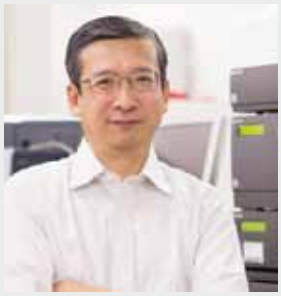
Bioinorganic Chemistry of Metal-Containing Sensor Proteins

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



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The widely studied biological function of heme is to act as a prosthetic group in hemeproteins that show a variety of functions, including oxygen storage and transport, electron transfer, redox catalysis, and sensing of gas molecules. Besides acting as a prosthetic group in a protein matrix, it has become apparent that free heme molecules can act as physiological effectors of several proteins, including transcriptional regulators, heme-regulated eIF2α kinase, and sensor kinases in two-component signal transduction systems. Reversible heme binding regulates the physiological function of these proteins. Though research on these proteins has shown new physiological functions of heme as a signaling molecule, the detailed molecular mechanisms by which heme regulates the functions of these proteins remain to be elucidated, mainly because the three-dimensional structures of these regulatory proteins have not yet been solved. Our research interests are focused on the elucidation of the molecular mechanisms of how heme molecule acts as a signaling molecule. We are also studying about the structure–function relationships of the heme-based gas sensor proteins.

1. Structural Basis for the Transcriptional Regulation of Heme Homeostasis in *Lactococcus lactis*[1)]

Though heme is a crucial element for many biological processes including respiration, heme homeostasis should be regulated strictly due to the cytotoxicity of free heme molecules. Numerous lactic acid bacteria, including *Lactococcus lactis*, acquire heme molecules exogenously to establish an aerobic respiratory chain. A heme efflux system plays an important role for heme homeostasis to avoid cytotoxicity of acquired free heme, but its regulatory mechanism is not clear. Here, we report that the transcriptional regulator HrtR senses and binds a heme molecule as its physiological effector to regulate the expression of the heme-efflux system responsible for heme homeostasis in *L. lactis*. To elucidate the molecular mechanisms of how HrtR senses a heme molecule and regulates gene expression for the heme efflux system, we determined the crystal structures of the apo-HrtR/DNA complex, apo-HrtR, and holo-HrtR at a resolution of 2.0, 3.1, and 1.9 Å, respectively. These structures revealed that HrtR is a member of TetR family of transcriptional regulators. The residue pair Arg46 and Tyr50 plays a crucial role for specific DNA-binding through hydrogen-bonding and a CH–π interaction with the DNA bases (Figure 1).

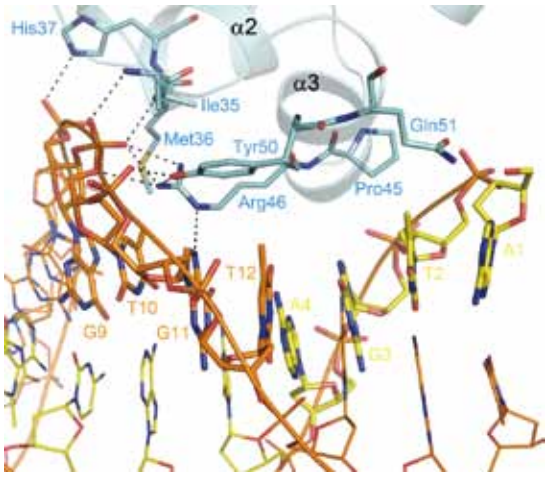

Figure 1. Interaction between apo-HrtR and DNA.

HrtR adopts a unique mechanism for its functional regulation upon heme-sensing. A comparison of the apo-HrtR/DNA complex and holo-HrtR structures revealed that heme-binding triggers a coil-to-helix transition at the α4a-α4b region (Figure 2 (A), (B)). In apo-HrtR, a loop (residues 68-71) intervenes between α4a and α4b helices. Glu70 in the middle of this intervening loop forms a hydrogen bond with Trp123 in apo-HrtR (Figure 2 (A)). Upon heme-binding, a coil-to-helix transition occurs in this intervening loop, which results in the formation of a long α4 helix in holo-HrtR. As the location of Glu70 is largely altered by this coil-to-helix transition, the hydrogen bond between Glu70 and Trp123 is lost in holo-HrtR as shown in Figure 2B. Heme-binding to HrtR causes a coil-to-helix transition of the α4 helix in the heme-sensing domain, which triggers a structural change of HrtR causing it to dissociate from the target DNA for de-repression of the genes encoding the heme efflux system. HrtR uses a unique heme-sensing motif with *bis*-His (His72 and His149) ligation to the heme, which is essential for the coil-to-helix transition of the α4 helix upon heme-sensing.

