Advanced Research Center for Beam Science
– Structural Molecular Biology –

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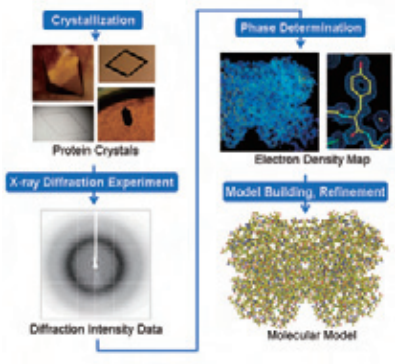
Scope of Research

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.

KEYWORDS

Crystal
X-ray Crystallographic Analysis
Maleylacetate Reductase
Resorcinol Catabolism
Enzymatic Reaction



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).

Open and Closed Subunit Structures of *Rhizobium* Maleylacetate Reductase

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. Therefore, much attention has been paid to the *Rhizobium* genes and gene products for their role in the regulation of symbiosis. Despite extensive studies on *Rhizobium*, 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 as a microorganism with a high level of γ-resorcylate (2,6-hydroxybenzoate) decarboxylase (EC 4.1.1.x) activity. During the cloning of the *graF* gene encoding the enzyme γ-resorcylate decarboxylase, the genes *graA*, *graB*, *graC*, and *graD* were found immediately upstream and downstream of *graF*. Enzymological studies showed that *graD*, *graA*, *graB*, and *graC* encode the reductase (GraD) and oxidase (GraA) components of resorcinol hydroxylase (EC 1.14.13.x), hydroxyquinol 1,2-dioxygenase (GraB) (EC 1.13.11.37), and maleylacetate reductase (GraC) (EC 1.3.1.32), respectively. In order to reveal their structures and functions, we have been performing X-ray structural studies of the enzymes.

Maleylacetate reductase (GraC) from *Rhizobium* sp. strain MTP-10005 catalyzes NADH- or NADPH-dependent reduction of maleylacetate to 3-oxoadipate. The polypeptide chain of the enzyme consists of 351 amino acid residues. The crystal was prepared by the sitting-drop vapour-diffusion method complemented with a microseeding technique. Good crystals were obtained at 293 K in 3days with protein solution at 8 mg/ml (in 50 mM Tris-HCl buffer, pH 8.0) and reservoir solution consisting of 1.4 M ammonium sulfate, 0.1 M sodium chloride, 2% (w/v) benzamidine HCl, and 0.1 M NaHEPES, pH 7.5. Diffraction data of the native crystal were collected at beamline BL6A, Photon Factory, Tsukuba, Japan with an X-ray wavelength of 1.000 Å at 100 K. The phase problem was solved with the multiwavelength anomalous diffraction method (MAD method) using the Hg-derivative crystal prepared by soaking the native crystal in the reservoir solution containing 0.025 mM ethylmercury thiosalicylate for 20 hours. The MAD data sets were collected at 3 Å resolution using X-rays at four wavelength-positions including the Hg-absorption edge. An initial electron density map was obtained at 3 Å resolution using MAD phases and interpreted with the help of the structure of lactaldehyde reductase (PDB ID=1RRM) which is homologous in sequence to GraC. The structure model was built by repeating the cycle of structure refinement, electron density calculation, and structure model improvement. The structure was refined at 1.96 Å resolution up to *R*= 0.165 and *R*free=0.212. The final structure model contains 696 of 702 amino acid residues corresponding to two polypeptide chains of GraC, 4 sulfate anions, 1 glycerol molecule, 1 benzamidine molecule and 381 water molecules.

GraC is dimeric in the crystal. Its subunit consists of two domains: the N-terminal NAD-binding domain (residues 1–159) adopting an α/β structure and the C-terminal α-helical domain (residues 160–351). The active site is located in the cleft between the domains of the subunit. The two subunits (Subunit-A & Subunit-B) have a little bit different structures from each other in the present crystal. The difference is clear by superposing two subunits based on not only whole subunit but also each domain (Figure 1). Superposition of the two subunits based on the corresponding Cα atoms for whole subunits, N-terminal domain region, and C-terminal domain region show the values of root means square deviations of 1.93 Å, 0.79 Å, and 0.52 Å, respectively. Subunit-A binds 2 sulfate anions, 1 benzamidine molecule and 1 glycerol molecule in the cleft. It has a closed conformation that may be adopted on binding the substrate with the cofactor. Subunit-B binds no ligand except 1 sulfate anion. It has an open conformation as is the case before the enzymatic reaction. Thus, the present crystal structure of GraC reveals the structures of maleylacetate reductase both in the substrate-binding state and in the ligand-free state. This suggests that the structure of GraC must change from the open conformation to the closed conformation in the course of enzymatic reaction. The active site structure of GraC shows several histidines residues in the cleft, some of which interact with binding sulfate anion (Figure 2). This suggests that these histidine residues may be involved in the enzymatic reaction or ligand binding.

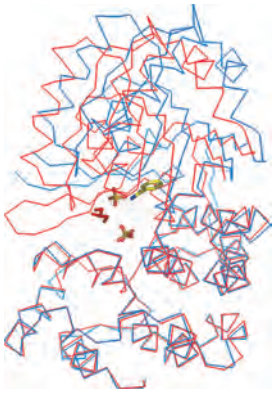


Figure 1. Superposition of GraC subunits. Subunit-A and Subunit-B are shown in red and blue, respectively.

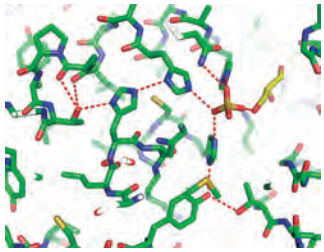


Figure 2. Active site structure of GraC. The dash lines represent hydrogen-bonds.