Bioinorganic Chemistry of Metalloproteins Responsible for Metal Homeostasis and Signal Sensing

Transition metal ions and metalloproteins play crucial roles in meeting the energy demands of the cell by playing roles in intermediary metabolism and in signal transduction processes. Although they are essential for biological function, metal ion bioavailability must be maintained within a certain range in cells due to the inherent toxicity of all metals above a threshold. This threshold varies for individual metal ions. Homeostasis of metal ions requires a balance between the processes of uptake, utilization, storage, and efflux and is achieved by the coordinated activities of a variety of proteins including extractoplasmic metal carriers, ion channels/pumps/transporters, metal-regulated transcription and translation proteins, and enzymes involved in the biogenesis of metal-containing cofactors/metalloproteins. In order to understand the processes underlying this complex metal homeostasis network, the study of the molecular processes that determine the protein-metal ion recognition, as well as how this event is transduced into a functional output, is required. My research interests are focused on the elucidation of the structure and function relationships of metalloproteins responsible for the regulation of biological homeostasis.

I am also working on gas sensor proteins. Gas molecules such as O₂, NO, CO and ethylene are present in the environment and are endogenously (enzymatically) produced to act as signaling molecules in biological systems. Sensing these gas molecules is the first step in their acting as signaling molecules. Sensor proteins are usually required. Input signals generated by gas sensing have to transduce to output signals that regulate biological functions. This is achieved by biological signal-transduction systems. Recognition of the cognate gas molecules is a general mechanism of functional regulation for gas sensor proteins. This induces conformational changes in proteins that controls their activities for following signal transductions. Interaction between gas molecules and sensor proteins is essential for recognition of gas molecules. Metal-containing prosthetic groups are widely used. In my research group, our research focuses on transition metal-based gas-sensor proteins and the signaling systems working with them.
1. Structural Characterization of Aldoxime Dehydrogenase OxdB That Catalyzes Dehydration Reaction of Aldoximes to Form Nitriles

Nitrile compounds are important intermediates in some industrial processes to produce nylon and acrylic fibers, insecticides, and pharmaceuticals. Though one of the most useful methods for nitrile production is dehydration of aldoxime, the chemical dehydration of aldoxime used in the industrial process requires harsh conditions. Therefore, a more environmentally benign process of aldoxime dehydration is expected to be established, for which a biological dehydration of aldoxime is a possible candidate. In nature, some microbes have “aldoxime-nitrile pathway,” where aldoximes are metabolized to the corresponding carboxylic acids through nitriles formed by dehydration of aldoximes with aldoxime dehydratase (Oxd; EC4.99.1.-). There are two pathways for the conversion of nitriles to carboxylic acids. One is hydrolysis of nitriles by nitrilase, and the other is the combination of the reactions catalyzed by nitrile hydratase and amidase. Nitriles are the important intermediate not only in some industrial processes but also in this biological system. The detail characterization of such a biological process to produce nitriles will give some useful information to develop an environmentally benign process for the production of nitriles in industrial field.

The crystal structures of OxdRE from Rhodococcus sp. N-771 and OxdA from Pseudomonas chlororaphis have been reported. Based on the biochemical characteristics of Oxds and the crystal structure of its Michaelis complex, a mechanism for the dehydration of aldoxime to the corresponding nitrile has been proposed. The active site of Oxds includes heme b as a cofactor and a catalytic triad, which consists of, for example, OxdA, arginine, histidine, and serine. The Fe$^{2+}$ ion is additionally coordinated to another histidine. When the substrate enters the active site, it becomes N-coordinated to Fe$^{2+}$. The hydroxyl moiety of aldoxime also forms hydrogen bonds with serine and histidine. Oxds share a common architecture to achieve this reaction, but show varying substrate selectivities. In particular, OxdB (Oxd from Bacillus sp. OxB-1) shows different enantioselectivities from those of OxdRE and OxdA when bulky compounds, such as racemic E/Z-2-(3-bromophenyl)-propanal oxime (1) and Z-2-(3-bromo-phenyl)-propanal oxime (1) complex is shown in slate color. 1 is shown in yellow stick model. Anomalous Fourier map (4σ) in active site is shown in blue mesh. Polder map (4σ) of I is shown in magenta mesh. (C) Superposition of substrate-free form and substrate-bound form is shown in green and slate color, respectively. Fo-Fc map (4σ) of substrate-free form is shown in blue mesh. (D) Superposition of OxdB-E85A and OxdRE in complex with butyraldoxime. OxdRE is shown in orange. Butyraldoxime is shown in cyan stick model.

OxdB. The catalytic triad were conserved in the structure of OxdB. Based on the crystal structure of OxdB, the molecular mechanism of the aldoxime dehydration in OxdB is as follows. When the substrate is bound to heme in OxdB, Thr202 forms a hydrogen bond with the hydroxyl group of the substrate. Dehydration of the substrate proceeds as a result of the proton supply by His306. His306 receives a proton from Glu126 or Arg159. The imidazole ring of His282 in OxdB was more perpendicular to heme than that of His299 in OxdRE and OxdA. This fact suggests that His282 in OxdB is highly nucleophilic toward heme iron. The experiments with mutagenesis on axial histidine and exogenous imidazole derivatives in OxdB have shown that the enzyme activity increases under conditions of high nucleophilicity by the axial ligand. In this context, the activity of OxdB is expected to be higher than that of OxdRE and OxdA.

In addition, the crystal structure of the Michaelis complex of OxdB and the diastereomERICally pure substrate Z-2-(3-bromophenyl)-propanal oxime implied the importance of several hydrophobic residues for substrate selectivity. Mutational analysis implicated Ala12 and Ala14 in the E/Z selectivity of bulky compounds. The N-terminal region of OxdB was shown to be shorter than those of OxdA and OxdRE, and have high flexibility. These structural differences possibly result in distinct preferences for aldoxime substrates based on factors such as substrate size.

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