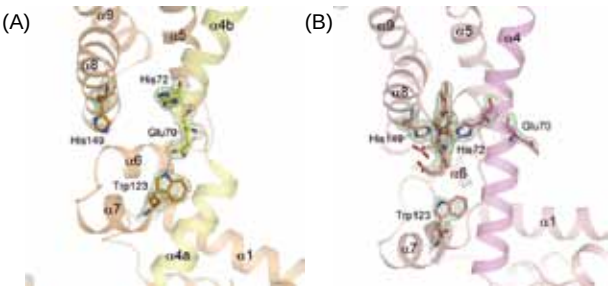

Figure 2. (A) Crystal structure of apo-HrtR. (B) Crystal structure of holo-HrtR.

2. A Model Theoretical Study on Ligand Exchange Reactions of CooA[2)]

Rr-CooA is a CO-sensor heme protein, where binding of CO with the heme group stimulates a transcriptional activator activity of CooA. In this process, the heme undergoes a series of ligand exchanges. In the ferric form, the heme has Cys75 and Pro2 as the axial ligands. In the reduced ferrous form, the heme has His77 instead of Cys75 as an axial ligand with Pro2. Only in the reduced form, CooA can bind CO that replaces Pro2. Model calculations are carried out to elucidate the ligand exchange reactions of CooA. The coordinated proline is found to be the neutral, protonated form. The ligand exchange of cysteine for histidine is reproduced by a relatively small model. This exchange would be mainly due to difference in stability of the non-bonding sulfur p-orbital in Cys75 between the ferric and ferrous states. The selectivity of gas molecules among CO, NO, and O_2 in the proteins is explained by the relative stability of products for Rr-CooA. This is also the case for Ch-CooA, where the amino group of the N-terminus and a histidine are coordinated to the iron ion both in the ferric and ferrous states. The ability to bind the gas molecules is a little stronger in Rr-CooA than in Ch-CooA. In the ferric form of Rr-CooA, heme is deformed to a ruffled form whereas heme is planar in the ferrous form, which leads to a red-shifted Q-band in the former.

3. Molecular Mechanisms of Heme Transport in Gram-Positive Bacteria

Iron, the second-most abundant metal in the Earth's crust, forms a water insoluble oxyhydroxide polymer in water under aerobic conditions, resulting in poor bio-availability. As it is an essential nutrient for prokaryotes and eukaryotes, they develop sophisticated iron acquisition systems to overcome this problem. A gram-positive bacterium *Listeria monocytogenes* has several iron (and iron complexes) transport systems, one of which is an ABC-type transporter (HupCGD) that transports heme as an iron source for this bacterium. HupC, HupG, and HupD proteins are an ATPase component, a membrane permease, and substrate (heme) biding protein, respectively. We have charezterized HupD protein to elucidate the molecular mechanisms of heme transport in *L. monocytogenes*.

HupD contains a typical N-terminal sequence of lipoprotein signal peptide. The amino acid sequence of LLASC is present at the C-terminus of the signal peptide, of which the cysteine residue is the target for lipid modification and becomes the first residue of the mature lipoprotein after cleavage by SPase II protease. When HupD with the signal peptide is expressed in *E. coli*, the recombinant HupD forms aggregates probably due to a hydrophobic signal peptide. Mature HupD lacking the signal peptide is expressed as a soluble protein, which is a mixture of apo- and holo-forms. Purified holo-HupD shows the Soret, α, and β peaks at 413, 565, and 530 nm, respectively, which are typical for 6-coordinated ferric heme proteins with two His as the axial ligands. Ferric HupD shows EPR signals with g = 3.27 and 2.08, which are consistent with a bis-His type ligation of the heme. Site-directed mutagenesis studies suggest that His105 and His259 act as the axial ligands of the heme in HupD. Crystallization experiments are now in progress to obtain structural information of HupD.

References

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2) T. Ishida and S. Aono, *Phys. Chem. Chem. Phys.* **15**, 6139–6148 (2013).

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