Bioinorganic Chemistry of Metal-Containing Sensor Proteins

Department of Life and Coordination-Complex Molecular Science
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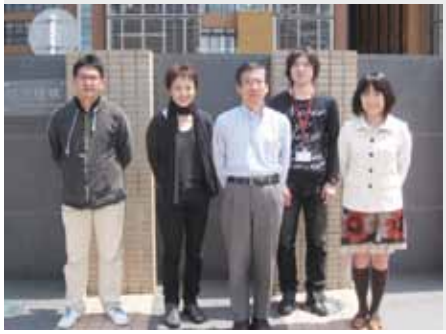

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Heme shows many biological functions. The most popular function is to be used as a prosthetic group in heme proteins. Heme proteins show a variety of functions including oxygen transport/storage, electron transfer, oxidase, peroxidase, oxygenase, catalase, 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.[1,2] 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. Our research interests are focused on the elucidation of the structure-function relationships of these heme-based sensor proteins and heme-sensing proteins.

1. Bacterial Gas Sensor Proteins Using Transition Metal-Containing Prosthetic Groups as Active Sites[3)]

Gas sensor proteins are involved in many biological regulatory systems, including transcription, chemotaxis, and other complex physiological processes. These regulatory systems consist of a sensor and regulatory protein, and, if any, signal transduction proteins that transmit the input signal sensed by the sensor protein to regulator proteins. Sensor proteins are the most upstream component in these regulatory systems, and the sensor and regulator proteins can be distinct molecules, or can sometimes exist in the same molecule as sensor and regulator domains. In both cases, the general scheme is as follows for biological regulatory systems by gas sensor proteins. Once a gas sensor protein/domain senses a gas molecule of its physiological effector, a conformational change of the sensor protein/domain is induced, and then intra- and/or inter-molecular signal transductions proceed to modulate the activity of the regulator protein/domain that is responsible for the regulation of the above biological functions.

Elucidating the mechanisms of gas sensing and signal transduction is required to understand the structure and function of gas sensor proteins. To do this, the following questions should be answered. How do gas sensor proteins discriminate a physiological gas molecule from other gas molecules? What conformational changes are induced upon gas sensing, and how? How are the conformational changes induced by gas sensing related to the subsequent signal transduction process? How do intra- and inter-molecular signal transduction take place between the sensor and regulator protein/domain?

Gas sensor proteins that contain metal centers are advantageous for answering these questions, because the metal centers can serve as excellent spectroscopic probes in various applications such as UV/Vis, electron paramagnetic resonance (EPR), resonance Raman, circular dichroism (CD), and X-ray absorption spectroscopy (XAS). These methods can provide detailed information on the reactivity of the metal center with a gas molecule, and structural information around the metal center. X-ray crystallography is also an important tool to study gas sensor proteins. We have elucidated the structure and function relationships of the heme-based sensor proteins by these techniques.

Aer2 is a new MCP responsible for aerotaxis of *Pseudomonas aeruginosa*, which consists of N-terminal three-unit poly-HAMP, PAS, di-HAMP, and MCP domains. The Aer2 PAS domain contains a heme that acts as the active site for sensing O_2. Once the PAS domain senses O_2, intramolecular signal transduction will proceed from the PAS domain to the MCP domain. Though the HAMP domains are assumed to be responsible for intramolecular signal transduction, detail mechanisms remain to be elucidated.

We have determined the X-ray crystal structures of Aer2-N384 (residues 1-384 that consists of three-HAMP, PAS, and di-HAMP) and Aer2-PH (residues 173-384 that consists of PAS and di-HAMP) to elucidate the mechanism by which

intramolecular signal transduction proceeds between the HAMP and PAS domains in Aer2. Aer2-N384 is a homodimer having a non-crystallographic 2-fold symmetry as shown in Figure 1. The three-unit poly-HAMP, PAS, and di-HAMP domains are ordered in linear configuration. Most of the C-terminal di-HAMP domain is disordered.

