Advanced Research Center for Beam Science
– Structural Molecular Biology –

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

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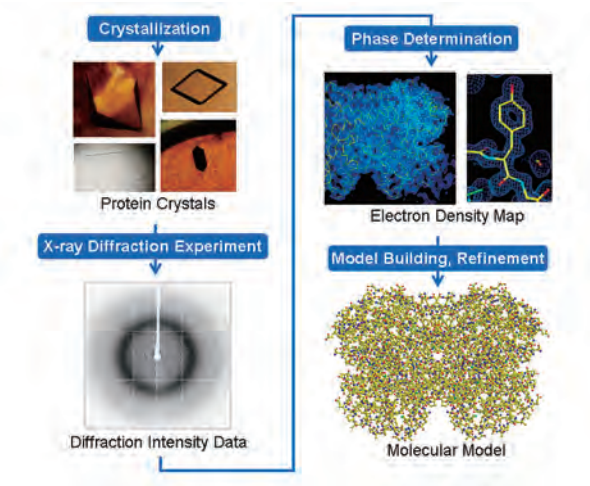
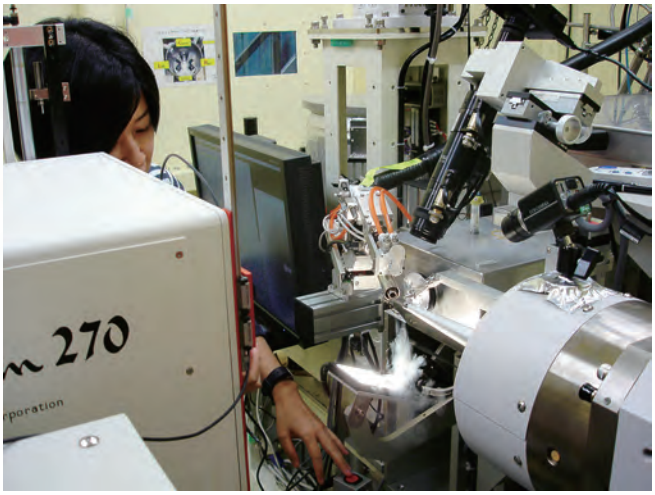
Scope of Research

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 NifS 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 Oxygenase Component of a Resorcinol Hydroxylase (GraA) 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 oxygenase component (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD. GraA is a tetramer and its subunit consists of 409 amino acid residues with the mass of 43,305 Da.

The N-terminal His-tagged GraA was used for crystallization. The protein solution consisted of 10 mg/ml GraA, 1 mM FAD, and 50 mM Tris-HCl pH 8.0. Crystals with size of 0.1 × 0.07 × 0.05 mm^3 were obtained in about 4 days by a sitting drop vapor diffusion method with a reservoir solution consisting of 20% (w/v) PEG3350 and 0.2 M KNO_3 (Figure 1). They belonged to the trigonal space group $P3_221$ with unit cell dimensions of $a=b=204.4$ Å, $c=299.9$ Å. Diffraction data were collected up to 3.2 Å resolution under cryogenic conditions at beamline BL5A, PF, Tsukuba, Japan. The structure was determined by molecular replacement and refined at 3.2 Å resolution.

In the holo-form crystal, four tetramers exist in the asymmetric unit and each subunit binds a FAD. GraA is a tetramer of four identical subunits related to one another by three molecular two-fold axes. A given pair of two subunits in the molecule form a close dimer with C-terminal α-helical domains crossed together around a molecular two-fold axis. Then, two of the close dimers form a loose dimer around another molecular two-fold axis crossing perpendicular to the former two-fold axis. Finally, the GraA tetrameric molecule adopts the structure of a dimer of dimers with three molecular two-fold axes perpendicular to one another. The subunit consists of three domains. The N-terminal domain (residues Met1–Ala121) has an α-structure mainly of antiparallel α-helices, the central domain has a β-structure of two β-sheets stacked together, and the C-terminal domain (residues Phe218–Tyr409) has a four-helix-bundle structure of long antiparallel α-helices involved in tetramer formation. The FAD is located in the space that is encompassed by these three domains (Figure 2). The FAD binds to the polypeptide chain via hydrophobic and hydrophilic interactions (Figure 3). The loop region of 13 residues (residues Gly271–Asn283), which is disordered in the apo-form, is ordered and covers FAD of another subunit (Figures 2 and 3). The turn portion of the loop occludes the entrance of the putative active site.

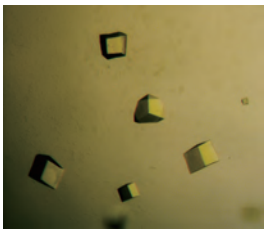


Figure 1. Crystals of the oxygenase component of a resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD.

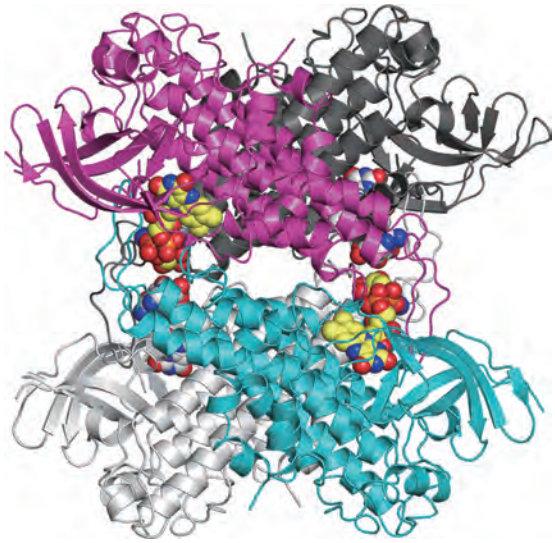


Figure 2. Tetrameric molecular structure of the oxygenase component of a resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD.

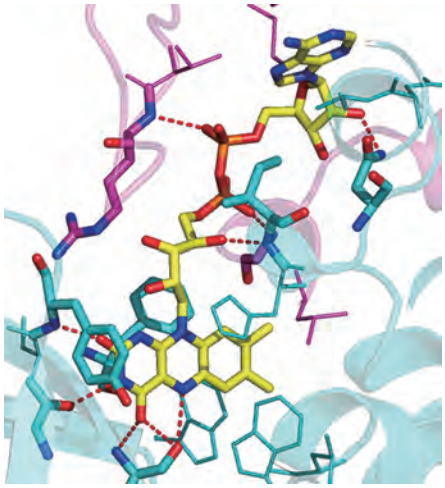


Figure 3. Structure of the FAD-binding site of the oxygenase component of a resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD.