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

The crystal structure of the dUTP pyrophosphatase (dUTPase) from feline immunodeficiency virus (FIV) has a similar fold to the E. coli enzyme (1) in which the C-terminal strand of an antiparallel b-sandwich participates in the b-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. Mg2+ 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 P63 crystal form containing two monomers per asymmetric unit (one trimer on the b4 screw axis and one trimer on the b3 axis). For two Hg derivatives the figure of merit is 0.67 to 2.7 A resolution (phasing power 2.99 and 2.97). Complete native data to 1.8 A resolution has been collected at 90K. Refinement of the structure is in progress. The P63 crystal form requires Mg2+ and also binds Sm3+, Gd3+ and Yb3+ at a site on the b3 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 UDP, an inhibitor of the enzyme. A third crystal form, in space group P61, contains 4 trimers per asymmetric unit. Complete data have been collected to 3.0 A resolution for this crystal form. Progress in the study of dUTPase Mg2+ substrate and inhibitor complexes in these crystal forms will be reported.


PS04.01.57 CRYSTAL STRUCTURE OF RNase-FORM I (COMPLEXED WITH NICKEL). Rama Balakrishnann, N. Ramasubbu*, K. I. Varughese, R. Parthasarathy,*, Center for Crystallographic Research and Biophysics Department, Roswell Park Cancer Institute, Buffalo, NY 14263, †Research Center of Oral Biology, SUNY at Buffalo, Buffalo, NY 14214, ‡Department of Biology, University of California at San Diego, CA 92039.

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 P212121 with unit cell parameters a = 44.0, b = 75.5, c = 37.51 Å, α = 90°, β = 90° and γ = 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 multicrystal detector up to 2.6 A resolution (Cu Ka). A total of 12,715 reflections were collected of which 12,103 are greater than 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.


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PS04.01.58 CRYSTALLOGRAPHIC STUDIES OF ALPHA-TOXIN (PHOSPHOLIPASE C) FROM CLOSTRIDIUM PERFRINGENS. A. K. Basak†, J. T. Eaton‡, D. S. Moss§, R. W. Titball,† Department Of Crystallography, Birkbeck College, UK, ‡Chemical & Biological 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 phosphatidylethanol preferring phospholipase C (PCPLC) 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 b-HELIX PROTEIN UDP-GLUCOSE PYRROPHOSPHORYLASE. Masami Kusunoki, Yasuyuki Kitagawa, Hisashi Naitou, Yukiteru Katsube, Yukio Sakamoto*, Katsuyuki Tanizawa* and Toshihiko 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 MglP, forming glucose-1-phosphate and Mg2UTP. 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 P212121 with cell dimensions a = 108.2, b = 124.7, c = 87.1 Å, Vcell = 28.8 Å3. The crystal structure was solved by multiple-isomorphous replacement with four heavy atom derivatives, K2Pt(CN)4, HgCl2,(CH3COO)2, UO2(NO3)2 and SnCl2(SO4)2. 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 “mplughe” in CCP4 pro-
gram 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 for-
mutual 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 crys-
tallographic asymmetric unit. The two molecules are related by
143 degree rotation around an axis almost parallel to the crystallo-
graphic z axis with some translation. Each molecule consists of
two domains. The N-terminal domain has five helices and seven
B-strands (αβ structure) with two additional long helices in N-
terminus. The C-terminal domain is mainly composed of left-hand-
ed β-helix similar to the structure of UDP-N-acetylglucosamine-
acyltransferase). The protein structure is under refinement with
program X-PLOR.


PS04.01.60 Calf spleen purine nucleoside phosphorylase in complex with an N(7)-acycloguanosine inhibitor. Gertraud Koellner, Marija Luic, Agnieszka Bzowska, David Stugur and Wolfram Sænger, Institut für Kristallographie, Freie Universität Berlin, Takustr. 6, D-14195 Berlin, Germany.

The complex of calf spleen purine nucleoside phosphorylase with an N(7)-acycloguanosine inhibitor was crystallized in the cubic space group P213 with an unit cell dimension a=94.02Å and one monomer in the asymmetric unit. The biologically active tri-
mer is formed by the crystallographic three-fold axis. The struc-
ture was solved by molecular replacement using the model of
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 (up-side-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 nitro-
gen of the acyclic chain (pKa=9.5) should be protonated. It fol-
los that it is the acyclic chain which is predominantly responsi-
ble for binding of the inhibitor.


PS04.01.61 Crystal structure of isozyme 4-4 & molecular modeling of isozyme 3-4 of class
mu glutathione S-transferases from rat liver.
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Glutathione S-transferases (GST) are a family of phase-II
detoxification enzymes that may also play a role as transport pro-
teins. To date, five different gene classes, alpha, mu, pi, theta and
sigma, of this dimeric enzyme have been identified. Several sub-
unit 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 ex-
ample 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 anal-
ysis of the interface interactions of the two homodimeric struc-
tures, and results of molecular modeling of the heterodimeric 3-4
mu GST isozyome will be to learn what features at the dimer interface
allow heterodimer formation will be presented.

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chemistry 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 phos-
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 inhibi-
tory 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 ac-
tive site through a hydrogen bond between its carbonyl group and
Tyrr55. Following binding to the PLP cofactor and aromatization
of the cyclohexadienyl ring, the inhibitor is sandwiched in a fa-
vorable stacked arrangement between two aromatic residues, Tyrr85
and Phe177.

PS04.01.63 The crystal structure of the HGXPRTase from the protozoan parasite T.
foetus. John R. Somoea, Marian Chin, Pamela J. Focia, Ching
C. Wang and Robert J. Fleiterick, Dept. of Biochemistry & Bio-
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0448

The crystal structure of the hypoxanthine-guanine-xanthine
phosphoribosyltransferase from Tritrichomonas foetus has been de-
termined 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 asymmet-
ic unit shows that the hinge region is flexible, and that ligand bind-
ing 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
HGXPRTase reveals that, while these enzymes retain a similar polypep-
tide fold, there are substantial differences between the active sites of
these two homologs. These differences suggest that it will be possi-
ble to find compounds that selectively inhibit the parasite enzyme.