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

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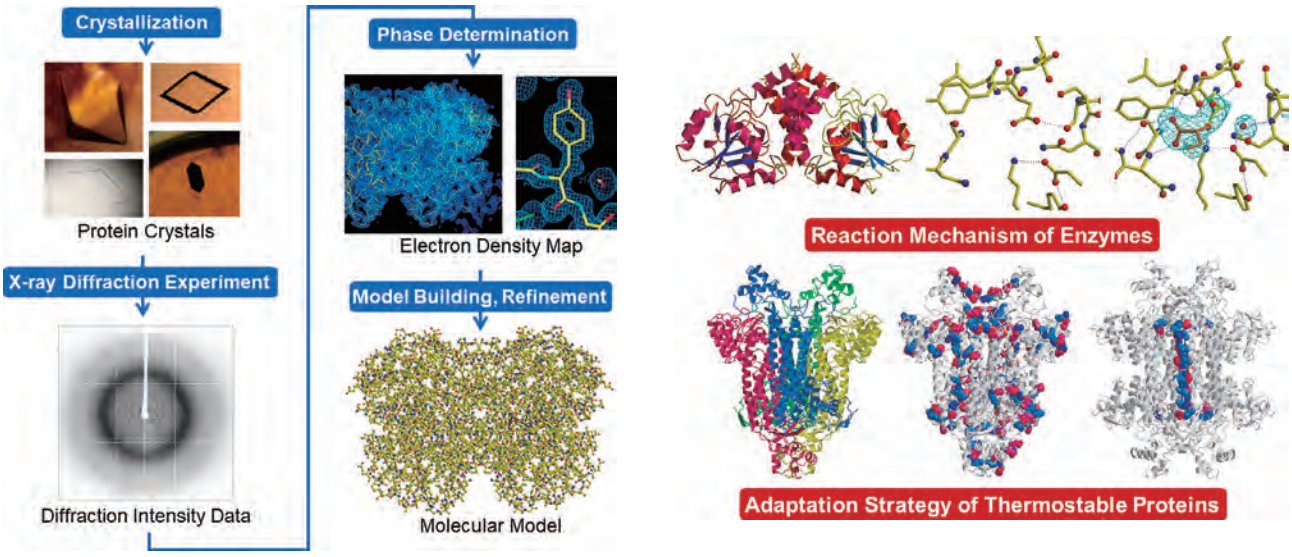
Scope of Research

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).

Crystal Structure Analysis of GraC from *Rhizobium* sp. Strain MTP-10005 in Complex with Coenzyme and Ligand

Rhizobium is a genus of tubercle-forming bacteria. It grows in symbiosis with the root of a plant 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 structures, functions, and properties of the enzymes involved in the metabolic pathways. *Rhizobium* sp. strain MTP-10005 was isolated from natural river water during a screening experiment. Enzymological and genetic studies showed that the translational products of the *graA*, *graB*, *graC*, and *graD* genes (GraA, GraB, GraC, and GraD, respectively) could be potentially involved in the resorcinol degradation pathway. To reveal the structure and function of all these proteins, we have performed X-ray structural studies of the proteins. In this study, we focused on maleylacetate reductase (GraC), which catalyzes the NADH- or NADPH-dependent reduction of maleylacetate to 3-oxoadipate and attempted to determine the structure of GraC-cofactor-ligand complex.

The GraC-NADH complex crystals were prepared using the sitting-drop vapor-diffusion method with a protein solution containing NADH and a reservoir solution containing PEG1500. The crystals were soaked in cryoprotectant solutions containing PEG1500, NADH, and each of several substrate analogs. Each soaked crystal was picked up in a cryoloop and frozen immediately in liquid nitrogen. Diffraction experiments were performed at Photon Factory, KEK, Japan. Diffraction data for each crystal were collected under cryogenic conditions. The electron density maps were calculated using the data collected for each crystal and the phases derived from the previously determined structural model of the GraC-NADH complex. The electron density map of the crystal soaked in the cryoprotectant solution containing 10 mM adipic acid dipotassium salt showed a blob of density at the active site. An adipate molecule was modeled in the blob, and the structure was refined at 1.9 Å resolution.

