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

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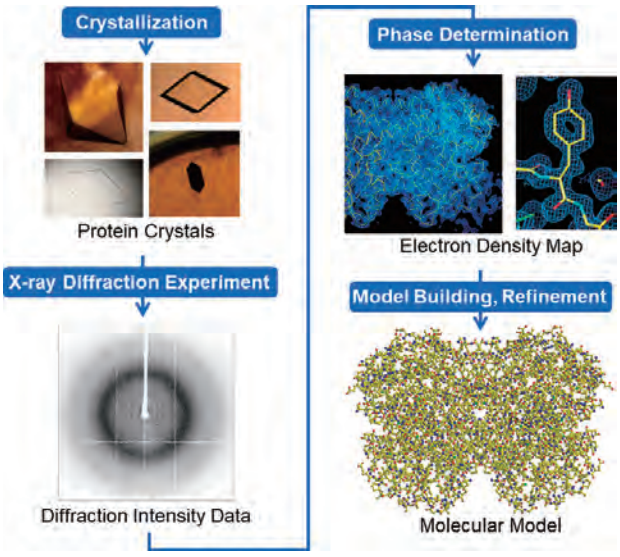
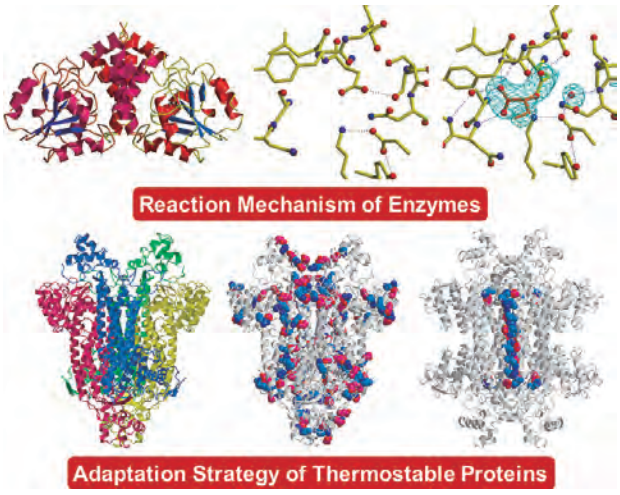
Assist Prof
FUJII, Tomomi
(D Sc)

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 GraC Protein from *Rhizobium* sp. Strain MTP-10005 in Complex with Coenzyme

Rhizobium is a genus of tubercle-forming bacteria. It grows in the root of a plant in symbiosis with other bacteria to fix nitrogen from the air. Although considerable attention has been paid to *Rhizobium* genes and gene products, there is still little information available on the molecular structure, function, and detailed properties of the enzymes involved in its metabolic pathways. In the course of a screening experiment, *Rhizobium* sp. strain MTP-10005 was isolated from natural river water. Enzymological and genetic studies showed that products of the *graA*, *graB*, *graC*, and *graD* genes in the bacterium are involved in the degradation pathway of resorcinol. In order to reveal the structures and functions, we have been performing X-ray structural studies of the enzymes. Maleylacetate reductase (GraC) catalyzes NADH- or NADPH-dependent reduction of maleylacetate to 3-oxoadipate. We have determined the GraC-coenzyme complex structure.

N-terminal His-tagged GraC was overexpressed in *Escherichia coli*, purified, and used for crystallization. The protein solution consisted of 2.5 mg/ml GraC, 5 mM NADH, and 50 mM Tris-HCl pH 8.0. Initial crystallization experiments were performed by the sitting-drop vapor-diffusion method using several screening kits. Small crystals were obtained after several days with several conditions. Particularly, thin plate-shaped adequately sized crystals for X-ray diffraction experiments were obtained using a reservoir solution consisting of 20% (w/v) PEG1500 and 0.1 M Bis-Tris pH 6.5. Diffraction experiments were performed on beamline BL-5A, Photon Factory, KEK, Japan. The crystal was mounted with a cryoloop and cooled with a cold stream of nitrogen. Diffraction data were collected up to 2.5 Å resolution. The crystal belonged to space group *P*1. The structure was determined by molecular replacement using an apo-type GraC crystal structure as a starting model and refined at 2.5 Å resolution.

In the present crystal, one homodimer GraC molecule exists in the unit cell and each subunit binds an NADH molecule (Figure 1). The subunit of GraC molecule consists of two domains: an N-terminal domain, residues 1–159, adopting an α/β structure and a C-terminal α-helical domain, residues 160–351. The active site is located in the cleft between the domains of the subunit. The NADH molecule is located in the active site cleft and mainly binds to the N-terminal domain (Figure 2). The subunit of the GraC-coenzyme complex has a closed conformation that may be adopted on binding the coenzyme. However, one subunit of apo-type GraC binds no other ligand except two sulfate anion. It has an open conformation, as is the case before the enzymatic reaction. Thus, the two types of GraC crystal structures reveal the structures of maleylacetate reductase both in the coenzyme-binding state and in the ligand-free state, which suggests that the structure of GraC must change from the open conformation to the closed conformation in the course of enzymatic reaction (Figure 3).

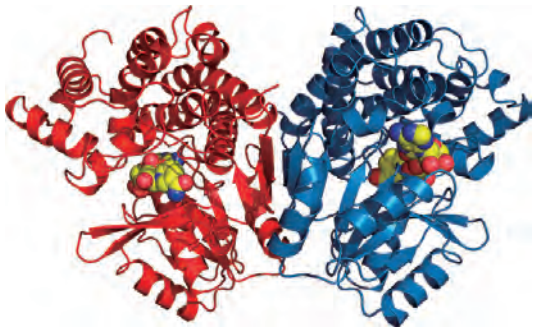


Figure 1. Dimeric molecular structure of GraC-coenzyme complex.

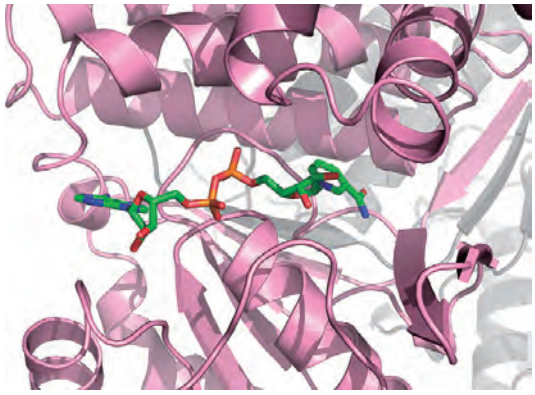


Figure 2. Structure of coenzyme-binding site of GraC-coenzyme complex.

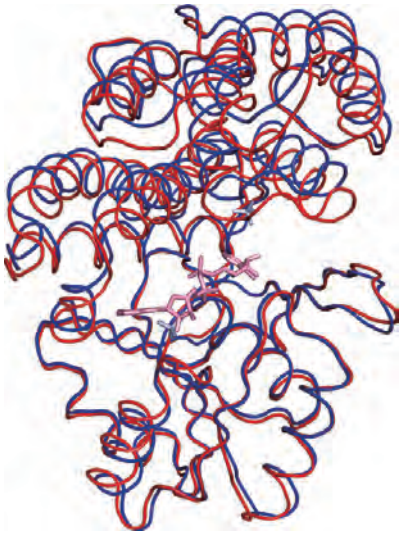


Figure 3. Superposition of GraC subunits. Subunit-A of GraC-coenzyme complex and Subunit-B of apo-type GraC are shown in red and blue, respectively.