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

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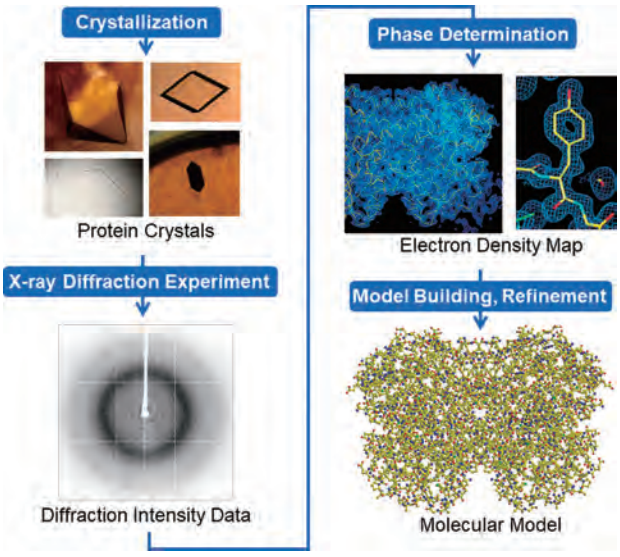
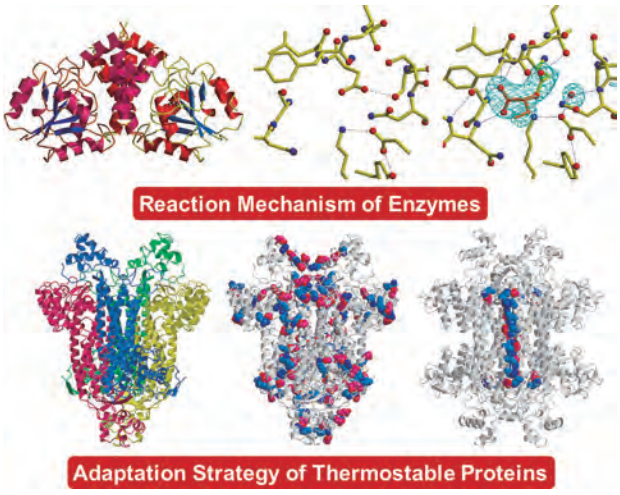
Scope of Research

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





Recent 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).

Refined Crystal Structure of the Oxygenase Component (GraA) of Resorcinol Hydroxylase

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 smaller component, flavin reductase, generates reduced flavin to allow the oxygenase component to oxygenate the substrate. Thus, the enzymatic reaction is executed in two steps. However, hydroxylation activity requires cooperation between both the components. To understand the structural basis of the catalytic mechanism, we performed a crystal structure analysis of the apo-form of the oxygenase component (GraA) from *Rhizobium* sp. strain MTP-10005. GraA is a tetramer, and its subunit consists of 409 amino acid residues with a mass of 43,305 Da.

N-terminal His-tagged GraA was used for crystallization. The protein solution consisted of 5 mg/ml GraA and 50 mM Tris-HCl pH 8.0. Crystals with suitable sizes for X-ray diffraction experiments were obtained over several days by a sitting drop vapor diffusion method, with a reservoir solution consisting of 17% (w/v) PEG3350 and 0.2 M K_2HPO_4. They belonged to the tetragonal space group $I4_122$ with unit cell dimensions of $a = b = 101.6$ Å, $c = 319.8$ Å and contained one GraA subunit in asymmetric unit. Diffraction data were collected up to 1.9 Å resolution under cryogenic conditions at beamline BL5A, PF, Tsukuba, Japan. The structure was determined by molecular replacement and refined at 1.9 Å resolution up to $R = 0.162$ and $R_{free} = 0.185$.

GraA is a tetramer of four identical subunits related to one another by three molecular two-fold axes which are identical to crystallographic 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 part of PEG3350 used as a precipitating agent for the crystallization is located in the space that is encompassed by these three domains (Figure 2). The PEG binds to both the binding site of a portion of isoalloxazine ring of FAD in the GraA-FAD complex and the putative substrate binding site (Figure 3). The loop region of 13 residues (residues Gly271–Asn283), which is ordered and covers the FAD of another subunit in the GraA-FAD complex, is disordered in this apo-form (Figures 2 and 3).

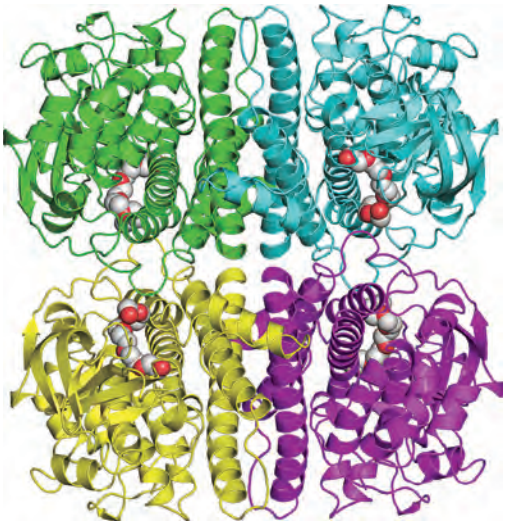


Figure 1. Tetrameric molecular structure of the apo-form of the oxygenase component of resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 with bound PEG.

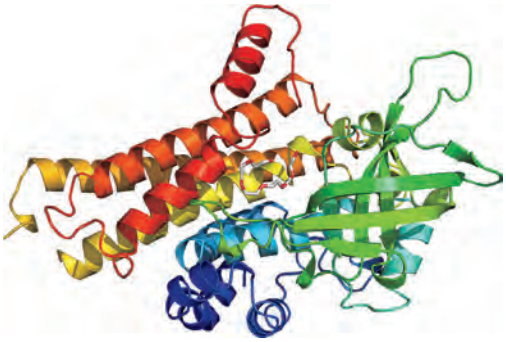


Figure 2. Subunit structure of the apo-form of the oxygenase component of resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 with bound PEG.

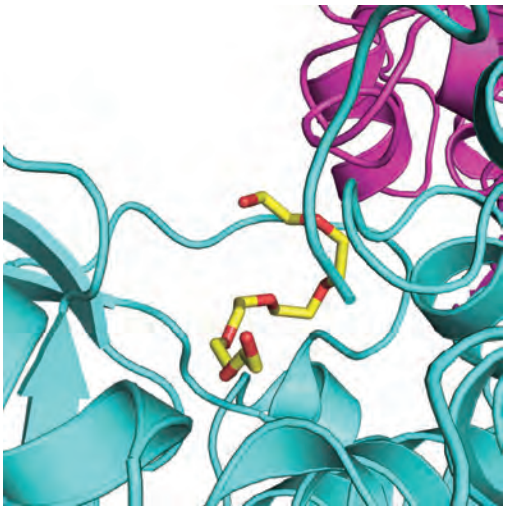


Figure 3. Structure of the active site cleft of the apo-form of the oxygenase component of resorcinol hydroxylase (GraA) from *Rhizobium* sp. strain MTP-10005 with bound PEG.