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.

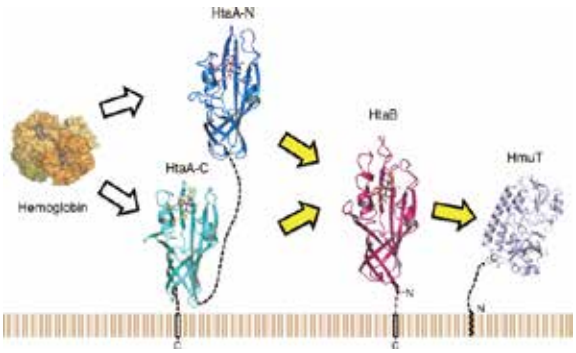

Figure 1. The protein machinery for heme uptake and transport in *Corynebacterium glutamicum*.

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*

Most commensal or pathogenic bacteria have heme acquisition systems for utilizing host heme as an iron source. The heme acquisition systems consist of cell surface localized heme binding proteins and heme transport proteins. In several Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus anthracis*, the iron regulated surface determinant (Isd) proteins capture and transfer heme to ABC transporter-type heme transport proteins. Though a similar ABC-type heme transporter consisting of HmuT, HmuU, and HmuV proteins is used by *Corynebacteria glutamicum* and *Corynebacterium diphtheriae*, they adopt different heme binding proteins, HtaA and HtaB, instead of Isd proteins (Figure 1). The ABC-type heme transporter system is widely used for heme acquisition, which consists of an ATP-binding protein, heme permease, and heme-binding protein (substrate-binding protein).

In this work, we have studied the structural and functional relationships of heme-binding and heme-trnasport proteins, HtaA, HtaB and HmuT in *Corynebacterium glutamicum*. Where HtaA and HtaB act as heme-binding and heme-transport proteins in the heme acquisition system of HtaAB-HmuTUV for *Corynebacterium glutamicum*, HmuT is a substrate (heme) binding protein for the ABC-type heme transporter HmuUV. Our working hypothesis is that heme captured in HtaA is transferred into cytoplasm by the ABC-type HmuUV heme transporter, but the detailed molecular mechanisms of heme transport in these systems remain to be elucidated. In this work, the molecular mechanisms of heme acquisition in *Corynebacterium glutamicum* have been elucidated based on the structural analyses of HtaA, HtaB, and HmuT.

Sequence analysis identified a conserved region (CR) of approximately 150 amino acids that is duplicated in HtaA and present in a single copy in HtaB. HtaA consists of two homologous CRs in the N- and C-terminal regions. We have determined the crystal structures of the N-terminal and C-terminal CRs of HtaA (HtaA-N and HtaA-C, respectively) at the resolution of 2.0 and 1.3 Å, respectively. The crystal structure of HtaB has also been determined at the resolution of 1.7 Å. HtaA-N consists of 11 β strands and two short α helices and accommodates one heme molecules with Tyr58 located in the first α helix as an heme axial ligand. Tyr58 forms a hydrogen bond with His111 (Figure 2A). A heme propionate forms hydrogen bonds with Ser54 and Tyr201. Heme is accommodated in an open pocket formed by hydrophobic amino acid residues including Phe55, Val63, Ile118, Leu119, Phe146, Phe197, and Phe200. Phe200 forms π-π stacking with heme and heme propionate forms a hydrogen bond with Ser54. These residues including the axial ligand and residues involved in the hydrogen bonding interactions are responsible for heme recognition by HtaA-N, which are conserved among HtaA-C and HtaB, as shown in Figure 2.

We have also determined the crystal structure of HmuT at the resolution of 1.4 Å. HmuT consists of structurally similar two domains located in the N-terminal and C-terminal regions connected a long α helix. A single heme molecule is bound in the cleft between these domains. Heme iron is ligated by His141 and Tyr240, and Tyr240 forms a hydrogen bond with Arg242. There is no amino acid residue interacting heme except for His141 and Tyr240 indicating that heme is recognized by the axial ligation in HmuT. Intriguingly, HmuT binds a heme with two different orientations. As the protoheme bound to HmuT has an asymmetric structure, there are two possible orientations of heme when it is accommodated in the heme-binding site of HmuT.

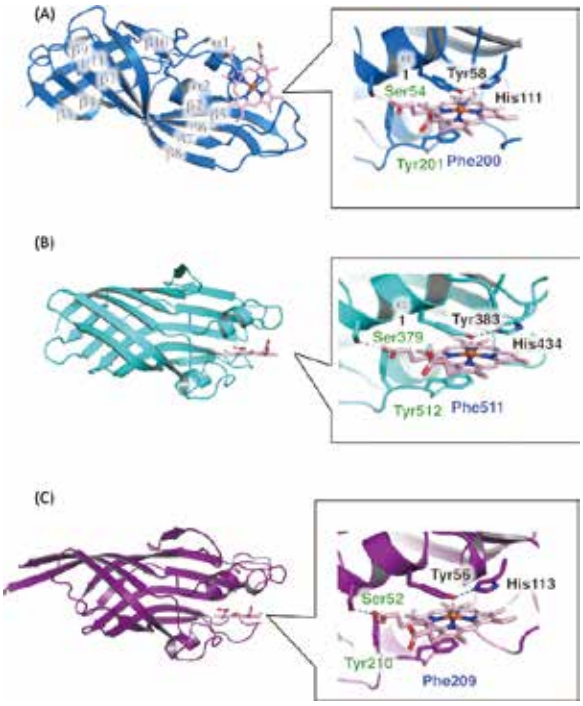

Figure 2. The crystal structures of (A) CgHtaA-N, (B) CgHtaA-C, and (C) CgHtaB with the close-up view of their heme binding sites.

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

We have studied the structure and function relationships of a novel photosensor protein CarH from *Thermus thermophilus* (*Tt*-CarH). In dark, adenosylcobalamin-bound CarH forms tetramer, which has a DNA binding ability. We have determined crystal structures of a C-terminal domain of *Tt*-CarH and full-length *Tt*-CarH at 2.5 Å and 3.0 Å resolution, respectively. *Tt*-CarH consists of an N-terminal DNA-binding domain and a C-terminal sensor domain. Adenosylcobalamin is bound in a cavity of the sensor domain with base-off form, where cobalt is coordinated by 5'-deoxyadenosine and His177 as axial ligands. The crystal structures suggest that interaction between adeonsyl group and surrounding amino acid residues plays a crucial role in photosensing by CarH.

* IMS International Internship Program