Advanced Research Center for Beam Science
- Structural Molecular Biology -

http://www.scl.kyoto-u.ac.jp/~hata/indexE.html

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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.

Research Activities (Year 2009)

Publications

Yamauchi T, Goto M, Wu H-Y, Uo T, Yoshimura T, Mihara H, Kurihara T, Miyahara I, Hirotsu K, Esaki N: Serine Racemase with Catalytically Active Lysinoalanyl Residue, *J. Biochem.*, **145**, 421-424 (2009).

Goto M, Yamauchi T, Kamiya N, Miyahara I, Yoshimura T, Mihara H, Kurihara T, Hirotsu K, Esaki N: Crystal Structure of a Homolog of Mammalian Serine Racemase from *Schizosaccharomyces pombe*, *J. Biol. Chem.*, **284**, 25944- 25952 (2009).

Presentations

Structure of Maleylacetate Reductase from *Rhizobium* sp. strain MTP-10005, Hata Y, Fujii T, Yoshida M, Oikawa T, AsCA'09 Beijing Conference of the Asian Crystallographic Association, 23 October 2009.

Psychrophilic Tetrameric Malate Dehydrogenase Has No Intersubunit Ion-pairs, Hata Y, Fujii T, Oikawa T, Soda K, 6th Asian Biophysics Symposium, 11–12 January 2009.

Crystal Structure of GraC Involved in Resorcinol Catabolism of *Rhizobium*

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 much attention has been paid to the *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 studies showed that the *graD*, *graA*, *graB*, and *graC* genes of the bacterium encode the reductase (GraD) and oxidase (GraA) components of resorcinol hydroxylase, hydroxyquinol 1,2-dioxygenase (GraB), and maleylacetate reductase (GraC), 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 amino acid sequence is deduced from the gene sequence.

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 by vapour-equilibrating drops of 1 μl protein solution at 8 mg ml^{-1} (in 50 mM Tris-HCl buffer, pH 8.0) and 1 μl reservoir solution against 500 μl 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 data set was collected at 1.96 Å resolution and has 44,689 independent reflections with completeness of 99.5%. 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 (EMTS) 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. Each of four data sets has about 12,800 independent reflections with completeness of over 99.5%. 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 (Sub A & Sub B) have a little bit different structures from each other in the present crystal. Sub A consists of 350 residues (residues 1–350), and 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. Sub B consists of 346 residues (residues 2–132, 134–324 and 327–350), and 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.

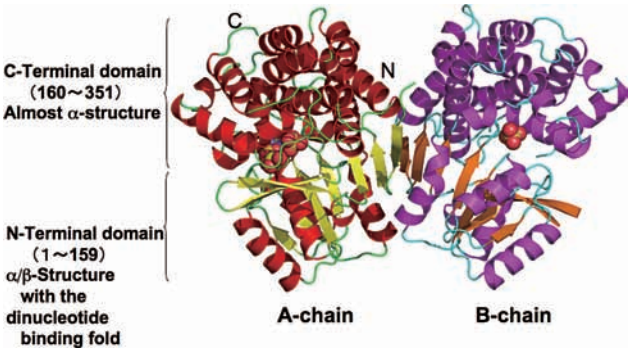

Figure 1. Structure of maleylacetate reductase (GraC) from *Rhizobium* sp. strain MTP-10005. GraC is a dimeric molecule composed of two identical subunits associating across each other.