Bioinorganic Chemistry of Metal-Containing Sensor Proteins

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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 mainly focused on the elucidation of the structure–function relationships of the biological systems in which a heme molecule takes part in maintaining cellular homeostasis.

Heme shows many biological functions. The most popular function is to be used as a prosthetic group in heme proteins. Heme proteins show a variety of functions including oxygen transport/storage, electron transfer, oxidase, peroxidase, oxygenase, catalase, and dehydratase. In addition to these functions, a new function of hemeprotein has been found recently, which is a sensor of diatomic gas molecules or redox change. In these heme-based sensor proteins, the heme acts as the active site for sensing the external signal such as gas molecules and redox change. Heme also shows a novel biological function as a signaling molecule for transcriptional and translational regulation. In these systems, heme-sensing proteins sense a heme molecule to regulate biological processes. We are now working on the heme-based gas sensor proteins, heme-dependent transcriptional regulation, and bacterial heme transport systems.

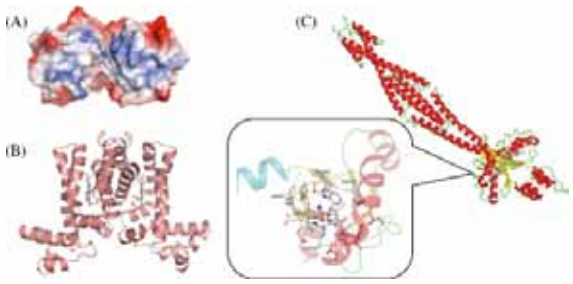

Figure 1. Structures of (A) the heme-transport protein HmuT, (B) the heme-sensing transcriptional regulator HrtR, and (C) the heme-based sensor protein Aer2.

Selected Publications

- 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).
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- S. Aono, "Novel Bacterial Gas Sensor Proteins with Transition-Metal-Containing Prosthetic Groups as Active Sites," *Antioxid. Redox Signaling* **16**, 678–686 (2012).

1. Molecular Mechanism for Heme-Mediated Inhibition of 5-Aminolevulinic Acid Synthase 1[1)]

Mammalian 5-aminolevulinic acid synthase 1 (ALAS1), an isozyme expressed in all cell types, catalyzes the first reaction in the heme biosynthetic pathway in mitochondria. Heme regulates ALAS1 function at multiple levels including the regulation of transcription, translation, mitochondrial import, protein degradation and enzyme activity to maintain intracellular heme concentrations at appropriate range. In this study, we elucidated the molecular mechanism of heme-mediated regulation of enzymatic activity for rat ALAS1. ALAS1 has three putative heme regulatory motifs (HRMs), two of which were found to be the ferric heme binding sites in ALAS1. Though the electronic absorption and resonance Raman spectroscopy have demonstrated that ^{110}Cys and ^{527}Cys are ferric heme binding sites in ALAS1, heme binding to ^{110}Cys and ^{527}Cys was not responsible for heme-mediated inhibition of ALAS1 activity. Rather, ALAS1 activity will be inhibited by heme binding with hydrophobic interactions to the succinyl-CoA binding pocket. The heme binding to the HRMs in ALAS1 was found not to be responsible for heme-mediated inhibition of ALAS1 activity. Though heme binding to ^{110}Cys and/or ^{527}Cys in the HRMs is not involved in the feedback regulation of ALAS1 activity, it would be responsible for the oxidative modification of ALAS1 that might regulate the heme-mediated proteolysis.

We also found that protoporphyrin IX (PpIX), a reaction intermediate of heme biogenesis, inhibited ALAS1 activity more efficiently compared with heme, indicating the presence of multiple pathways for the feedback regulation of ALAS1 activity. The intracellular accumulation of PpIX, which may be caused by unbalance between PpIX synthesis and iron chelation to PpIX to produce heme, causes severe damage to cells. Thus, it makes sense that PpIX functions as an effector of the feedback regulation of ALAS1 to shut down its activity to prevent from accumulation of PpIX under iron limitation.

2. Heme-Binding Properties of HupD Functioning as a Substrate-Binding Protein in a Heme-Uptake ABC-Transporter System in *Listeria monocytogenes*[2)]

Iron is an essential element for all organisms, which is used as a component of iron-containing proteins such as hemoproteins and iron-sulfur proteins responsible for a variety of biological processes. Pathogenic bacteria need to thieve iron from host organisms for their growth. Since heme iron involved in hemoglobin is the most abundant iron source for pathogenic bacteria infected in vertebrate, these bacteria have evolved sophisticated acquisition systems of heme from their host organisms.

Recently, it is reported that a Gram-positive pathogen *L. monocytogenes* that cause listeriosis has three Fur-regulated heme-uptake systems: the *srtB* region coding sortase-anchored proteins and a putative ABC transporter, the *fhu* and *hup* operons coding putative ABC transporters for ferric hydroxamates and hemin respectively. An ABC-type transporter system HupDGC is the primary heme transporter system and is most crucial to virulence for *L. monocytogenes*. HupD, the substrate-binding protein in the HupDGC transporter, is reported to be specific for heme, but the detailed properties of HupD for heme-binding remains to be elucidated. In this work, heme-binding properties of HupD from *L. monocytogenes* were characterized by spectroscopic and mutagenesis studies.

Titration of apo-tHupD with hemin revealed that apo-HupD takes up 1 mol equivalent of heme to form a 1:1 complex, as shown in Figure 2. UV-vis absorption, EPR, and resonance Raman spectroscopy have revealed that HupD binds a heme with two histidine residues as the axial ligand. ^{105}His and ^{259}His are identified as the axial ligands by site-directed mutagenesis. HupD is the first example of the heme-binding protein having bis-histidine coordination environment among heme-binding proteins working in the bacterial heme acquisition systems. While mutation of ^{259}His to Ala resulted in a loss of heme-binding ability of HupD, the H105A variant of HupD retained heme-binding ability with lower heme-binding affinity compared with wild type. These results suggest that ^{259}His is an essential ligand for heme acquisition by HupD and that ^{105}His might be responsible for the regulation of heme-binding affinity of HupD during heme transport process.

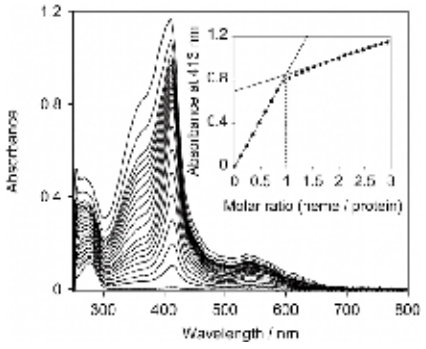

Figure 2. Spectral changes upon titration of apo-HupD with hemin. Inset: Titration curve of hemin binding to apo-HupD measured at 413 nm in 50 mM Tris-HCl (pH 7.4).

References

1) C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, *Bull. Chem. Soc. Jpn.* **87** (2014), in press.

2) Y. Okamoto, H. Sawai, M. Ogura, T. Uchida, K. Ishimori, T. Hayashi and S. Aono, *Bull. Chem. Soc. Jpn.* **87** (2014), in press.