Advanced Research Center for Beam Science – Atomic and Molecular Structures –

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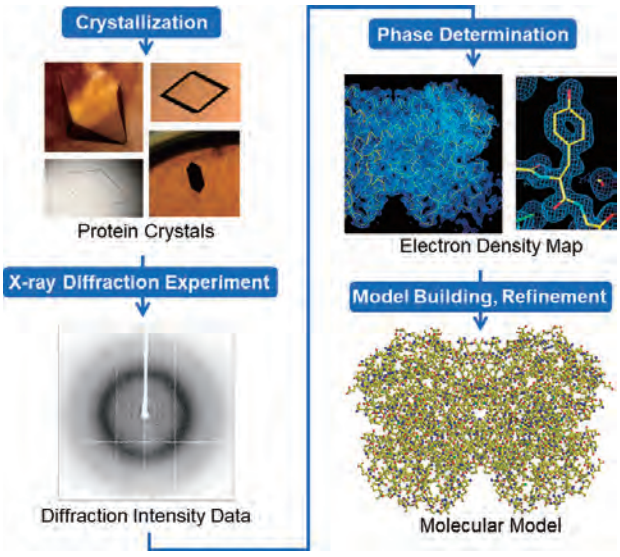
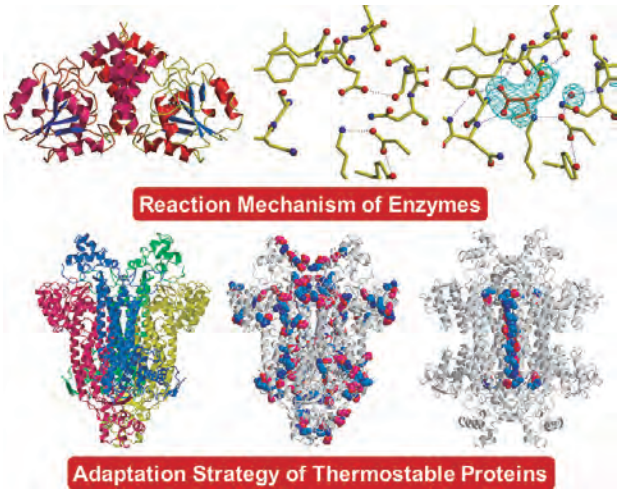
Scope of Research

This laboratory analyzes X-ray crystallographic structures of biological macromolecules and studies the structural biology about the relationships between protein structures and their functions and properties based on the crystal structures. The main research themes are elucidation of the reaction mechanism of enzymes, the relationship between the multiform conformation and the functional variety of proteins, the structural basis for the domain-arrangements of multi-domain proteins or protein-protein interactions, structure determination for structure-based protein engineering and industrial application, and the adaptation strategy of proteins from thermophilic or cold-adapted bacteria.

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





Selected Publications

Fujii, T.; Sato, A.; Okamoto, Y.; Yamauchi, T.; Kato, S.; Yoshida, M.; Oikawa, T.; Hata, Y., The Crystal Structure of Maleylacetate Reductase from *Rhizobium* sp. Strain MTP-10005 Provides Insights into the Reaction Mechanism of Enzymes in Its Original Family, *Proteins: Structure, Function, and Bioinformatics*, **84,** 1029-1042 (2016).

Fujii, T.; Yamauchi, T.; Ishiyama, M.; Gogami, Y.; Oikawa, T.; Hata, Y., Crystallographic Studies of Aspartate Racemase from *Lactobacillus sakei* NBRC 15893, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **71,** 1012-1016 (2015).

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).

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.; 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).

Crystal Structure Analysis of the Oxygenase Component of Resorcinol Hydroxylase (GraA) in Complex with FAD and Nitrate Ions

Resorcinol hydroxylase is involved in the first step of the resorcinol catabolic pathway and catalyzes the hydroxylation of resorcinol to hydroxyquinol. This enzyme belongs to the two-component flavin-diffusible monooxygenase (TC-FDM) family and comprises 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 executed in two steps. However, hydroxylation activity is exhibited in the cooperative presence of both components. To understand the structural basis for the catalytic mechanism, we performed a crystal structure analysis of the oxygenase component (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD and nitrate ions. GraA is a tetramer, and its subunit consists of 409 amino acid residues.

