Laboratory for Biological Structural Chemistry: Structure-Function Relationship of Enzymes and Protein Crystallography

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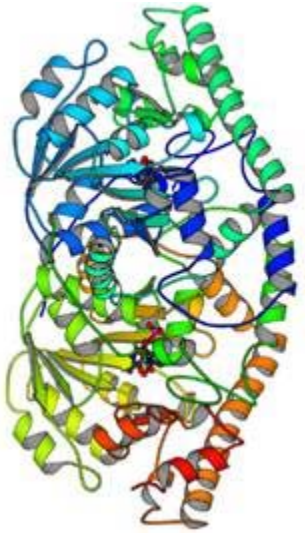
http://www.sci.osaka-cu.ac.jp/chem/chem.html

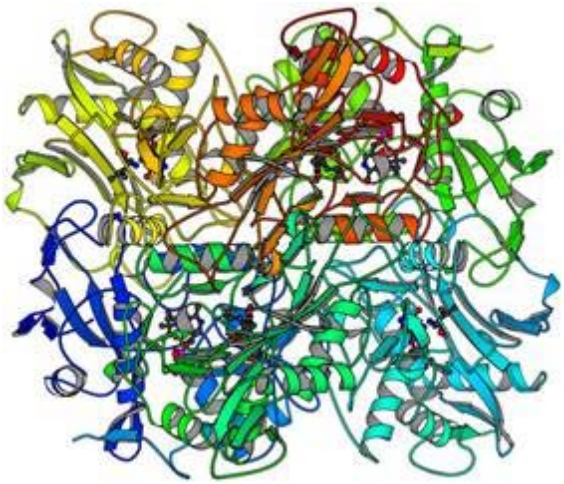
1. Current Research and Principal Research Interests

We are engaged in structure analysis of proteins by X-ray crystallography. The overall aim of our research is to learn the relationship of the three-dimensional structure of protein to its biochemical function and the basic concept of the molecular design. One area of interest is the mechanism of the induced fit and substrate recognition of proteins. A second is to understand the electron and proton transfer among protein, cofactor and substrate through the catalytic process. A third area of interest is the structural and evolutionary relationship between and within families of proteins. The tools used are purification, crystallization, X-ray analysis, computer graphics and molecular biology (especially protein expression and site-directed mutagenesis). A strong X-ray source given by a third generation synchrotron facility (SPring-8) and a focused X-ray from in- house equipment with a two-dimensional detector have particularly raised the level of macromolecular crystallography. Now we can determine not only the precise structure of a protein, but also the structure of its reaction intermediate. A series of proteins related to one another such as a family of vitamin B6 dependent enzymes and all proteins coded on a structural gene can be subjected to X-ray crystallographic studies.

For a long time, we had been working on small molecule crystallography. We then began macromolecular crystallography as part of our research activity, and about three years ago shifted almost completely to this area.

