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.
M. V. King, J. Bello, E. H. Pignataro and D. Harker (1962), *Acta Cryst*, **15**, 144 147.
M. V. King (1964) *Biochemica et Biophysica Acta*, **79**, 388-392.
*Supported by Grant DE08240 from USPHS

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

three-dimensional intensity data were collected by imaging plate diffractometer of RIGAKU RAXIS IIc. The average figure of merit at 3.0 Å resolution is 0.43, using "mlphare" in CCP4 program package. The phase improvement was carried out by "dm" in CCP4, resulting in a 2.2 Å resolution density map. The density modification includes histogram-mapping, constraint of Sayer formula, non-crystallographic averaging and solvent flattening, with assumption of 41% solvent content. The final free-R-value was 0.27 at 2.2 Å resolution. Two identical molecules are in the crystallographic asymmetric unit. The two molecules are related by 143 degree rotation around an axis almost parallel to the crystallographic z axis with some translation. Each molecule consists of two domains. The N-terminal domain has five helices and seven β-strands (α/β structure) with two additional long helices in N-terminus. The C-terminal domain is mainly composed of left-handed β-helix similar to the structure of UDP-N-acetylglucosamine-acyltransferase[1]). The protein structure is under refinement with program X-PLOR.

1) C. R. H. Raetz and S. L. Roderick, Science, 270, 997-1000(1995)

PS04.01.60 CALF SPLEEN PURINE NUCLEOSIDE PHOSPHORYLASE IN COMPLEX WITH AN N(7)-ACYCLOGUANOSINE INHIBITOR. Gertraud Koellner, Marija Luic, Agnieszka Bzowska, David Shugar & Wolfram Saenger, Institut fur Kristallographie, Freie Universitat Berlin, Takustr. 6, D-14159 Berlin, Germany

The complex of calf spleen purine nucleoside phosphorylase with an N(7)- acycloguanosine inhibitor was crystallized in the cubic space group $P2_13$ with an unit cell dimension a=94.02Å and one monomer in the asymmetric unit. The biologically active trimer is formed by the crystallographic three-fold axis. The structure was solved by molecular replacement using the model of the human erythrocyte enzyme [Ealick et al., Proc. Nat. Acad. Sci. USA 88, 11540 11544 (1990)]. The complexed calf spleen PNP crystallizes at pH 8.2-8.5 from PEG, which is almost optimal for enzyme activity [Kulikowska et al., Biochim. Biophys. Acta 874, 355- 363 (1986)]. N(7)-acycloguanosine binds in an inverted ('upside-down') orientation with respect to guanosine in the human PNP. The acyclic chain is engaged in several hydrogen bonds. Since the crystals were grown at pH 8.2-8.5. the secondary nitrogen of the acyclic chain (pKa~9.5) should be protonated. It follows that it is the acyclic chain which is predominantly responsible for binding of the inhibitor.

Agnieszka Bzowska, Marija Luic, Werner Schröder, David Shugar, Wolfram Saenger, Gertraud Koellner. (1995) FEBS Letters, 367, 214-218.

PS04.01.61 CRYSTAL STRUCTURE OF ISOZYME 4-4 & MOLECULAR MODELING OF ISOZYME 3-4 OF CLASS MU GLUTATHIONE S-TRANSFERASES FROM RAT LIVER. Gaoyi Xiao,[1] Xinhua Ji,[1,2] Richard Armstrong,[3] and Gary L. Gilliland[1]. [1]Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and the National Institute of Standards and Technology, 9600 Gudelsky Drive, Rockville, MD 20850. [2]NCIFCRDC, P.O. Box B, Frederick, MD 21702. [3]Department of Biochemistry and the Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232

Glutathione S-transferases (GST) are a family of phase-II detoxification enzymes that may also play a role as transport proteins. To date, five different gene classes, alpha, mu, pi, theta and sigma, of this dimeric enzyme have been identified. Several subunit types have been found for the different gene classes in many different organisms. Heterodimers composed of different subunits of the same gene class are commonly isolated. Interclass heterodimers, however, have not been observed [1]. We report here the crystal structure of the 50 kDa 4-4 isozyme of the rat liver mu GST. The three-dimensional structure was determined at 3.5 Å resolution by the molecular replacement method using the 3-3 isozyme of the rat liver mu GST [2]. This represents the first example of the structure of a second GST subunit type from the same gene class. Details of the 4-4 mu GST structure, results of an analysis of the interface interactions of the two homodimeric structures, and results of molecular modeling of the heterodimeric 3-4 mu GST isozyme to learn what features at the dimer interface allow heterodimer formation will be presented.

[1] Armstrong, R., (1994) *Advances in Enzymology & Related Areas in Molecular Biology* 69, 1-44.
[2] Ji, X., Zhang, P., Armstrong, R. N. and Gilliland, G. L. (1994) *Biochemistry* 31, 10169-10184.

PS04.01.62 HIGH RESOLUTION CRYSTAL STRUCTURE OF ORNITHINE AMINOTRANSFERASE COMPLEXED WITH THE NEUROTOXIN GABACULINE. Sapan A. Shah, Betty W. Shen and A.T. Brunger. The Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA.

Ornithine aminotransferase (OAT) is a 45kD pyridoxal phosphate-dependent enzyme that catalyzes the transfer of the delta amino group of ornithine to an alpha ketoglutarate substrate. OAT and gamma aminobutyric acid transaminase (GABA-AT) belong to the same subgroup of transaminases, and in addition to sharing high sequence homology, are inactivated by common inhibitors. One such inhibitor is the neurotoxin gabaculine (5-amino- 1,3-cyclohexadienylcarboxylic acid), a cyclic analogue of the inhibitory neurotransmitter GABA. We present here a 2.3 angstrom structure of the OAT/gabaculine complex, solved using phases from the native structure (Shen et al, manuscript in preparation). The complex reveals the structural basis for the "suicide" binding of gabaculine to the active site. Gabaculine is positioned in the active site through a hydrogen bond between its carboxyl group and Tyr55. Following binding to the PLP cofactor and aromatization of the cyclohexadienyl ring, the inhibitor is sandwiched in a favorable stacked arrangement between two aromatic residues, Tyr85 and Phe177.

PS04.01.63 THE CRYSTAL STRUCTURE OF THE HGXPRTASE FROM THE PROTOZOAN PARASITE T. FOETUS. John R. Somoza, Marian Chin, Pamela J. Focia, Ching C. Wang and Robert J. Fletterick, Dept. of Biochemistry & Biophysics, University of California at San Franscisco, CA 94143-0448

The crystal structure of the hypoxanthine-guanine-xanthine phosphoribosyltransferase from Tritrichomonas foetus has been determined and refined against data to 1.9 angstrom resolution. T. foetus HGXPRTase crystallizes as an asymmetric dimer, with GMP bound to only one of the two molecules that form the asymmetric unit. Each molecule of HGXPRTase is formed by two lobes joined by a short "hinge" region, and the GMP binds in a cavity between the two lobes. A comparison of the two molecules in the asymmetric unit shows that the hinge region is flexible, and that ligand binding affects the relative positions of the two lobes. The binding of GMP brings the two lobes closer together, rotating one lobe by about 5 degrees relative to the other.

T. foetus appears to depend on HGXPRTase for its supply of GMP, making this enzyme a target for anti-parasite drug design. A comparison of the structures of T. foetus HGXPRTase and human HGPRTase reveals that, while these enzymes retain a similar polypeptide fold, there are substantial differences between the active sites of these two homologs. These differences suggest that it will be possible to find compounds that selectively inhibit the parasite enzyme.