The N-terminal His-tagged GraA was used for crystallization. The protein solution consisted of 12 mg/ml GraA, 1 mM FAD, 6 mM resorcinol, and 49 mM Tris-HCl pH 8.0. Crystals with suitable sizes for X-ray diffraction experiments were obtained in several days by a sitting drop vapor diffusion method with a reservoir solution consisting of 20% (w/v) PEG3350 and 0.2 M KNO_3. These crystals belonged to the monoclinic space group *C*2 with unit cell dimensions of a = 155.4 Å, b = 102.8 Å, c = 128.2 Å, β = 104.6°. Diffraction data were collected up to 2.0 Å resolution under cryogenic conditions at beamline BL-1A, PF, Tsukuba, Japan. The structure was determined by molecular replacement and refined at 2.0 Å resolution.

In the crystal, a tetramer exists in the asymmetric unit and each subunit binds an FAD and a nitrate ion. GraA is a tetramer of four identical subunits related to one another by three molecular two-fold axes (Figure 1). A given pair of two subunits in the molecule forms a close dimer and two of the close dimers form a loose dimer. The GraA tetrameric molecule adopts the structure of a dimer of dimers. The subunit consists of three domains (Figure 2). 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 encompassed by these three domains and binds to the polypeptide chains via hydrophobic and hydrophilic interactions. The loop region of 13 residues (residues Gly271–Asn283), which is disordered in the apo-form structure, is ordered and covers the FAD of another subunit. The turn portion of the loop occludes the entrance of the active site. The nitrate ion and three water molecules bind to the active site via hydrogen bonds (Figure 3). Side chains of Tyr127, His364, Ser386, and Gln408 take part in the interactions. These residues might be involved in substrate binding or catalytic reactions.

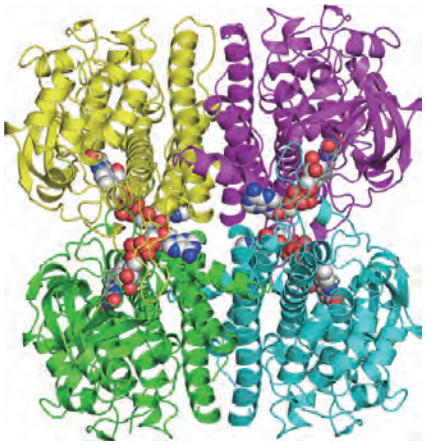


Figure 1. Tetrameric molecular structure of the oxygenase component of resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD and nitrate ions.

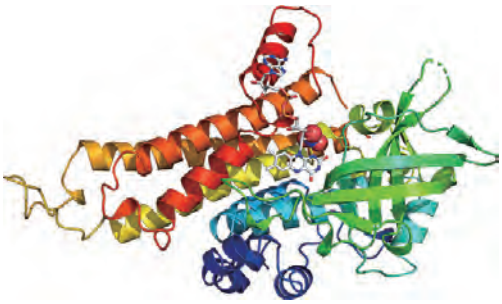


Figure 2. Subunit structure of the oxygenase component of resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD and nitrate ions.

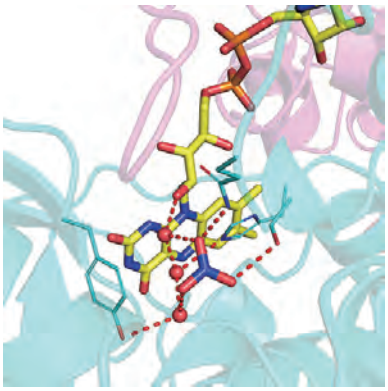


Figure 3. Structure of the active site of the oxygenase component of resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 in complex with FAD and nitrate ions.