Figure 1. X-ray crystal structure of Aer2-N384 in cyano-met form.

Most of the di-HAMP domain is also disordered in the structure of Aer2-PH. A heme exists in a hydrophobic pocket in the PAS domain. His234 serves as the proximal ligand of the heme as shown in Figure 2. The 6-propionate forms a salt bridge with NE2 atom of His251, and the 7-propionate has hydrogen bonds with a water molecule, the main chain N atom of Lys235, and NE2 atom of Gln240. The heme-bound CN^- interacts with the side chain of Trp283. The distance is 2.9 Å between the N atom of CN^- and the NE2 atom of Trp283. CN-bound form of the heme can be thought to be a model of O_2-bound heme. The structure of Aer2-PH suggests that the heme-bound O_2 forms a hydrogen bond with Trp283. Trp283 is located on the C-terminal of the strand β5. This strand connects to the helix α5, which is the starting region of the C-terminal di-HAMP domain, suggesting that the hydrogen bond between Trp283 and O_2 are responsible for intramolecular signal transduction.

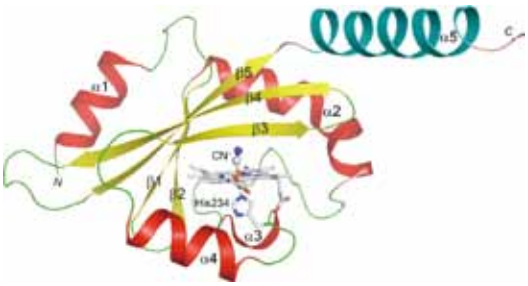

Figure 2. X-ray crystal structure of Aer2-PH in cyano-met form.

2. Structural Basis for the Transcriptional Regulation of Heme Homeostasis in *Lactococcus lactis*

Though a lactic acid bacterium *Lactococcus lactis* lacks heme biosynthesis genes, it can uptake and use heme molecules provided externally to grow by oxygen respiration. As free heme molecules are toxic for cells, cellular concentrations of heme should be controled strictly. *L. lactis* controls cellular heme concentrations by operating a heme efflux system. The expression of the heme efflux system is regulated by a heme-sensing transcriptional regulator HesR (heme efflux system regulator). In this work, we have determined X-ray crystal structures of HesR in heme-binding (holo-) and heme-free (apo-) forms to elucidate the structure and functions relationships of HesR.

HesR is a homo-dimer in the both of apo- and holo-forms as shown in Figure. HesR monomer consists of the N-terminal DNA-binding domain and the C-terminal heme binding domain that binds one heme molecule. Global fold of HesR is similar to that of TetR family transcriptional regulators. HesR is the first example of heme-sensing TetR family transcriptional regulator. A change in the relative orientation of the DNA-binding domain is induced upon heme-binding, which results in the regulation of DNA-binding activity of HesR.

Figure 3. (Left) X-ray crystal structure of apo-HesR.
Figure 4. (Right) X-ray crystal structure of holo-HesR.

The HesR recognition sequence, ATGACACAGTGTCAT, is a perfect palindrome sequence as shown in the underlined sequence.We have found that apo-HesR can bind the target DNA, but holo-HesR can not. DNA-binding affinity of apo-HesR is determined to be *Kd* = 0.7 nM by fluorescence anisotropy measurements. These results indicate that heme molecule acts as a physiological effector of HesR to regulate its DNA-binding activity.

HesR shows a very high affinity for heme binding. When apo-HesR is mixed with holo-myoglobin, it can extract heme from myoglobin. The estimated heme binding affinity of HesR is comparable to that of myoglobin.

References

1) S. Aono, *Dalton Trans.* 3125–3248 (2008).
2) S. Aono, *Acc. Chem. Res.* **36**, 825–831 (2003).
3) S. Aono, *Antioxid. Redox Signaling* in press (2011).