03-Crystallography of Biological Macromolecules

03-03.05.17 Crystal Structure of a NADH Oxidase from *Thermus thermophilus* Reﬁned at 2.3 Å Resolution


*Gesellschaft für Biologische Forschung*, Departments of Molekularer Struktur und Funktion und *Enzyme* Technology, Maschaköpf Weg 1, D-3381 Braunschweig, FRG

NADH oxidase (EC 1.6.5.3) from *Thermus thermophilus* is a flavoenzyme that mediates the oxidation of NADH by molecular oxygen to NADP(+) and H2O2. The enzyme is a dimer with a molecular weight of 22000 per monomer and accepts as cofactor FAD and FMN. The monomeric crystalline in spacegroup P42121 with lattice dimensions a=94.8 Å and c=659.4 Å. The orientation 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 16.8% for reflections between 8.0 and 2.3 Å resolution. The main part of the molecule is formed by a four stranded antiparallel β-sheet covered by four helices. A Ω-turn excurs of the second molecule of the dimer form a fifth parallel strand to this β-sheet. The interface between the monomers forms a deep cleft with contacts between the monomers 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-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.

03-03.05.19 STRUCTURE ANALYSIS ON CHIMERIC 3-ISOPROPYLMALATE HYDROGENASE AND ITS THERMOSTABLE MUTANTS. by M. Sakurai, S. Kusuda, K. Onodera, K. Moriyama, N. Tanaka, K. Izikawa, Y. Katsube*, and T. Ohashi, 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 (107) and *Bacillus subtilis* IPMDH (109), and its thermostable mutants. The 2T2M67 is constructed by replacing 20-408 region from N-terminal of 107 to the corresponding polypeptide. The 2T2M67 is sensitive to the heat. The mutants, 8S28 and 19H, 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 107 that belong to space group P321 with the unit cell dimension of a=b=78.3 Å, c=158.6 Å. The diffraction data were collected by K-AXIS IIc. We successfully applied the molecular replacement method to solve the structure. The structural refinements were performed by the PHENIX and K-AXIS. The final R-factors within 2.1 Å resolution for 2T2M67, 2S28, and 19H are 19, 19, and 18% respectively. The three compounds take almost the same structure to intact 107 with their r.m.s. displacement of Ca atoms are 0.16 Å. The distributions of temperature factors are also same, however 19H shows the more low values. In the details, 2T2M67 have geometrical repulsion at 1e93 against Arg94. In the 2S28 the repulsion had removed. 2S28 have a new hydrogen bond between Arg92 and Glu87 through water molecule. These small but significant structural changes make the mutant compound thermostable.

03-03.05.18 CRYSTAL STRUCTURE OF Peroxidase FROM A FUNGUS *ARUMOSUS* RUMITOSUS. By N. Kanaizuma*, K. Fukuyama, H. Hatanao*, 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, Siamamato-cho, Minohgun, Osaka 618, Japan.

Peroxidases (domain: H2O2, oxidoreductase [EC 1.11.1.7]) are a class of heme enzymes that catalyze the electron oxidation of a variety of compounds by hydrogen peroxide. The peroxidase isolated from a fungus, *Arum osus* Ruminatus, (ARP) has higher activity than that of horseradish peroxidase. ARP consists of 334 amino acid residues and a non-collinearly 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 P4212. The unit cell dimensions are a=b=74.57 Å, c=177.47 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 I2 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.

03-03.05.20 CRYSTALLOGRAPHIC AND SOLUTION STUDIES OF H-FRAGMENT FROM THE GLYCINE DECARBOXYLASE COMPLEX IN PEA MITOCHONDRIA. C. Cohen-Albali*, S. Pare* (1), L. Sieker(3), M. Neuburger(2), R. Douce(2), G. Zaccai(1)

(1) Inst. Biologique Structure, 41 av. des Martyrs, Grenoble 38027 Cédex 1, France (2) Dept. de Biologie, LBS, PCV, C.E.N.G., BP38X, 38041 Grenoble, France (3)Dept. of Biol. Structure, SM-20, Univ. of Washington, Seattle, WA 98195, USA

The oxidation of glycine by plant mitochondria represents an important step in the metabolic pathway of photosynthesis 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 13,360 kDa lipoprotein-containing H-protein. Its sequence consists of 131 amino acids (Macherel, Lebrum, 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 crystallography of a lipoprotein-dependent protein. Crystals of H-protein were grown by vapor diffusion against 2M (NH4)2SO4 solution at pH 5.2. The space group is P321 (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-Albali, Neuburger & Douce, 1994). X-ray intensities were collected on a crystal of the native protein using a FAST/Enraf-Nonius area detector to 2.7 Å resolution.