Advanced Research Center for Beam Science
– Structural Molecular Biology –

http://www.scl.kyoto-u.ac.jp/~hata/indexE.html

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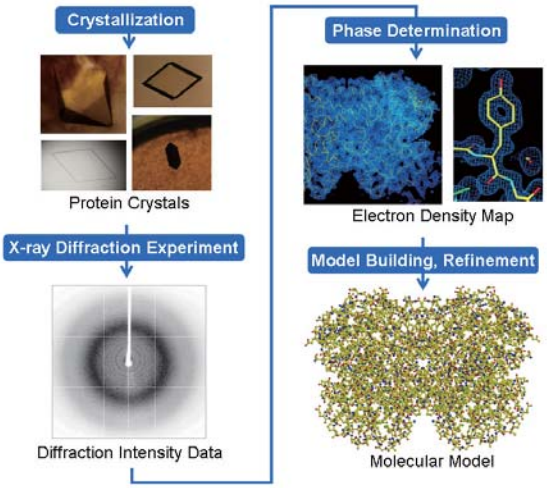
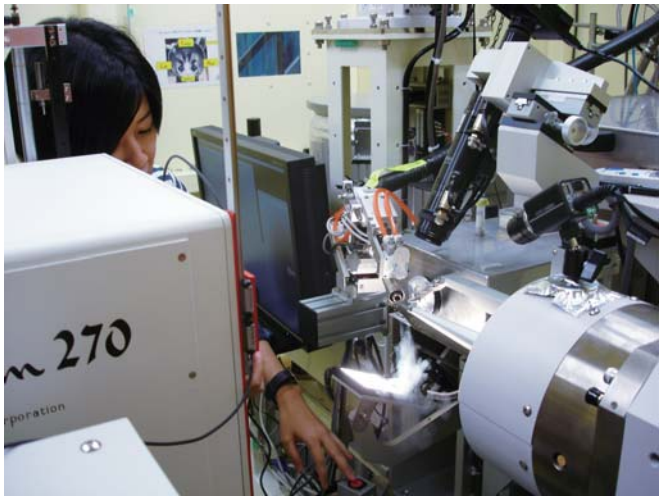
Scope of Research

KEYWORDS
Crystal
X-ray Crystallographic Analysis
Structural Biology
Protein Crystallography
Structure and Function

The research activities in this laboratory are performed for X-ray structural analyses of biological macromolecules and the investigation of the electronic state in materials as follows: The main subjects of the biomacromolecular crystallography are crystallographic studies on the reaction mechanism of enzymes, the relationship between the multiform conformation and the functional variety of proteins, and the mechanism of thermostabilization of proteins. In the investigation of the chemical state in materials, the characteristics of the chemical bonding in the atom and molecules are investigated in detail using a newly developed X-ray spectromator with a high-resolution in order to elucidate the property of materials. The theoretical analysis of the electronic states with DV-Xα and WIEN2k, and the development of new typed X-ray spectrometer with ultra high-resolution have also been carried out.





Selected Publications

Fujii, T.; Maeda, M.; Mihara, H.; Kurihara, T.; Esaki, N.; Hata, Y., Structure of a Nif S Homologue: X-ray Structure Analysis of CsdB, an *Escherichia coli* Counterpart of Mammalian Selenocysteine Lyase, *Biochemistry*, **39,** 1263-1273 (2000).

Fujii, T.; Sakai, H.; Kawata, Y.; Hata, Y., Crystal Structure of Thermostable Aspartase from *Bacillus* sp. YM55-1: Structure-based Exploration of Functional Sites in the Aspartase Family, *J. Mol. Biol*., **328,** 635-654 (2003).

Hayashida, M.; Fujii, T.; Hamasu, M.; Ishiguro, M.; Hata, Y., Similarity between Protein-Protein and Protein-Carbohydrate Interactions, Revealed by Two Crystal Structures of Lectins from the Roots of Pokeweed, *J. Mol. Biol*., **334,** 551-565 (2003).

Fujii, T.; Oikawa, T.; Muraoka, I.; Soda, K.; Hata, Y., Crystallization and Preliminary X-ray Diffraction Studies of Tetrameric Malate Dehydrogenase from the Novel Antarctic Psychrophile *Flavobacterium frigidimaris* KUC-1, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun*., **63,** 983-986 (2007).

Fujii, T.; Goda, Y.; Yoshida, M.; Oikawa, T.; Hata, Y., Crystallization and Preliminary X-ray Diffraction Studies of Maleylacetate Reductase from *Rhizobium* sp. Strain MTP-10005, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun*., **64,** 737-739 (2008).