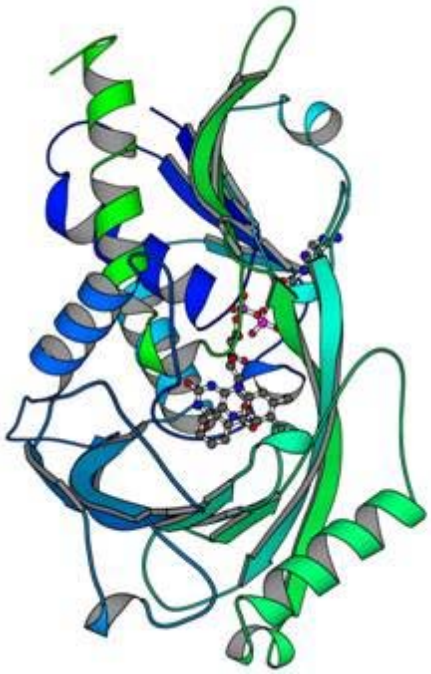


aspartate aminotransferase



branched-chain amino acid aminotransferase

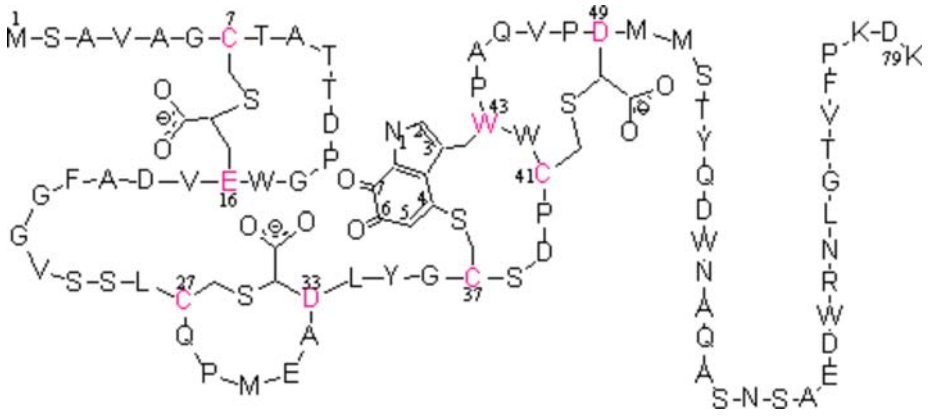
For many years we have worked on the structure-function relationship in a series of pyridoxal 5'-phosphate (vitamin B6) dependent enzymes. Vitamin B6 enzymes play important roles in the amino acid metabolism, and catalyze a wide range of amino acid transformation (transamination, decarboxylation, deamination, racemization and aldol cleavages) by labilizing one of three bonds at C carbon atom of an amino acid substrate. Importantly, one enzyme usually catalyzes one reaction with a substrate specificity. The vitamin B6 dependent enzymes are classified into fold type I-IV, indicating that the enzymes evolved from four common ancestors, and the versatility of enzymes has been attained by divergent and convergent molecular evolution. The aim of this work is to uncover the underlying mechanism in the catalytic events of vitamin B6 enzymes, and to understand the action of B6 enzymes in general. The structures of many aminotransferases such as aspartate, branched-chain, histidinol phosphate, and aromatic amino acid aminotranferases have been determined to provide some insight into the substrate recognition, the role of the active site residues, the reaction mechanism, and the evolutionary relationship of the vitamin B6 dependent enzymes. In addition to transaminase, we are studying a number of vitamin B6 enzymes such as decarboxylase, deaminase, lyase and racemase.



D-amino acid oxidase

We are also interested in the catalytic action of flavin (vitamin B2) dependent enzymes. D-Amino acid oxidase catalyzes the oxidative deamination of D-amino acids. (DAO) Although DAO has been one of the most extensively investigated flavoenzymes, the catalytic mechanism of DAO was a matter yet to be settled. X-ray structure of DAO revealed that there was no presumed protein base to abstract the -proton of a substrate amino acid, allowing us to propose new catalytic mechanisms on the active-site model of enzyme-substrate complex. Two possible mechanisms for the reductive-half reaction are the electron-proton-electron transfer mechanism and the ionic mechanism. The structure of the purple intermediate of DAO, which is a complex between the dehydrogenated product and the reduced DAO, was solved by cryo-X-ray crystallography. It was shown that the oxidative half-reaction of DAO is optimized through the alignment of the product with respect to reduced flavin. The X-ray studies on another flavoenzymes are ongoing.

A new and exciting project involves a quinoprotein that has a presumed quinone cofactor, and two hemes as redox active group. The X-ray structure of quinohemo-



Novel cofactor (cysteine tryptophylquinone) and hitherto unknown cross-links

Figure 3 Two-photon reaction via hot benzene produce 1,3-hexadiene-5-yne as a dominant photoproduct. The corresponding vibrational temperature of benzene with two-photon energy of ArF laser light is evaluated to be as high as 5900 K.

