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.

