Bioinorganic Chemistry of Novel Hemeproteins

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions

AONO, Shigetoshi	Professor
YOSHIOKA, Shiro	Assistant Professor
ISHIKAWA, Haruto	IMS Research Assistant Professor
SAWAI, Hitomi	JSPS Post-Doctoral Fellow
TANIZAWA, Misako	Secretary

Hemeproteins are a typical metalloprotein, which show a variety of functions including oxygen storage/transport, electron transfer, redox catalysis with various substrates. Besides these traditional functions of hemeproteins, several new functions of hemeproteins have been found recently. Heme-based sensor proteins show a novel function of the heme prosthetic group, in which the heme acts as the active site for sensing the external signal such as diatomic gas molecules and redox change. Aldoxime dehydratase is another novel hemeprotein, in which the heme prosthetic group tethers the substrate for its dehydration reaction. Our research interests are focused on the elucidation of the structure-function relationships of these novel hemeproteins.

1. Structure and Function of Aldoxime Dehydratase Containing a Heme as the Active Site for Dehydration Reaction

Aldoxime dehydratase (Oxd) is a new heme-containing enzyme that works as a hydro-lyase. The enzymatic activity of Oxd is dependent on the oxidation state of the heme iron, though the reaction catalyzed by Oxd is not a redox reaction. Ferrous Oxd containing a Fe^{2+}-heme shows the enzymatic activity, but ferric Oxd containing a Fe^{3+}-heme does not. Previous spectroscopic analyses reveal a novel mechanism, where the change in the coordination mode of the substrate plays a crucial role for the regulation of the enzymatic activity. While the oxygen atom of aldoxime is coordinated to the ferric heme, the nitrogen atom of aldoxime is coordinated to the ferrous heme. The dehydration reaction proceeds only via N-coordinated substrate in the ferrous heme. The organic substrate is directly coordinated to the heme iron in dehydration of aldoxime, which is a unique example among heme enzymes though the coordination of O_2 or H_2O_2 to the heme is well known in the heme-containing oxygenases, catalases, and peroxidases. It is proposed that the dehydration reaction of the heme-bound aldoxime proceeds in a general acid-base catalysis with a histidine working as a catalytic residue in the distal heme pocket. However, the detail reaction mechanisms remained to be elucidated mainly because the structural information of Oxd was lacking.

We have determined the crystal structures of Oxd from *Rhodococcus* sp. N-771 (OxdRE) in the substrate-free and substrate-bound forms. OxdRE formed a homodimer with non-crystallographic two-fold symmetry, consistent with previous gel filtration analyses results. Each monomer contained one heme molecule. The α10 helix of one monomer interacted with the α10 helix of the other to create the dimer interface, which was stabilized by hydrogen bonds and electrostatic interactions.

We could determine the crystal structure of the Michaelis complex of OxdRE by means of the unique property of OxdRE for the substrate binding. As the reaction spontaneously proceeds when mixing ferrous Oxd and the substrate, the crystallization of the substrate-bound OxdRE in the ferrous form (the Michaelis complex of OxdRE) is not possible by usual methods. However, we could prepare the crystal of the Michaelis complex of OxdRE by the reduction of the crystal of the substrate-ferric Oxd complex using X-ray irradiation under cryogenic temperature. The structures of the resting state and the Michaelis complex provide the structural insights into the mechanisms of substrate recognition and the catalysis of OxdRE.

