Bioinorganic Chemistry of Metalloproteins Responsible for the Homeostasis Control

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Keywords Bioinorganic Chemistry, Metalloproteins, Sensor Protein

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 extracytoplasmic 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.

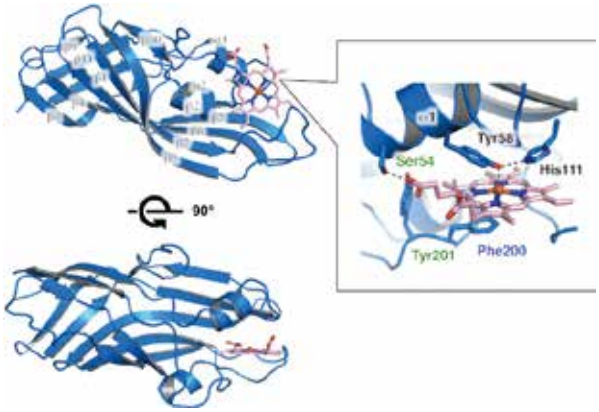

Figure 1. The crystal structure of CgHtaA-N and the close-up view of heme binding site in CgHtaA-N.

Selected Publications

- N. Muraki, C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Structural Characterization of Heme Environmental Mutants of CgHmuT that Shuttles Heme Molecules to Heme Transporters," *Int. J. Mol. Sci.* **17**, 829 (2016).
- N. Muraki and S. Aono, "Structural Basis for Heme Recognition by HmuT Responsible for Heme Transport to the Heme Transporter in *Corynebacterium glutamicum*," *Chem. Lett.* **45**, 24–26 (2015).
- C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Molecular Mechanism for Heme-Mediated Inhibition of 5-Aminolevulinic Acid Synthase 1," *Bull. Chem. Soc. Jpn.* **87**, 997–1004 (2014).
- Y. Okamoto, H. Sawai, M. Ogura, T. Uchida, K. Ishimori, T. Hayashi and S. Aono, "Heme-Binding Properties of HupD Functioning as a Substrate-Binding Protein in a Heme-Uptake ABC-Transporter System in *Listeria monocytogenes*," *Bull. Chem. Soc. Jpn.* **87**, 1140–1146 (2014).
- S. Aono, "The Dos Family of Globin-Related Sensors Using PAS Domains to Accommodate Haem Acting as the Active Site for Sensing External Signals," *Adv. Microb. Physiol.* **63**, 273–327 (2013).
- H. Sawai, M. Yamanaka, H. Sugimoto, Y. Shiro and S. Aono, "Structural Basis for the Transcriptional Regulation of Heme Homeostasis in *Lactococcus lactis*," *J. Biol. Chem.* **287**, 30755–30768 (2012).

1. Structure and Function of Heme Uptake Machinery in *Corynebacterium glutamicum*

As iron is an essential trace element for most of organisms, they develop sophisticated iron acquisition systems. Pathogenic bacteria can use heme as an iron source partly because heme is the most abundant iron species in their host. However, there is little free heme molecule as most of heme molecules are tightly bound to hemoproteins as a prosthetic group. Therefore, some heme acquisition system is required to use heme in hemoproteins as an iron source.

In Gram-negative bacteria, hemophores that are secreted to the extracellular medium acquire heme from hemoproteins and transport it to a specific outer membrane receptor. The outer membrane receptor transports heme across the outer membrane to the periplasmic space, where a periplasmic heme-binding protein binds heme to transport it to an ABC-type heme transporter. On the other hand, in Gram-positive bacteria, heme uptake occurs by direct interaction between hemoproteins or heme and the membrane anchored proteins responsible for heme binding and transport. In a Gram-positive bacterium *Corynebacterium glutamicum*, heme is captured by the membrane anchored heme binding proteins, HtaA and HtaB proteins, and then heme is transferred to HmuT, which is a heme-binding protein for the ABC-type heme transporter HmuUV. Heme is transported into cytoplasm by this ABC transporter. While this heme uptake process is proposed based on the genetic and microbiological studies, the molecular mechanisms of heme uptake/transport are not obvious mainly due to a lack of structural information of these proteins. We have characterized HtaA, HtaB, and HmuT from *Corynebacterium glutamicum* (CgHmuT) by X-ray crystallography to elucidate the molecular mechanism of heme transport and uptake.

