishnan[a], N. Ramasubbu[b*], K. I. Varughese[c], R. Parthasarathy[3], [a]Center for Crystallographic Research and Biophysics Department, Roswell Park Cancer Institute, Buffalo, NY 14263, [b]Research Center of Oral Biology, SUNY at Buffalo, Buffalo, NY 14214, [c]Department of Biology, University of California at San Diego, CA 92093.

The orthorhombic crystal form of Ribonuclease (Form I; King 1964) has been crystallized in the presence of six-fold excess of Nickel. The crystals belong to the space group $P2_12_12_1$ with unit cell parameters a = 44.0, b = 75.5, c = 37.51Å, $\alpha = 90°$, $\beta = 90°$ and $\gamma = 90°$. In 1956, Harker and coworkers (King *et al* 1956, 1962) showed that RNase could be crystallized in several forms indicating the versatility of packing of RNase molecules. Although a structure solution for these forms including form I was attempted earlier, only the monoclinic form (Form II) was successfully solved. We have initiated the structure solution of Form I because of our interest in Ni binding sites and the rarity of Ni in proteins. We have collected three-dimensional x-ray diffraction data of this crystal using the multiwire detector up to 2.6Å resolution (Cu Ka). A total of 12,715 reflections were collected of which 12,103 are > 2σ (Rsymm = 4.7%). The structure was solved by Molecular Replacement method using a phosphate-free RNase structure as starting model and using AmoRe and was refined using X-PLOR and PROLSQ to an R-factor of 20%. Further fitting of the electron density and refinement is in progress. Details of structure solution, refinement, and the effect of this metal on the structure will be investigated.

M. V. King, B. S. Magdoff, M. B. Adelman and D. Harker (1956), *Acta Cryst.*, **9**, 460 - 465.
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PS04.01.58 CRYSTALLOGRAPHIC STUDIES OF ALPHA-TOXIN (PHOSPHOLIPASE C) FROM *CLOSTRIDIUM PERFRINGENS*. A. K. Basak[1], J. T. Eaton[1], D. S. Moss[1], R. W. Titball[2], [1]Department Of Crystallography, Birkbeck College, UK, [2]Chemical & Bological Defence Establishment, Porton Down, UK.

A wide variety of gram-positive and gram-negative bacteria produce phospholipase C's and these enzymes have markedly different biophysical properties. The enzymes hydrolyse different phospholipids with varying efficiencies and only some of them have haemolytic and lethal properties. The enzyme alpha-toxin of *Clostridium perfringens* is the most toxic phospholipase C characterized to date, but in spite of that it is still not clear why this enzyme (and some other phospholipases C) is toxic whereas others such as phosphatidylcholine preferring phospholipase C (PC-PLC) from *Bacillus cereus,* are non-toxic. The N-terminal two-thirds of the protein (1-249 residues) show amino acid sequence homology with the entire *B. cereus* PC-PLC. It is also known that the C-terminal domain of the protein confers the haemolytic and lethal properties of the phospholipase C.

In order to investigate the molecular basis of the toxicity of the alpha-toxin we are currently determining the crystal structure of the enzyme. The protein is composed of a single polypeptide chain of 370 amino acid residues and has a molecular weight of 42.5kDa The protein has been expressed in *E. Coli* and purified in two different strains. Three different crystal forms suitable for X-ray diffraction analysis have been grown from these strains.

Initial attempts to solve the structure by molecular replacement methods using the known *B. cereus* phospholipase C structure as a model were not successful. Subsequently phases have been determined (from one of these three crystal forms) using three different heavy atom derivatives and an initial solvent-flattened electron density map at 3.5Å resolution shows the secondary structural elements of the molecule. Interpretation of the electron density map, phase extension are currently in progress to provide an accurate structure.

PS04.01.59 LEFT-HANDED β-HELIX PROTEIN UDP-GLUCOSE PYROPHOSPHORYLASE. Masami Kusunoki, Yasuyuki Kitagawa, Hisashi Naitou, Yukiteru Katsube, Yukiyo Sakamoto*, Katsuyuki Tanizawa* and Toshio Fukui*, Institute for Protein Research, Osaka University, Suita Osaka 565 JAPAN *The Institute of Scientific and Industrial Research, Osaka University, Ibaraki Osaka 567 JAPAN

UDP-glucose pyrophosphorylase catalyzes the reversible uridylyl transfer from UDP-glucose to $MgPP_i$ forming glucose-l-phosphate and MgUTP. We isolated and purified cDNA encoding UDP-glucose pyrophosphorylase from potato tuber. It has 477 amino acid residues and no apparent sequence homology to other proteins. The enzyme was crystallized by the hanging-drop vapor diffusion method with the precipitant ammonium sulfate. The space group is $P2_12_12_1$ with cell dimensions a=108.2, b=124.7, c=87.1 Å, V_M=2.8 Å^3/dalton. The crystal structure was solved by multiple-isomorphous replacement with four heavy atom derivatives, $K_2Pt(CN)_4$' $Hg(CH_3COO)_2$, $UO_2(NO_3)_2$ and $Sm_2(SO_4)_3$. The