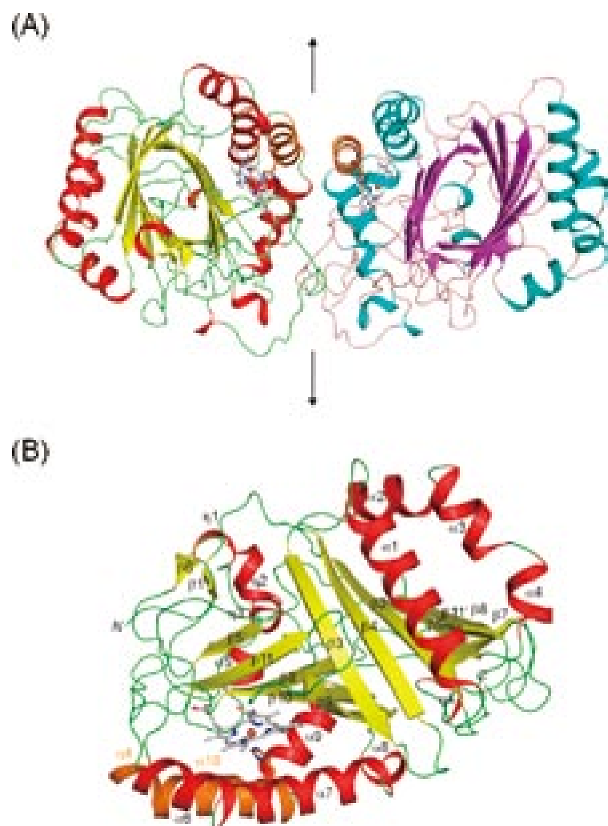

Figure 1. Crystal Structure of OxdRE in substrate-free form. The structures are colored based on the secondary structural elements. The heme is represented by a silver-colored stick model. The heme proximal helix (α10), the subsequent 3_{10} helix (η4), and the axial His299 are colored in orange. (A) Structure of the OxdRE dimer. Two subunits are related by non-crystallographic two-fold symmetry. (B) Closed-up view of the monomer.

2. Regulation of Enzymatic Activity of a Heme-containing Diguanylate Cyclase (HemDGC) by O_2 Binding to the Heme in the Sensor Domain

Bis-(3',5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is a ubiquitous bacterial second messenger involved in the regulation of cell motility, differentiation, development, virulence, and biofilm formation. This second messenger generally regulates transitions between the free-living, motile lifestyle and the sessile life style. Low concentrations of c-di-GMP promote motile growth, while high concentrations promote sessile growth with biofilm formation. The intracellular concentrations of c-di-GMP are controlled by the balance between synthesis and hydrolysis of c-di-GMP, which are catalyzed by the enzymes, diguanylate cyclases (DGCs) and phosophodiesterases (PDEs), respectively.

DGCs contain the GGDEF domain, named from the conserved sequence motif (Gly-Gly-Asp-Glu-Phe) that constitutes part of the active site of the enzymes. The GGDEF domain is typically found coupled to a variety of other sensor and/or regulator domains within multidomain proteins.

We have studied the structural and enzymatic properties of a diguanylate cyclase from an obligatory anaerobic bacterium *Desulfotalea psychrophila*, which consists of the N-terminal sensor domain and the C-terminal diguanylate cyclase domain. The sensor domain shows an amino acid sequence homology and spectroscopic properties similar to those of the sensor domains of the globin-coupled sensor proteins containing a protoheme. This heme-containing diguanylate cyclase catalyzes the formation of cyclic di-GMP from GTP only when the heme in the sensor domain binds molecular oxygen. When the heme is in the ferric, deoxy, CO-bound, and NO-bound forms, no enzymatic activity is observed. Resonance Raman spectroscopy reveals that Tyr55 forms a hydrogen bond with the heme-bound O_2, but not with CO. Instead, Gln81 interacts with the heme-bound CO. These differences of hydrogen bonding network will play a crucial role for the selective O_2 sensing responsible for the regulation of the enzymatic activity.

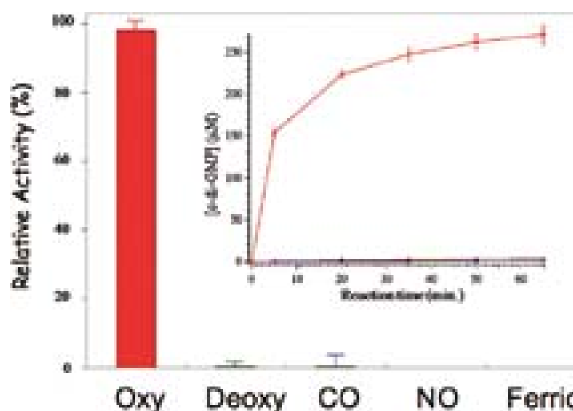

Figure 2. The enzymatic activity of HemDGC is regulated by the coordination state of the heme in its sensor domain. Only when O_2 is bound to the heme, HemDGC shows the activity for the formation of cyclic di-GMP from GTP. Inset: the time-course of the formation of cyclic di-GMP.