2. Selected Publications

1. “Crystal Structure of Argininosuccinate Synthetase from *Thermus thermophilus* HB8: Structural Basis for the Catalytic Action”, M. Goto, Y. Nakajima, and K. Hirotsu, *J. Biol. Chem.*, **277**, 15890-15896 (2002).

2. “Three-dimensional Structure of the Flavoenzyme Acyl-CoA Oxidase-II from Rat Liver, the Peroxisomal Counterpart of Mitochondrial Acyl-CoA Dehydrogenase”, Y. Nakajima, I. Miyahara, K. Hirotsu, Y. Nishina, K. Shiga, C. Setoyama, H. Tamaoki, and R. Miura, *J. Biochem.*, **131**, 365-374 (2002).

3. “Effects of hydrogen bonds in association with flavin and substrate in flavoenzyme D-amino acid oxidase. The catalytic and structural roles of Gly313 and Thr317”, C. Setoyama, Y. Nishina, H. Tamaoki, H. Mizutani, I. Miyahara, K. Hirotsu, K. Shiga, and R. Miura, *J. Biochem.*, **131**, 59-69 (2002).

4. “Crystal Structure of Quinohemoprotein Amine Dehydrogenase from *Pseudomonas putida*, Identification of a novel quinone cofactor encaged by multiple thio-ether cross-bridges”, A. Satoh, J-K. Kim, I. Miyahara, B. Devreese, I. Vandenberghe, A. Hacisalihoglu, T. Okajima, S. Kuroda, O. Adachi, J.A. Duine, J. Beeumen, K. Tanizawa, and K. Hirotsu, *J. Biol. Chem.*, **277(4)**, 2830-2834 (2002).

5. “Substrate recognition mechanism of thermophilic dual-substrate enzyme”, H. Ura , T. Nakai, Si. Kawaguchi , I. Miyahara, K. Hirotsu, and S. Kuramitsu. *J. Biochem.*, **130**, 89-98 (2001).

6. “Structures of *Escherichia coli* Branched-Chain Amino Acid Aminotransferase and Its Complexes with 4-Methylvalerate and 2-Methylleucine: Induced Fit and Substrate Recognition of the Enzyme”, K. Okada, K. Hirotsu, H. Hayashi, and H. Kagamiyama, *Biochemistry*, **40**, 7453-7463 (2001).

7. “Porcine kidney D-amino acid oxidase: The three-dimensional structure and its catalytic mechanism based on the enzyme-substrate complex model”, R. Miura, C. Setoyama, Y. Nishina, K. Shiga, I. Miyahara, H. Mizutani, and K. Hirotsu, *J. Mol. Catal. B, Enzym.*, **12**, 43-52 (2001).

8. “Structures of *Escherichia coli* Histidinol-Phosphate Aminotransferase and Its Complexes with Histidinol-Phosphate and N-(5’-Phosphopyridoxyl)-L-Glutamate: Double Substrate Recognition of the Enzyme”, K. Haruyama, T. Nakai, I. Miyahara, K. Hirotsu, H. Mizuguchi, H. Hayashi, and H. Kagamiyama, *Biochemistry*, **40**, 4633-4644 (2001).

9. “Three-Dimensional Structure of the Purple Intermediate of Porcine Kidney D-Amino Acid Oxidase. Optimization of the Oxidative Half-Reaction through Alignment of the Product with Reduced Flavin”, H. Mizutani, I. Miyahara, K. Hirotsu, Y. Nishina, K. Shiga, C. Setoyama, and R. Miura, *J. Biochem.*, **128**, 73-81 (2000).

10. “Three-Dimensional Structure of 4-Amino-4-deoxychorismate Lyase from *Escherichia coli*”, T. Nakai, H. Mizutani, I. Miyahara, K. Hirotsu, S. Takeda, K.-H. Jhee, T. Yoshimura, and N. Esaki, *J. Biochem.*, **128**, 29-38 (2000).

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