03-Crystallography of Biological Macromolecules

Ornithine transcarbamoylase (OTCase) is a critical enzyme in the urea cycle. OTCase deficiency, inherited as an X-linked trait, is the most prevalent genetic defect of ureagenesis, causing severe neonatal hyperammonemia. Point mutations of the OTCase gene account for about 80-85% of the patients.

We have purified both recombinant E. coli and human liver OTCase following the procedure described previously. Single crystals of E. coli OTCase suitable for X-ray analysis (0.5-0.8 mm long) have been grown. The crystallization of the human liver OTCase is in progress.

The space group of E. coli OTCase has been determined as either $P3_1$ or $P3_2$. There is one homotrimer of 36.8 kDa subunits in the asymmetric unit. A complete native data set to 2.8 Å has been collected. The flash freezing technique using liquid nitrogen was employed during the data collection to prevent radiation damage. The unit cell dimensions are a=b=103.43 Å and c=86.45 Å. Data have also been collected on several potential derivatives to allow a multiple isomorphous replacement solution of the structure.

The result of the self-rotation function calculation indicates that there is three-fold non-crystallographic symmetry in the crystals of E. coli OTCase. At a later stage, we can use this information to average electron density and therefore improve map quality.

PS–03.05.27 RICIN A REFINED AT 1.7A

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Ricin is an exceptionally toxic heterodimeric protein from the seeds of the castor plant, *Ricinus communis*. The B-chain is a 262-residue lectin which binds eukaryotic cell surfaces. The A-chain is a 267-residue glycosidase which enters the cytoplasm by an unknown mechanism and attacks ribosomes: it removes a specific adenine base from rRNA, thereby inhibiting protein synthesis and killing the cell. The ricin A-chain is being developed as an anticancer immunotoxin.

We have grown ricin A crystals of a new tetragonal crystal form with favourable diffraction characteristics ($P4_12_12$, $a=b=68.8$ A, $c=141.2$ A). We solved the structure by molecular replacement methods using a 2.5 A model of the ricin dimer (Katzin *et al, PROTEINS* 10, 251, 1991) available in the Protein Data Bank. Data extending to 1.7A resolution were collected at the Daresbury synchrotron on a FAST area detector. We are refining the model using simmulated annealing and least-squares methods, and we will present the refined high resolution structure.

PS–03.05.28 X-RAY STRUCTURE ANALYSIS OF ABRIN-A - A RIBOSOME-INACTIVATING PROTEIN FROM SEEDS OF *ABRUS PRECATORIUS*.

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Abrin-a from seeds of *Abrus Precatocius* is a ribosome inactivating protein which catalyze endohydrolysis of the N-glycosidic bond at one adenosine on the 28S rRNA (EC 3.2.2.22) consisting of A and B chains of 250 and 247 amino acid residues, respectively. The three-dimensional structure of abrin-a has been determined by X-ray crystallography to a resolution 2.8Å. Crystals of this abrin-a grow in monoclinic space group $P2_1$ at room temperature with cell dimensions a=85.1Å, b=73.1Å, c=48.2Å, and β=96.6°. The intensity data were collected on San Diego area detector system. Initial phases were determined by the method of molecular replacement using a starting model built from QUANTA protein package with ricin structure as a template. The molecule containing 4842 atoms in the asymmetric unit has been refined by using X-PLOR to a crystallographic $R = 0.218$ for 21128 data with $I \geq 1.5\sigma(I)$. The analysis of the detailed structure is in progress.

PS–03.05.29 FLUORIDE INHIBITION OF ENOLASE: CRYSTALLOGRAPHIC STUDIES AND ELECTROSTATIC POTENTIAL CALCULATIONS. By L. Lebioda*, K. Lewinski, E. Zhang, Department of Chemistry and Biochemistry, University of South Carolina and J. M. Brewer, Department of Biochemistry, University of Georgia, U.S.A.

Enolase in the presence of its physiological cofactor Mg^{2+} is inhibited by fluoride and phosphate ions in a strongly cooperative manner (Nowak, T. & Maurer, P. *Biochemistry* 20, 6901, **1981**). The structure of the quaternary complex yeast enolase - Mg^{2+}-F^--Pi has been determined by x-ray diffraction and refined to an R=16.9% for those data with $F/\sigma(F) \geq 3$ to 2.6Å resolution with a good geometry of the model. The movable loops of Pro35-Ala45, Val153-Phe169 and Asp255-Asn266 are in the closed conformation found previously in the precatalytic substrate-enzyme complex. Calculations of molecular electrostatic potential show that this conformation stabilizes binding of negatively charged ligands at the Mg^{2+} ion more strongly than the open conformation observed in the native enolase. This closed conformation is complementary to the transition state, which also has a negatively charged ion, hydroxide, at Mg^{2+}. The synergism of inhibition by F^- and Pi most probably is due to the requirement of Pi for the closed conformation. It is possible that other Mg^{2+} dependent enzymes that have OH^- ions bound to the metal ion in the transition state also will be inhibited by fluoride ions.

