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 P63 or P6p. There is one homotrimer of 36,6 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=163.43 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 a 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 1.7Å
S. A. Weston, A. D. Tucker, D. R. Thatcher and R. A. Pauptit

Ricin is an exceptionally toxic heterodimeric protein from the seeds of the castor plant, Ricinus communis. The B-chain is a 362-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 adenosine base from mRNA, 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 (P4212, 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 (Katzen et al., PROTEINS 10, 251, 1991) available in the Protein Data Bank. Data extending to 1.7Å 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 RHODOSOME INACTIVATING PROTEIN FROM SEEDS OF ABRUS PRECATORIUS.
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Aribin-a from seeds of Abrus Precatorius is a ribosome-inactivating protein which catalyzes endonucleohydrolase of the N-glycosidic bond at one adenine 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 arbin-a has been determined by X-ray crystallography to a resolution 2.8Å. Crystal of this arbin-a grow in monoclinic space group P21 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 21129 data with I ≥ 1.5σ(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. Lehotay, 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 Mg2+ is inhibited by fluoride and phosphate ions in a strongly cooperative manner (Nowak, T. & Mauer, P. Biochemistry 20, 6001, 1981). The structure of the quaternary complex yeast enolase-Mg2+-F-Pi has been determined by X-ray diffraction and refined to a R=16.9% for those data with F(0)(F) > 3 to 2.6 Å resolution with a good geometry of the model. The movable loops of Pro55-Arg54, Val53-Val169 and Asp255-Asn266 are in the closed conformation found previously in the pre-catalytic substrate-enzyme complex. Calculations of molecular electrostatic potential show that this conformation stabilizes binding of negatively charged ligands at the Mg2+-ion more strongly than the open conformation observed in the native enzyme. This closed conformation is complementary to the transition state, which also has a negatively charged ion, hydroxide, at Mg2+. 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 Mg2+-dependent enzymes that have OH- 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 amino-transferase by Ikemizu, S.; Rehse, P.A.; Watanabe, N.; Sakabe, K.; Sakabe, N.; and Yoshida, K.
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