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 $\beta=96.6^0$. 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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