

## 03-Crystallography of Biological Macromolecules

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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 1.7 Å

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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 ( $P4_12_12$ ,  $a=b=68.8$  Å,  $c=141.2$  Å). We solved the structure by molecular replacement methods using a 2.5 Å model of the ricin dimer (Katzin *et al*, *PROTEINS* 10, 251, 1991) available in the Protein Data Bank. Data extending to 1.7 Å resolution were collected at the Daresbury synchrotron on a FAST area detector. We are refining the model using simulated 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 Precatorius* 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 2.8 Å. Crystals of this abrin-a grow in monoclinic space group  $P 2_1$  at room temperature with cell dimensions  $a=85.1$  Å,  $b=73.1$  Å,  $c=48.2$  Å, and  $\beta=96.6^\circ$ . 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<sup>-</sup>-P<sub>i</sub> has been determined by x-ray diffraction and refined to an  $R=16.9\%$  for those data with  $F/\sigma(F) \geq 3$  to 2.6 Å 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<sup>-</sup> and P<sub>i</sub> most probably is due to the requirement of P<sub>i</sub> for the closed conformation. It is possible that other  $Mg^{2+}$  dependent enzymes that have OH<sup>-</sup> 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  $\omega$ -amino acid:pyruvate aminotransferase by Ikemizu, S.<sup>1</sup>\*, Rehse, P.<sup>2</sup>, Watanabe, N.<sup>2</sup>, Sakabe, K.<sup>3</sup>, Sakabe, N.<sup>1,2</sup> and Yonaha, K.<sup>4</sup>

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