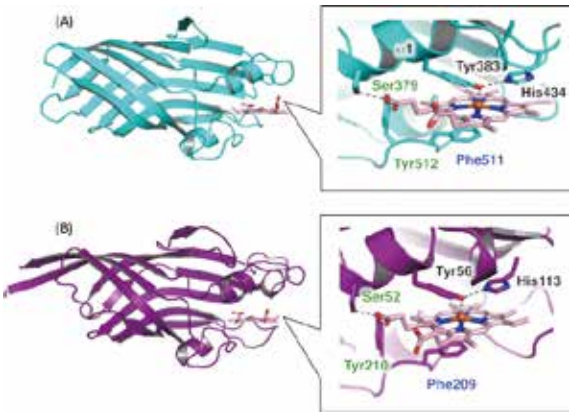

Figure 2. The crystal structures of (A) CgHtaA-C and (B) CgHtaB with the close-up view of their heme binding sites.

HtaA consists of two homologous domains, HtaA-N and HtaA-C. We have determined the structure of CgHtaA-N at a resolution of 2.0 Å (Figure 1). HtaA-N consists of 11 β-strands and 2 short α-helices and binds a heme molecule with Tyr58 as an axial ligand in a hydrophobic pocket. Tyr58 forms a hydrogen bond with His111, which may regulate the heme binding affinity of HtaA-N. Phe200 forms π-π stacking with heme and heme propionate forms a hydrogen bond with Ser54.

The crystal structures of CgHtaA-C and CgHtaB have also been determined as shown in Figure 2. The whole structures of CgHtaA-N, CgHtaA-C, and CgHtaB are superimposable and the heme environmental structures are highly conserved among them including the axial ligand, hydrogen bonding interaction between Tyr and His, and heme propionate and Ser, and π-π stacking of heme and Phe.

2. A Novel Photosensor Protein CarH Using Vitamin B12 as a Photosensing Unit

Vitamin B12 is well known as a cofactor for the B12-dependent enzymes that catalyze carbon skeleton rearrangement or elimination reactions, where Co–C bond hemolysis takes place to form the radical species as the reaction intermediate. Recently, a novel biological function of vitamin B12 has been reported: A photosensor protein CarH utilizes adenosylcobalamin (vitamin B12) as its senor unit for light sensing. We have determined the crystal structure of CarH from *Thermus thermophilus* to elucidate the molecular mechanisms of photosensing and signal transduction of CarH (Figure 3). CarH forms homo-tetramer and each subunit binds an adenosylcobalamin (AdoCbl). The protomer of CarH consists of the

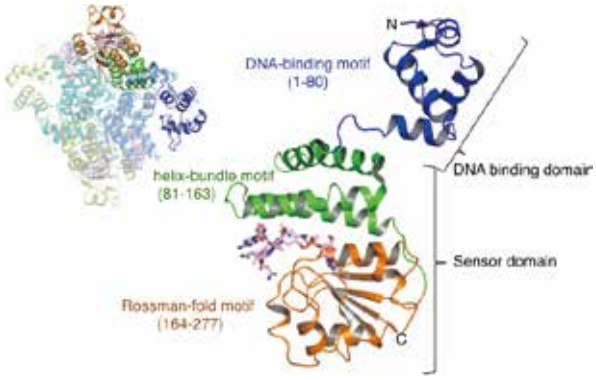

Figure 3. The crystal structures of AdoCbl-bound CarH.

DNA-binding and sensor domains as shown in Figure 3. The sensor domain consists of the helix-bundle motif and Rossman-fold motif, between of which AdoCbl is accommodated.

AdoCbl-bound CarH is photosensitive and dissociation of tetramer to monomer takes place upon photo irradiation. The adenosyl group is dissociated from Co ion upon photosensing, which is a trigger of the change of CarH quaternary structure.

* IMS International Internship Program