**PS-03.05.12** 1.8 Å CRYSTAL STRUCTURE OF COLLAGENASE FROM INSECT LARVAE HYPODERMA LINEATUM

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Collagenases are proteolytic enzymes which degrade collagen under physiological conditions of pH and temperature. They belong to metallo- and serine-proteinase families. Collagenases (230 aminoacids) purified from insect larvae *Hypoderma lineatum* which are endoparasistes of cattle, is a trypsin-like enzyme of the later family. Crystals, obtained without inhibitor at neutral pH, belong to I422 space group with a=b=111.7 and c=165.8 Å and two molecules in the asymmetric unit. High resolution data were recorded using synchrotron radiation (LURE/Orsay, France) and Mark II area detector.
Using high resolution data, structure of some loops were rebuilt. Refinement was carried out using X-Plor followed by PROLSQ. Final R-factor is 19.7% at 1.8 Å resolution with 39,231 reflexions and 267 water molecules. There is a pseudo binary axis between the two molecules of the asymmetric unit. Description of the active site and packing interactions will be given.

**PS-03.05.13 ACTIVE SITES REVEALED BY THE REFINEMENT AT 2.4Å RESOLUTION OF METHANOL DEHYDROGENASE FROM BACTERIUM W3A1 USING THE DNA-DERIVED AMINO ACID SEQUENCE.** By Z.-X. Xia*, W.-W. Dai, Y.-F. Zhang, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China; V.L. Davidson, Department of Biochemistry, University of Mississippi Medical Center, Jackson, MS 39216, USA; G. Boyd, F.S. Mathews, Department of Biochemistry, Washington University School of Medicine, St. Louis, MO 63110, USA.

Methanol dehydrogenase (MEDH) from bacterium W3A1 is a quinoprotein of molecular weight about 138KDa, containing two heavy (H) and two light (L) subunits and two molecules of non-covalently associated pyrroloquinoline quinone (PQQ). The three-dimensional structure of MEDH from W3A1 has been determined at 2.6Å resolution (Xia et al., J. Biol. Chem., 1992, **267**, 22289-22297) by using X-ray data of MEDH from W3A1 to refine the structural model of the isomorphous MEDH from Methylophilus methylotrophus which was determined by multiple isomorphous replacement method. Both structures were determined based on the amino acid sequences of homologous proteins, MEDH from Paracoccus denitrificans for the H subunit and from Methylobacterium extorquens AM1 for the L subunit. The structural model contains 579 and 57 amino acid residues, respectively, for each of H and L subunit. It was preliminarily refined and PQQ was fitted into the resulting (2Fo-Fc) map, and further refinement led to an R-factor of 0.266. This is the first reported structure of a PQQ-containing enzyme.
The amino acid sequence of MEDH from W3A1 is being determined by DNA sequencing method. The sequence of the N-terminal 545 residues of the H subunit has been determined showing 63% identity with that from P. denitrificans. These residues have been refitted into the (2Fo-Fc) map. Based on the refitted model the crystallographic refinement at 2.4Å resolution is in progress and the current R-factor without any manual adjustment is 0.233 in the resolution range 5.0-2.6Å with the rms deviation of 0.025Å from ideal bond lengths. The model refitting and refinement using the DNA-derived sequence for about 90% of the amino acid residues has confirmed the reported course of the polypeptide chain and the noteworthy structural feature that the main disk-shaped body of the H subunit contains eight circularly arranged β-sheets, each composed of four antiparallel β-strands.
The refined model showed the active site structure of the enzyme. PQQ is located in the funnel-shaped central channel at the top of the disk containing the eight β-sheets. The two carbonyl oxygen atoms of PQQ make hydrogen bonds to Thr155 and Ser170, respectively. The three carboxyl groups of it interact with several charged and polar residues, such as Glu57, Arg111, Asn257, Asp299, Arg326 and Asn389. Four tryptophans, i.e. Trp239, Trp261, Trp469 and Trp533, are located around PQQ and at least two of them interact with the carboxyl groups. Glu173 is close to PQQ and may be involved in the interaction with PQQ as well. All of these residues are conserved in the four MEDHs with known sequences. The sequence determination is in progress and the structure will be fully refined after the sequence is complete.