In the present crystal, one homodimeric GraC molecule exists in the *P*1 unit cell. Each subunit binds one NADH molecule, while only one subunit binds one adipate molecule (Figure 1). The subunit of GraC molecule consists of two domains: an N-terminal domain with an α/β structure formed by residues 1–159 and a C-terminal α-helical domain formed by residues 160–351. The adipate molecule is located in the vicinity of NADH bound to the active site cleft between the domains of the subunit (Figure 2). The superposition of subunit Cα atoms between GraC-NADH-adipate and GraC-NADH complexes results in the root mean square deviations of 0.49 Å. No significant conformational changes are observed in the protein backbone upon the binding of adipate to the GraC-NADH complex. In the active site, one of the carboxyl groups of the adipate molecule forms hydrogen bonds with the side-chains of Asn170, His243, His253, and His257. These residues might be involved in substrate binding or catalysis (Figure 3).

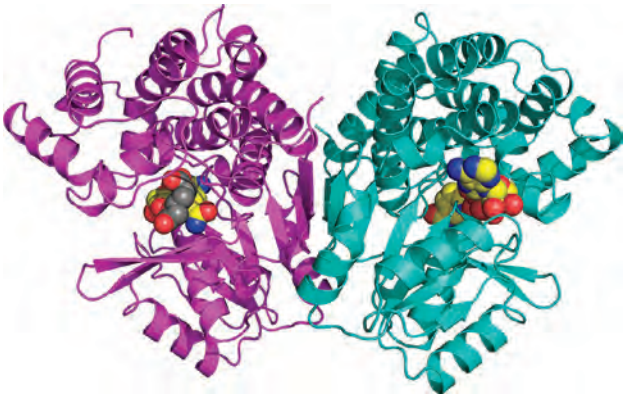


Figure 1. Dimeric molecular structure of GraC-coenzyme-ligand complex.

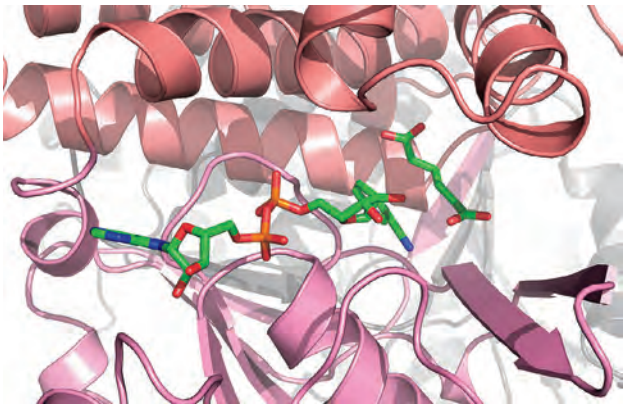


Figure 2. The active-site cleft of GraC-coenzyme-ligand complex.

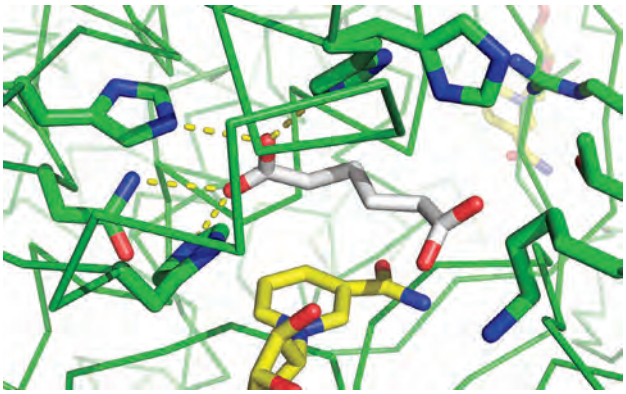


Figure 3. The active-site structure of GraC-coenzyme-ligand complex.