PS-03.05.17 Crystal Structure of a NADH Oxidase from *Thermus thermophilus* Refined at 2.3 Å Resolution

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NADH oxidase (EC 1.6.99.3) from Thermus thermophilus is a flavoenzyme that catalyses the oxidation of NAD(P)H by molecular oxygen to NAD(P) and H_2O_2. The enzyme is a dimer with a molecular weight of 22000 D per monomer and accepts as cofactor FAD and FMN. The molecule crystallizes in spacegroup $P4_12_12$ with lattice dimensions a=94.8 Å and c=49.0 Å. The structure of the enzyme has been determined by isomorphous replacement using 3 derivatives and has been refined to 2.3 Å resolution. The present crystallographic R-value is 19.6 % for reflections between 8.0 and 2.3 Å resolution. The main part of the molecule is formed by a four stranded antiparallel ß-sheet covered by four helices. A C-terminal excursion of the second molecule of the dimer forms a fifth parallel strand to this ß-sheet. The interface between the monomers forms a deep clèft with contacts between the subunits mainly via a long bent helix. The FAD-binding site is situated at this interface between the monomers. As expected from the absence of the Gly-X-Gly-X-X-Gly consensus sequence for the nucleotide binding site this binding site is different from other FAD-binding proteins. The active site involves residues from both subunits of the dimer.

PS-03.05.18 CRYSTAL STRUCTURE OF PEROXIDASE FROM A FUNGUS *ARTHROMYCES RAMOSUS*. By N. Kunishima*, K. Fukuyama, H. Hatanaka†, Y. Shibano†, T. Amachi†, and H. Matsubara, Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan, and †Institute for Fundamental Research, Suntory Limited, Shimamoto-cho, Mishima-gun, Osaka 618, Japan.

Peroxidases (donor : H_2O_2 oxidoreductase [EC 1.11.1.7]) are a class of heme enzymes that catalyse the two-electron oxidation of a variety of compounds by hydrogen peroxide. The peroxidase isolated from a fungus, *Arthromyces ramosus*, (ARP) has higher activity than that of horseradish peroxidase. ARP consists of 344 amino acid residues and a non-covalently bound iron protoporphyrin IX. ARP has a sequence homology of about 20 % to other peroxidases. In order to elucidate the reaction mechanism of peroxidases as well as to compare the structure of ARP with that of cytochrome *c* peroxidase (CCP), X-ray analysis of ARP has been undertaken. ARP crystals belong to tetragonal, the space group of $P4_22_12$. The unit cell dimensions are a=b=74.57 Å, c=117.47 Å. Platinum, iodine, and mercury derivatives were prepared by soaking method. Native data to 1.9 Å and derivative data to 2.5 Å were collected with a screenless Weissenberg camera for macromolecular crystals using synchrotron radiation. The structure was solved by the multiple isomorphous replacement method. The overall figure of merit was 0.66 for the data to 2.5 Å resolution. The best map has shown that the spatial arrangement of α helices in CCP is basically conserved in ARP but the orientation and the length of some α helices are different. In the iodine derivative, an I_3^- is bound to ARP at the distal side of the heme. The proximal and distal ligands of the heme are His184 and His56, respectively. Crystallographic refinement is currently under way.

PS-03.05.19 STRUCTURE ANALYSIS ON CHIMERIC 3-ISOPROPYLMALATE DEHDROGENASE AND ITS THERMOSTABLE MUTANTS. by M. Sakurai, S. Kadono, K. Onodera, H. Moriyama, N. Tanaka, K. Imada*, Y. Katsube*, and T. Ooshima, Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, and Institute for Protein Research, Osaka University, Japan.

We are studying on chimeric 3-isopropylmalate dehydrogenase that are fusion of *Thermus thermophilus* IPMDH (10T) and *Bacillus subtilis* IPMDH (10M), and its thermostable mutants. The 2T2M6T is constructed by replacing 20-40% region from N-terminal of 10T to the corresponding mesophile. The 2T2M6T is sensitive to the heat. The mutants, S82R and I93L, recovered the thermostability. Crystallizations of the proteins were achieved by the hanging drop vapor diffusion method utilizing ammonium sulfate. The crystals of all proteins were isomorphous with that of the intact 10T that belong to space group $P3_221$ with the unit cell dimension of a=b=78.3A, c=158.6A. The diffraction data were collected by R-AXIS IIc. We successfully applied the molecular replacement method to solve the structure. The structural refinements were performed by the PROLSQ and X-PLOR. The final R-factors within 2.1 A resolution for 2T2M6T, S82R, and I93L are 19, 19, and 18 % respectively. The three compounds take almost the same structures to intact 10T with their r.m.s. displacement of Ca atoms are 0.16A. The distributions of temperature factors are also same, however I93L shows the more low values. In the detail, 2T2M6T have geometrical repulsion at Ile93 against Arg94. In the I93L the repulsion had removed. S82R have a new hydrogen bond between Arg82 and Glu87 through water molecule. These small but significant structural changes make the mutant compound thermostable.

PS-03.05.20 CRYSTALLOGRAPHIC AND SOLUTION STUDIES OF H-PROTEIN FROM THE GLYCINE DECARBOXYLASE COMPLEX IN PEA MITOCHONDRIA.

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The oxidation of glycine by plant mitochondria represents an important step in the metabolic pathway of photorespiration in leaf tissue and is catalyzed by the glycine decarboxylase complex. The complex, purified from pea leaf mitochondria, contains four proteins, P,T,H and L (Bourguignon, Neuburger & Douce, 1988). The pivotal enzyme is the 13.3kDA lipoamide-containing H-protein. Its sequence consists of 131 amino-acids (Macherel, Lebrun, Grignon, Neuburger and Douce, 1990). Its structure determination has been undertaken to further our understanding of the mechanisms involved in this multi-enzyme complex. This is the first report of the crystallization of a lipoic-dependent protein.
Crystals of H-protein were grown by vapour diffusion against 2M $(NH_4)_2SO_4$ solution at pH 5.2. The space group is $P3_121$ (a=57.22, c=136.8 Å) with two independent molecules in the asymmetric unit and a crystal solvent content of about 46% (Sieker, Cohen-Addad, Neuburger & Douce, 1991). X-ray intensities were collected on a crystal of the native protein using a FAST/Enraf-Nonius area detector to 2.7Å resolution

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