Bioinorganic Chemistry of Novel Hemeproteins

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

AONO, Shigetoshi	Professor
YOSHIOKA, Shiro	Assistant Professor
SAWAI, Hitomi	JSPS Post-Doctoral Fellow
NISHIMURA, Muneto	Graduate Student
TANIZAWA, Misako	Secretary

Heme-based sensor proteins show a novel function of the heme prosthetic group, in which the heme acts as the active site for sensing the external signal such as diatomic gas molecules and redox change. Aldoxime dehydratase is another novel hemeprotein, in which the heme prosthetic group tethers the substrate for its dehydration reaction. Our research interests are focused on the elucidation of the structure-function relationships of these novel hemeproteins.

1. Hydrogen Bonding Interaction on the Heme-Bound Ligand in the Heme-Based O_2 Sensor Protein[1)]

HemAT is a signal transducer protein that is a member of methyl accepting chemotaxis proteins (MCPs), which is responsible for aerotaxis control of some bacteria and archaea. HemAT consists of two domains, the N-terminal sensor domain containing a heme and the C-terminal signaling domain that interacts with CheA, a component of CheA/CheY two-component system regulating the rotational direction of a flagellar motor in response to an input signal into MCPs. The heme in the sensor domain of HemAT acts as the active site for sensing its physiological effector, O_2. When O_2 binds to the heme in the sensor domain, it is thought that a specific conformational change will occur, and then signal transduction will proceed from the sensor domain to the signaling domain. As a result, the self-kinase activity of CheA is regulated by a change in the interaction between HemAT and CheA via the specific conformational change of HemAT.

HemAT should discriminate O_2 from other gas molecules such as NO and CO, for which the heme environmental structure plays a crucial role. To elucidate the mechanism of selective O_2 sensing by HemAT, structural, mutagenesis, and spectroscopic studies were carried out for HemAT from *Bacillus subtilis* (HemAT-Bs). The interaction between the heme-bound ligand and the surrounding amino acid residue(s) plays a crucial role for selective sensing of O_2 and signal transduction by HemAT. In this work, we elucidated by resonance Raman spectroscopy how O_2 and CO interact with HemAT-Hs and HemAT-Rr, HemAT from *Halobacterium salinarum* and *Rhodospirillum rubrum*, respectively. HemAT-Hs and HemAT-Rr showed three conformers in the O_2-bound form, as is the case of HemAT-Bs, HemAT from *Bacillus subtilis*. Though the hydrogen bonding patterns observed in the three conformers were same among HemAT-Bs, HemAT-Hs, and HemAT-Rr, the involved residues for the hydrogen bonding interaction were different from one another.

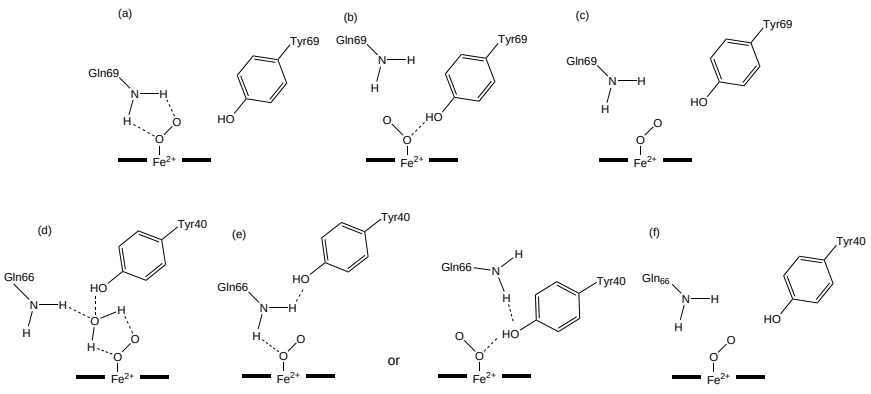

Figure 1. Hydrogen bonding network of the oxygen-bound form of HemAT-Hs (a-c) and HemAT-Rr (d-f).

2. Metal-Containing Sensor Proteins Sensing Diatomic Gas Molecules[2)]

All of the living organisms have a variety of regulatory systems to confront the change in the environmental conditions, which should be essential for maintaining the homeostasis in the cells, organs, and whole bodies. The chemical or physical stimuli act as signals for them to sense the change in the environmental conditions. These regulatory systems are responsible for the control of cell motility, gene expression, and/or enzymatic activity in response to the external stimuli. Sensing the external stimuli is the first step for these regulatory systems to work. Sensor (receptor) proteins should be required to sense these signals in biological systems, which sense the cognate signals in a specific manner.

Recently, it becomes apparent that diatomic gas molecules such as O_2, CO, and NO can act as signaling molecules for many biological processes. The functional role as external signals is a new one of these gas molecules in biological systems while it is well known that gas molecules are involved in biological systems as substrates and/or reaction products of many enzymatic reactions.

The gas sensor proteins usually use some prosthetic group to sense diatomic gas molecules. Heme, iron-sulfur cluster, and non-heme iron are known as the active center for these gas sensor proteins. When the gas sensor proteins sense their effector gas molecules, intramolecular and intermolecular signal transductions take place to regulate many physiological functions including gene expression, aerotaxis, and change in metabolic pathways, *etc*. The metal-containing prosthetic groups in these sensor proteins play a crucial role for selective sensing of their effectors.

In this perspective, I will discuss the structure and function of some O_2-, CO-, and NO-sensor proteins, especially focus on the structural, biochemical and biophysical properties of the active centers of these sensor proteins.

3. Protein Conformation Changes of HemAT-Bs upon Ligand Binding Probed by Ultraviolet Resonance Raman Spectroscopy[3)]

HemAT from *Bacillus subtilis* (HemAT-*Bs*) is a heme-based O_2 sensor protein that acts as a signal transducer responsible for aerotaxis. HemAT-*Bs* discriminates its physiological effector (O_2) from other gas molecules (CO and NO), although all of them bind to a heme. To monitor the conformational changes in the protein moiety upon binding of different ligands, we have investigated ultraviolet resonance Raman (UVRR) spectra of the ligand-free and O_2-, CO-, and NO-bound forms of full-length HemAT-*Bs* and several mutants (Y70F, H86A, T95A, and Y133F) and found that Tyr^{70} in the heme distal side and Tyr^{133} and Trp^{132} from the G-helix in the heme proximal side undergo environmental changes upon ligand binding. In addition, the UVRR results confirmed our previous model, which suggested that Thr^{95} forms a hydrogen bond with heme-bound O_2, but Tyr^{70} does not. It is deduced from this study that hydrogen bonds between Thr^{95} and heme-bound O_2 and between His^{86} and heme 6-propionate communicate the heme structural changes to the protein moiety upon O_2 binding but not upon CO and NO binding. Accordingly, the present UVRR results suggest that O_2 binding to heme causes displacement of the G-helix, which would be important for transduction of the conformational changes from the sensor domain to the signaling domain.

References

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2) S. Aono, *Dalton Trans.* 3137–3146 (2008).
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Award

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