

## 03-Crystallography of Biological Macromolecules

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The crystal system is orthorhombic and space group is  $P2_12_12_1$ . The unit cell dimensions are  $a=70.0\text{\AA}$ ,  $b=70.0\text{\AA}$ , and  $c=355.0\text{\AA}$ . Assuming two trimers of a molecular weight of 110,000 per asymmetric unit,  $V_m$  was calculated to be  $1.99\text{\AA}^3/\text{Da}$ ; the solvent volume fraction is 39%. X-ray diffraction data from native and  $\text{HgCl}_2$  derivative crystals were collected on a Weissenberg camera specially designed for macromolecules using on synchrotron radiation at the Photon Factory. The self-rotation function has been calculated using the native data, and non-crystallographic three- and four-fold axes were observed. The former suggests a symmetry within the trimer molecule, and the latter corresponds to a symmetry between the trimer molecules.

**PS-03.05.15** HIGH-TEMPERATURE CRYSTALLOGRAPHY OF HIGHLY THERMOSTABLE ENZYME, 3-ISOPROPYLMALATE DEHYDROGENASE (IPMDH) FROM *THERMUS THERMOPHILUS* HB8.

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The crystal structure of IPMDH from *T. thermophilus* has been analyzed at 10 °C, 20 °C, 38 °C and 45 °C to elucidate the structure-thermostability relationship. The structure at 20 °C was solved by m.i.r. method and refined at 2.2 Å resolution to a final R value of 0.18 (Imada *et al.*, *J. Mol. Biol.*, 1991, **222**, 725-738). For rapid deterioration of the crystal by X-rays at high temperatures, fast data-collection was carried out by an R-AXIS IIC imaging-plate area-detector system using a rotating-anode X-ray generator (Sato *et al.*, *J. Appl. Cryst.*, 1992, **25**, 348-357). Intensity distributions of the crystals at 10 °C and 38 °C were similar to that of the crystal at 20 °C, but the diffraction data from the crystal at 45 °C showed significant difference from the data at 20 °C, indicating temperature-induced structure change occurs in crystal between 38 and 45 °C. In fact, enzymatic activity of this enzyme increases with temperature until 75 °C with an turning-point in Arrhenius plot around 40 °C. All the structures at 10 °C, 39 °C and 45 °C were refined at resolutions of 2.2 Å, 2.5 Å and 2.5 Å, respectively by the program PROLSQ on the basis of the structure at 20 °C, refined at 2.2 Å resolution. The final R values are 0.19, 0.17 and 0.19 for the structures at 10 °C, 38 °C and 45 °C, respectively. These structures were almost the same as that of the structure at 20 °C, but showed noticeable behavior between 38 and 45 °C in temperature factors. Several amino-acid residues become more mobile with increase of temperature from 38 to 45 °C, although they are less mobile at 38 °C, when comparing with the neighboring two residues. These residues are localized in the tertiary structure, indicating crucial correlation with thermostability.

**PS-03.05.16** Structural Studies of Active-site Mutants of *Escherichia Coli* Aspartate Aminotransferase

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Aspartate Aminotransferase (AspAT) plays an important role in amino acid metabolism by catalyzing the reversible trans-amination from L-aspartate to 2-oxoglutarate. The enzyme is an  $\alpha_2$  dimer with 396 residues per subunit. Each subunit consists of the large and the small domains and binds the coenzyme, pyridoxal 5'-phosphate (PLP) forming a Schiff base with a Lys residue. The active site is comprised of the residues from both subunits. In the active site, Asp222 is situated with a salt bridge formation to N1 of the pyridine ring of PLP. In order to understand the role of Asp222, the structures of mutant enzymes (D222E and D222A) have been determined by X-ray methods.

The crystals suitable for X-ray analysis were obtained by hanging drop method with ammonium sulfate as a precipitant. Intensity data were collected at a resolution of 2.0 Å using synchrotron radiation at Photon Factory (KEK), Japan, with a large Weissenberg camera and Imaging plates. Crystals of all mutants were isomorphous with that of wild type enzyme (space group  $C222_1$  and one subunit in the asymmetric unit). Refinements, were performed using the coordinates of the wild type AspAT or the wild type AspAT • 2-methylaspartate by the program XPLOR.

The replacement of Asp222 by Glu (D222E) retained 30% of the overall activity. The active-site structure of D222E is quite similar to that of the wild type AspAT with E222 interacting with N1 of PLP, although the residues around E222 slightly change their orientations to accommodate the longer side chain of E222 than that of D222. The activity of D222A was decreased to less than 0.1%. Interestingly, the catalytic activity was recovered partially by the reconstitution of D222A with N1-methylated PLP, while the orientation of PLP in the active site is considerably different from that of the wild type enzyme as is shown in Fig. 1. X-ray results of D222E and D222A suggest the following role of D222: D222 tethers the PLP in a productive mode within the active site and stabilizes the positive charge at N1 of PLP to enhance the function of PLP as an electron sink.

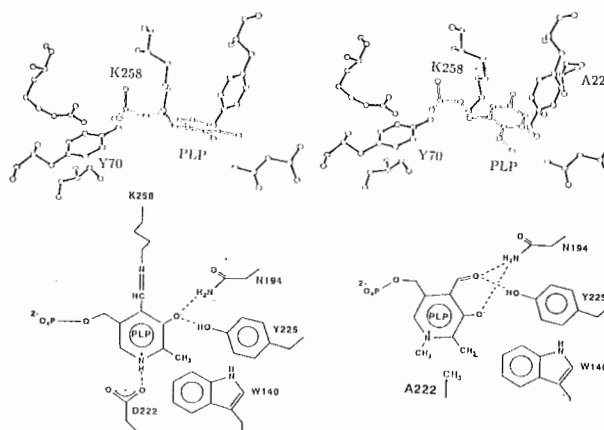


Fig. 1 ORTEP drawings and schemes of the active site regions. Wild type (left) and D222A(N-MePLP) (right)