Advanced Research Center for Beam Science – Structural Molecular Biology –

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

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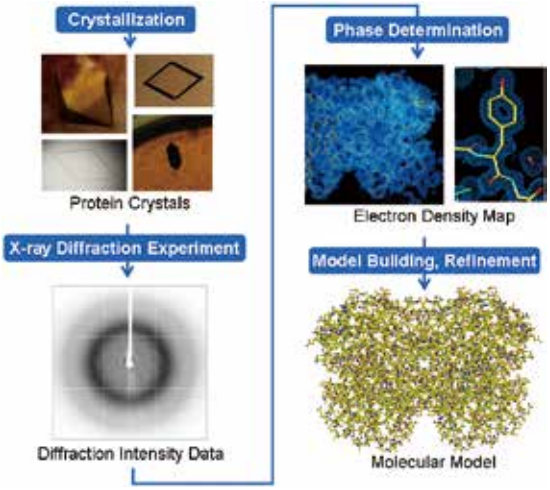
Scope of Research

KEYWORDS
Crystal
X-ray Crystallographic Analysis
Structural Biology
Protein Crystallography
Structure and Function

The research activities in this laboratory are on X-ray structural analyses of biological macromolecules and the investigation of the electronic state in materials as follows: 1) the main subjects of the biomacromolecular crystallography are crystallographic studies on the reaction mechanism of enzymes, 2) the relationship between the multiform conformation and the functional variety of proteins, and 3) the mechanisms underlying the 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 spectrometer with 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 a new type of X-ray spectrometer with ultra high resolution have also been carried out.



Selected Publications

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).

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).

Crystallographic Structure Analysis of Aspartate Racemase from *Thermococcus litoralis* DSM 5473

Amino-acid racemases are responsible for the racemization of amino acids and can be mainly grouped into two families, pyridoxal 5'-phosphate (PLP) dependent and PLP independent. Aspartate racemase catalyzes the interconversion between L- and D-aspartate and belongs to the PLP-independent racemase group. The enzyme is thought to employ a two-base mechanism to catalyze both the directions of racemization and utilize two cysteine residues as the conjugated catalytic acid and base in the catalytic reaction. To elucidate the structure-function relationship and the thermostability of aspartate racemase, we have determined the crystal structure of aspartate racemase from *Thermococcus litoralis* DSM 5473 (TlAspR). TlAspR has maximum activity at 95 °C.

Initial crystallization experiments were performed by the sitting-drop vapour-diffusion method using Crystal Screen, Crystal Screen 2, PEGRx 1, PEGRx 2 (Hampton Research), Wizard I, and Wizard II (Emerald BioSystems). Crystals were obtained after several days with the solution PEGRx 2 #17. The crystallization conditions were optimized based on those of the solution. The final conditions produced plate-shaped crystals with approximate dimensions of 0.15 × 0.06 × 0.03 mm at 20 °C in 1 week using the sitting-drop vapour-diffusion method with seeding technique (Figure 1). Drops of 1 µl protein solution at 12 mg/ml (in 20 mM Tris-HCl buffer pH 8.0, 0.01%(v/v) β-mercaptoethanol) and 1 µl reservoir solution were equilibrated against 500 µl reservoir solution consisting of 24% (w/v) PEG1500, 0.2 M L-proline, and 0.1 M HEPES pH 7.5.

Figure 1. Crystal of aspartate racemase from *Thermococcus litoralis* DSM 5473.

Diffraction experiments were performed at beamline BL-5A, Photon Factory (Tsukuba, Japan). The crystal was flash-cooled in a nitrogen stream at 100 K. Diffraction data were collected at a wavelength of 1.000 Å using a Quantum 315r CCD detector set to 142.3 mm in a crystal-to-detector distance. The crystals belonged to space group $P2_12_11$ with unit cell parameters of a = 90.26, b = 125.78, and c = 40.64 Å. The data set was collected at 1.6 Å resolution and has 65,774 independent reflections with 98.3% completeness. The asymmetric unit contained one dimeric molecule of TlAspR with a corresponding crystal volume per protein mass (V_M) of 2.32 Å^3/Da and a solvent content of 47%. The crystal structure has been determined by molecular replacement. The current model was refined at 1.6 Å resolution to an R-factor of 19.3% (R_{free} = 24.0%).

In crystals, TlAspR adopts a homodimeric form. The subunit consists of two domains: the N-terminal domain (residues 1–101 and 213–228) and the C-terminal domain (residues 102–212). In each domain, a central four-stranded parallel β-sheet is flanked by six α-helices. The spatial arrangement of the strictly conserved residues Cys83 and Cys194 strongly indicates that the active site of TlAspR must be located in the cleft between the two domains.

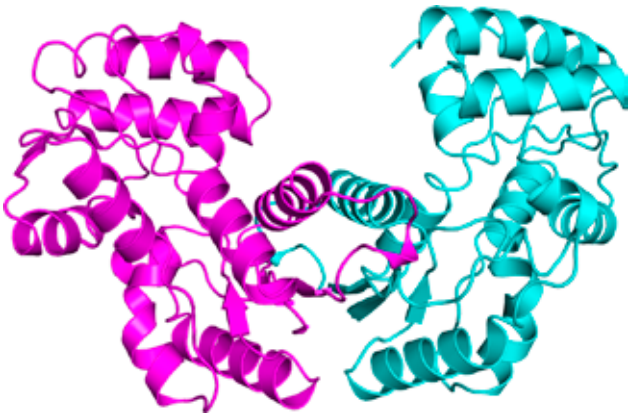


Figure 2. Dimeric molecular structure of aspartate racemase from *Thermococcus litoralis* DSM 5473.

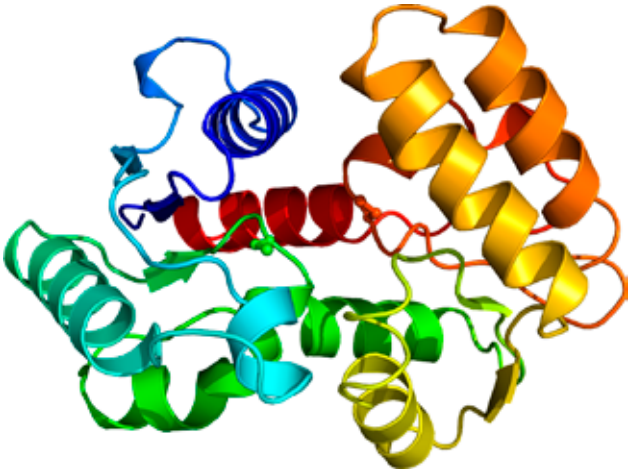


Figure 3. Subunit structure of aspartate racemase from *Thermococcus litoralis* DSM 5473. The strictly conserved residues Cys83 and Cys194 are shown as ball-and-stick models.