PS–03.05.30 Crystallographic studies on substrate complexes of ω-amino acid:pyruvate aminotransferase by Ikemizu, S.[1,*], Rehse, P.[2], Watanabe, N.[2], Sakabe, K.[3], Sakabe, N.[1,2] and Yonaha, K.[4]

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ω-Amino acid:pyruvate aminotransferase(ω-APT) from *Pseudomonas* sp. F126 is a Pyridoxal 5'-phosphate(PLP) dependent enzyme. This enzyme catalyzes the reversible transamination of the ω-amino group from β-alanine to pyruvate and produces malonic semialdehyde and L-alanine. The structure of this enzyme was solved at 2.0Å resolution using MIRA method and was refined with 1.8Å resolution data(N. Watanabe et al, Acta Cryst. A suppl., 1990, C19). In order to determine the mechanism, crystals of the enzyme complex with various substrates were prepared by cocrystallization and solved structures were compared.

For each data set, intensity data greater than 1.8Å resolution was collected using synchrotron radiation at the BL-6A2 station, PF, with a Weissenberg camera and Fuji Imaging Plate(N. Sakabe, Nucl. Instrum. Method, 1991, **A303**, 448-463). Data were processed using the "WEIS" program system(Higashi, T., *J. Apple. Cryst.*, 1989, **22**, 9-18). With the L-alanine/ω-APT cocrystals, data to 1.4Å resolution was collected yielding 76,761 unique reflections with a R-merge(I) of 5.64%. The structure was extensively refined using PROLSQ with 8.0-1.6Å data to a R-value of 15.5%. Difference fourier maps were calculated and the substrate model was built in. This result shows that L-alanine and PLP form a Shiff base, indicating a radically different reaction mechanism of ω-APT from aspartate aminotransferase. This interpretation is supported by other amino donor substrate complexes collected and refined to 1.8Å resolution.

A detailed comparison among the liganded and unliganded ω-APT structures has been carried out. There are no large conformational change in the structure of the protein but there are major changes of solvent structure around the ligand binding region.

The substrate side chains are classified into three groups; linear, branch and aromatic. The regularities of side chain stacking will be discussed with respect to the hydrogen bond and hydrophobic interactions.

PS-03.05.31 CRYSTAL STRUCTURE OF D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM PALINURUS VERSICOLOR AT 1.8Å RESOLUTION BY Shi-ying Song*, Jun Li, Zheng-jiong Lin and Chen-lu Tsou National Laboratory of Biomacromolecules, Institute of Biophysics, Beijing, China

D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an important enzyme in glycolysis and shows cooperativity in NAD^+ binding and half-of-the-sites properties toward reaction with some thiol modification reagents.
The enzyme was extracted from the tail muscle of South China sea Lobster P.versicolor and crystallizes in a form different from that for the Atlantic lobster H.americanus enzyme. The space group is C2 with cell dimensions a=128.11Å, b=99.61Å, c=80.69Å and ß=114.4° with half of the molecule per asym. unit[Song, et al., (1983), J.Mol.Biol. 171, 225-228]. The structure was determined by the molecular replacement method using the atomic coordinates of the known structure of H.americanus solved by Rossmann's group and 2.7Å 4-circle diffractomator data.
The high resolution data was collected by synchrotron radiation-Fuji imaging plate-Sakabe's Weissenberg camera system at BL6A2 of photon factory in KEK. The whole data set contains 172806 reflections (55850 unique reflections) with R-merge of 5.77%. The structure refinement was carried out using the programs XPLOR and PROLSQ and model building techniques on PS 390 based on the 2.7Å model. The current model containing 2 NAD^+ molecules, 2 sulphate ions and 153 ordered water molecules gives a crystallographic R-factor of 0.216 for 50031 reflections with F>2.5 r within 5.0-1.8 Å resolution and a stereochemistry with r.m.s. deviations from ideal geometry of 0.018Å for bond lengths and 3.42° for bond angles.
The folding of subunit resembles closely the known structure of H. americanus GAPDH and B. stearothermophilus GAPDH. The structure similarity between B.stearothermophilus GAPDH and P.versicolor GAPDH in catalytic domain is higher than that in NAD^+-binding domain. The conformation of the adenine about the glycosidic bond is anti for the ribose ring not only in the red subunit but also in the green subunit. The r.m.s. differences in atomic positions between the green and red subunits are 0.39Å for the C^a atoms and 1.27Å if other atoms are included, showing the existence of minor side chain asymmetry. Detailed analysis of the structure after further refinement will be presented in the Congress.
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PS-03.05.32 THE CRYSTAL STRUCTURE OF HMG-CoA REDUCTASE FROM PSEUDOMONAS MEVALONII. By C. Martin Lawrence[1], Victor W. Rodwell[2] and Cynthia V. Stauffacher[1]*, [1]Department of Biological Sciences, [2]Department of Biochemistry, Purdue University, West Lafayette, IN 47907

Pseudomonas mevalonii HMG-CoA reductase is a four electron oxidoreductase that catalyzes the interconversion of HMG-CoA and mevalonate, the first committed step in polyisoprenoid biosynthesis. In mammals this reaction is the rate-limiting step in the synthesis of cholesterol and the enzyme is the target of anti-cholesterol drugs. We have crystallized HMG-CoA reductase in the cubic space group $I4_132$, a=229.4 Å, with two monomers (45 kD/monomer) per asymmetric unit. Native and derivative data sets have been collected to 2.8 Å resolution on a Xuong-Hamlin area detector. Gold and mercury derivatives were used to produce a 3.0 Å MIR map in which clear secondary structure was evident. The phases were then