

Advanced Research Center for Beam Science - Structural Molecular Biology -

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Prof McLEOD, Cameron University of Sheffield, UK, 26–27 November 2007

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 spectrometer with a high-resolution in order to elucidate the property of materials. The theoretical analysis of the electronic states with DV-X α and WIEN2k, and the development of new typed X-ray spectrometer with ultra high-resolution have also been carried out.

Research Activities (Year 2007)

Publication

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 Cryst.*, **F63**, 983-986 (2007).

Structural Features of Psychrophilic Malate Dehydrogenase Adapting to the Extreme Environment, Hata Y, Fujii T, Oikawa T, Soda K, The 8th Conference of Asian Crystallographic Association (AsCA'07 Taipei), 6 November 2007, Taipei, Taiwan.

Presentations

Cold-adapting Structure of Psychrophilic MDH, Hata Y, Fujii T, Oikawa T, Muraoka I, Soda K, 2007 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 27 March 2007, Tokyo, Japan.

Grant

Ito Y, Development of Basic Technologies for New Functional Particle Materials, Kyoto Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST, 1 December 2004–31 December 2008.

X-Ray Crystallographic Analysis of Tetrameric Malate Dehydrogenase from Novel Antarctic Psychrophile

Various psychrophilic microorganisms, which grow at low temperatures unsuitable for most of other organisms, produce various psychrophilic and thermolabile enzymes in order to survive and grow effectively under such extreme environments. These enzymes have generated considerable interest, since they can be used to improve the efficiency of industrial processes and for environmental applications. In order to understand the structural basis of cold adaptation of psychrophilic enzymes, we have initiated X-ray structural studies on tetrameric malate dehydrogenase (MDH) from a novel psychrophilic bacterium *Flavobacterium frigidimaris* KUC-1, which was isolated from Antarctic seawater, as a model of structurally unknown psychrophilic enzyme. Malate dehydrogenase catalyzes the reversible oxidation of malate to oxaloacetate in the presence of NAD^+ . The overall molecular weight of the present MDH is about 130 kDa as a tetramer and the subunit mass about 33 kDa. The subunit consists of 311 amino acid residues. The crystal structure of the tetrameric MDH is probably different in intersubunit organization and network of interactions from already determined crystal structure of dimeric MDH from a psychrophile *Aquaspirillum arcticum*. The structure determination of the present MDH and intensive comparisons tetrameric MDH structures of microorganisms living in different environments are expected to provide more detailed structural information on the mechanism of cold adaptation.

Crystallization was performed using the hanging-drop vapour-diffusion method. The protein concentration was adjusted to 17 mg ml^{-1} in 10 mM potassium phosphate buffer pH 7.0. Each of the drops was prepared by mixing $1 \text{ }\mu\text{l}$ protein and $1 \text{ }\mu\text{l}$ reservoir solutions. After extensive investigation of conditions, rod-shaped crystals of the enzyme with maximum dimensions of $1.1 \times 0.15 \times 0.05 \text{ mm}$ were obtained at 288 K within 2–3 days by equilibrating the $2 \text{ }\mu\text{l}$ protein drop against $500 \text{ }\mu\text{l}$ reservoir solution (1.4 M $(\text{NH}_4)_2\text{SO}_4$, 5% (v/v) MPD, 2 mM NAD^+ , 50 mM sodium citrate buffer pH 5.5) (Figure 1). The crystal belonged to trigonal space group $P3_221$ with unit cell dimensions of $a=b=147.8$ and $c=165.1 \text{ }\text{\AA}$. It contained one tetrameric molecule per asymmetric unit and a solvent content of 69.1% .

X-ray diffraction data collection was performed at the beamline BL-5A of Photon Factory, Tsukuba, Japan. A crystal with size of $0.35 \times 0.15 \times 0.05 \text{ mm}$ was soaked in



Figure 1. Crystals of psychrophilic MDH from *F. frigidimaris* KUC-1. The dimensions of the largest crystal were $1.0 \times 0.15 \times 0.05 \text{ mm}$.

the reservoir solution containing 30% (v/v) glycerol for several ten seconds, then mounted in a nylon loop and flash-cooled in a nitrogen stream at 100 K . Diffraction data up to $1.8 \text{ }\text{\AA}$ resolution were collected at a wavelength of $1.000 \text{ }\text{\AA}$ using a Quantum 315 CCD detector (ADSC) set to the crystal-to-detector distance of 249.2 mm . The oscillation steps was 1.0° over a range of 180° . The exposure time was 1.5 seconds per frame. All diffraction images were processed with the program *MOSFLM* in the *CCP4* program suit. $2,099,880$ observed reflections were merged to $192,407$ independent reflections (completeness of 100%) with redundancy of 10.9 . The R_{sym} value was 8.0% .

The structure determination of the enzyme was carried out by molecular replacement (MR) using the program *MOLREP* in *CCP4*. The structure of a hybrid consisting of a thermophilic and a mesophilic MDHs was used as a search model. MR calculations gave one solution for search model of the tetramer and four solutions for that of the subunit. These results coincide with the presence of one tetrameric MDH per asymmetric unit. The current structure of *F. frigidimaris* MDH is shown in Figure 2.

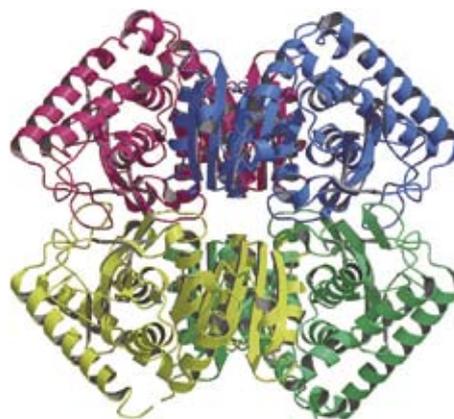


Figure 2. Ribbon drawing of tetrameric MDH.