Bioinorganic Chemistry of Metalloproteins Responsible for the Homeostasis Control

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

AONO, Shigetoshi
Professor
[aono@ims.ac.jp]

Education
1982 B.S. Tokyo Institute of Technology
1987 Ph.D. Tokyo Institute of Technology

Professional Employment
1988 Postdoctoral Fellow, Georgia University
1989 Assistant Professor, Tokyo Institute of Technology
1994 Associate Professor, Japan Advanced Institute of Science and Technology
2002 Professor, Institute for Molecular Science
Professor, Okazaki Institute for Integrative Bioscience
Professor, The Graduate University for Advanced Studies

Member
Assistant Professor
YOSHIOKA, Shiro
IMS Research Assistant Professor
MURAKI, Norifumi
Visiting Scientist
RILHAC, Vincent*
Secretary
NAKANE, Kaori

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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.

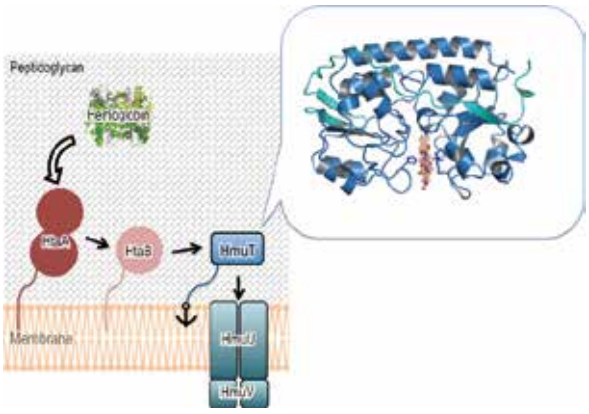

Figure 1. Schematic view of heme uptake system in *Corynebacterium glutamicum* and the crystal structure of HmuT that transports heme to the heme transporter HmuUV.

Selected Publications

- 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).
- H. Sawai, H. Sugimoto, Y. Shiro and S. Aono, "Structural Basis for Oxygen Sensing and Signal Transduction of the Heme-Based Sensor Protein Aer2 from *Pseudomonas aeruginosa*," *Chem. Commun.* **48**, 6523–6525 (2012).
- S. Aono, "Novel Bacterial Gas Sensor Proteins with Transition-Metal-Containing Prosthetic Groups as Active Sites," *Antioxid. Redox Signaling* **16**, 678–686 (2012).

1. Structure and Function of CgHmuT that is a Heme Binding Protein for the ABC-Type Heme Transporter CgHmuUV

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 HmuT from *Corynebacterium glutamicum* (CgHmuT) by X-ray crystallography to elucidate the molecular mechanism of heme transport by CgHmuT.

The structure of CgHmuT was determined at a resolution of 1.42 Å. CgHmuT showed a basket handle shape, where a long α helix is connected the N- and C-terminal domains (Figure 1). There was a cleft between the N- and C-terminal domains, in which one heme molecule was accommodated with His141 and Tyr240 as axial ligands that were located at the loop regions in the N- and C-terminal domains, respectively. Intriguingly, it was shown that heme was accommodated in the heme-binding site of CgHmuT with two different orientations. As protoheme bound to CgHmuT has an asymmetric structure, there are two possible orientations of heme when it is accommodated in the heme-binding site of CgHmuT. When a single orientation of heme was assumed in the model refinement, the residual electron densities were observed in the F_O-F_C map. On the other hand, good fitting of the model into the electron densities was obtained without any residual electron densities when 1:1 mixture of two orientations of heme was assumed, indicating the existence of the two different orientation of heme in CgHmuT.

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 are now working on CarH from *Thermus thermophilus* to elucidate the molecular mechanisms of photosensing and signal transduction of CarH.

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