Crystal Structure Analysis of the Reductase Component of a Resorcinol Hydroxylase (GraD) in Complex with FAD

The resorcinol hydroxylase is involved in the first step of the resorcinol catabolic pathway and catalyzes hydroxylation of resorcinol to hydroxyquinol. The enzyme belongs to the two-component flavin-diffusible monooxygenase (TC-FDM) family and consists of two components: an oxygenase and a flavin reductase. It uses molecular oxygen and reduced flavin for hydroxylation and NAD(P)H for flavin reduction. The small component, flavin reductase, generates reduced flavin for the oxygenase component to oxygenate the substrate. Thus, the enzymatic reaction is separated into two steps. However, hydroxylation activity is exhibited in the cooperative presence of both the components. To understand the structural basis for the catalytic mechanism, we performed the crystal structure analysis of the reductase component (GraD) from *Rhizobium* sp. strain MTP-10005 in complex with FAD. GraD is a dimer and its subunit consists of 179 amino acid residues with the mass of 19,429 Da.

The N-terminal His-tagged GraD was used for crystallization. The protein solution consisted of 2.0 mg/ml GraD, 0.1 mM FAD and 50 mM Tris-HCl pH 8.0. Crystals with size of 0.15 mm × 0.1 mm × 0.1 mm were obtained in about 6 days by a sitting drop vapor diffusion method with a reservoir solution consisting of 100 mM sodium citrate pH 5.6, 13–16% (w/v) PEG2000, 6–9 % (v/v) 2-propanol, 10 mM dithiothreitol and 0.2 mM FAD (Figure 1). The crystals of GraD were deep yellow in color, indicated that GraD crystals contain oxidized FAD cofactor. Diffraction data were collected up to 1.8 Å resolution under cryogenic conditions at beamline NW12A, Photon Factory PF-AR, Tsukuba, Japan. The GraD crystal belongs to the tetragonal space group $P4_12_12$ with unit cell dimensions of $a = b = 77.8$ Å and $c = 124.2$ Å. Assuming that the crystal contains two subunits in the asymmetric unit, the V_M value is 2.17 Å^3/Da and a solvent content is 43%, which is acceptable for a protein crystal. The structure was determined by molecular replacement and refined at 1.8 Å resolution.

Figure 1. Crystals of the reductase component of a resorcinol hydroxylase (GraD) from *Rhizobium* sp. strain MTP-10005 in complex with FAD.

In the crystal, one homodimer exists in the asymmetric unit and each subunit binds an FAD molecule (Figure 2). The subunit consists of twelve β-strands, three α-helices and two 3_{10} helices. The core of the GraD subunit is a seven-stranded anti-parallel β-barrel capped by one α-helix. The subunit adopts a FMN-binding split barrel fold (Greek key architecture), related to the ferredoxin reductase like FAD-binding domains. The FAD molecule is located in the groove in the surface of the enzyme. The N5-atom of the isoalloxazine ring is hydrogen bonded to the hydroxyl group of the side chain of Thr50. The dimethyl benzene moiety of isoalloxazine ring is located in a hydrophobic pocket formed by hydrophobic amino acid residues which are highly conserved in flavin reductases. The O2, O4 and N3 atoms of the pyrimidine moiety of isoalloxazine ring are hydrogen bonded to backbone atoms of protein (Figure 3).

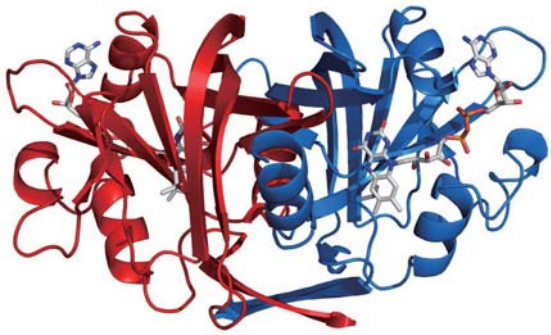


Figure 2. Dimeric molecular structure of the reductase component of a resorcinol hydroxylase (GraD) from *Rhizobium* sp. strain MTP-10005 in complex with FAD.

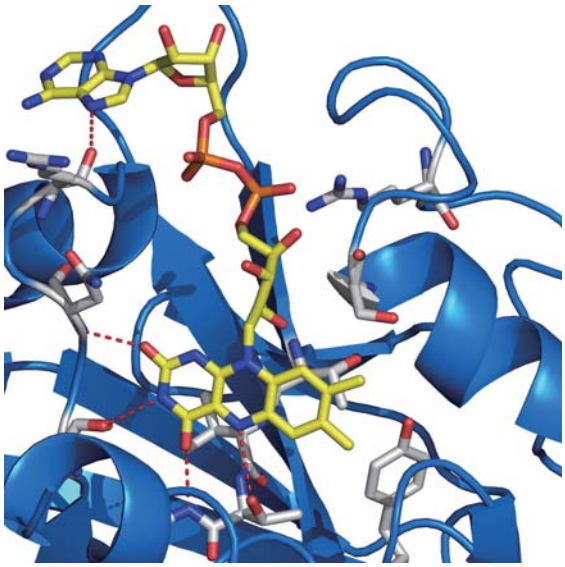


Figure 3. Structure of FAD-binding site of the reductase component of a resorcinol hydroxylase (GraD) from *Rhizobium* sp. strain MTP-10005 in complex with FAD.