**PS-03.05.14** Crystallization and X-ray Structural Studies on ACC Deaminase

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1-Aminocyclopropane-1-carboxylic acid (ACC) is a cyclic amino acid isolated from several plant tissues including pears and apples. It is a key intermediate in the biosynthesis of ethylene, a fruit-ripening hormon in plants. ACC deaminase isolated from a soil bacterium and from yeast catalyzes the cleavage of ACC to α-ketobutyrate and ammonia. The introduction of this enzyme into plants by the gene technology has been proved to be useful in the agricultural chemistry; it provides a way to regulate ACC levels and ethylene biosynthesis.
ACC deaminase purified from a bacterium *Pseudomonas* has an estimated molecular weight of 110,000 and is composed of three identical subunits. Each consists of a single polypeptide chain of 338 amino acid residues with a molecular weight of 36,500 and contains tightly bound pyridoxal 5'-phosphate as a cofactor. For better understanding of the enzymatic function in the atomic resolution, we initiated X-ray structure analysis of this enzyme.
Crystals of ACC deaminase were grown by the hanging-drop vapour diffusion method with MPD as a precipitant.

  

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The crystal system is orthorhombic and space group is $P2_12_12_1$. The unit cell dimensions are a=70.0Å , b=70.0Å , and c=355.0Å . Assuming two trimers of a molecular weight of 110,000 per asymmetric unit, $V_m$ was calculated to be 1.99 $Å^3$/Da; the solvent volume fraction is 39%. X-ray diffraction data from native and $HgCl_2$ 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.

By K. Imada, M. Sato*, Y. Katsube and T. Oshima†, Institute for Protein Research, Osaka University, Japan, †Tokyo Institute of Technology, Japan.

The crystal structure of IPMDH from *T. thermophilus* has been analyzed at 10 ℃, 20 ℃, 38 ℃ and 45 ℃ to elucidate the structure-thermostability relationship. The structure at 20 ℃ 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 ℃ and 38 ℃ were similar to that of the crystal at 20 ℃, but the diffraction data from the crystal at 45 ℃ showed significant difference from the data at 20 ℃, indicating temperature-induced structure change occurs in crystal between 38 and 45 ℃. In fact, enzymatic activity of this enzyme increases with temperature until 75 ℃ with an turning-point in Arhenius plot around 40 ℃. All the structures at 10 ℃, 39 ℃ and 45 ℃ 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 ℃, refined at 2.2 Å resolution. The final R values are 0.19, 0.17 and 0.19 for the structures at 10 ℃, 38 ℃ and 45 ℃, respectively. These structures were almost the same as that of the structure at 20 ℃, but showed noticeable behavior between 38 and 45 ℃ in temperature factors. Several amino-acid residues become more mobile with increase of temperature from 38 to 45 ℃, although they are less mobile at 38 ℃, when comparing with the nighboring 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 2.0Å 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.

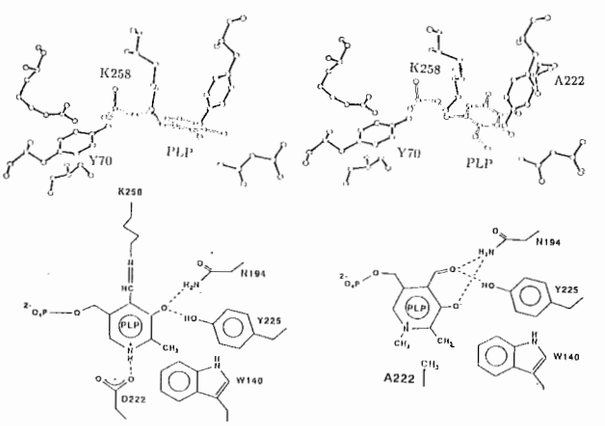


Fig .1 ORTEP drawings and schemes of the active site regions. Wild type (left) and D222A(N-MePLP) (right)