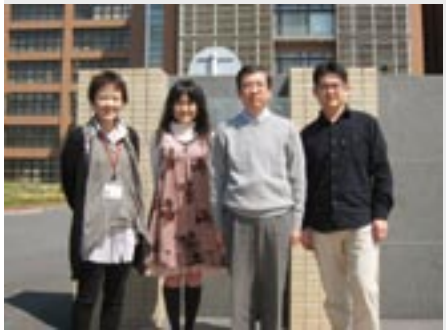
Bioinorganic Chemistry of Metal-Containing Sensor Proteins

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



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Hemeproteins are a typical metalloprotein, which show a variety of functions including oxygen storage/transport, electron transfer, redox catalysis with various substrates. Besides these traditional functions of hemeproteins, a new function of hemeprotein has been found recently, which is a sensor of diatomic gas molecules or redox change.[1)] 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. Our research interests are focused on the elucidation of the structure-function relationships of these heme-based sensor proteins. We are also studying about an iron-sulfur (Fe-S) cluster-containing sensor protein responsible for the transcriptional regulation of nitrogenase gene.

1. Oxygen Sensor Proteins Responsible for Aerotaxis (Chemotaxis toward Molecular Oxygen) Control in Bacteria

Chemotaxis is a major biological signal transduction system, which consists of signal transducer proteins and several chemotaxis proteins including CheA, CheY, and CheW responsible for intermolecular signal transduction to control direction of flagellar rotation. Signal transducer proteins, sometimes called as MCPs (methyl-accepting chemotoxis proteins), bind a repellant or attractant in their sensor domain. Many chemical and physical stimuli act as a repellant or attractant, among which molecular oxygen is a typical gaseous signaling molecule. In this work, we have studied the structure and function relationships of MCPs, Aer2 and HemAT, that sense molecular oxygen for aerotaxis (chemotaxis toward molecular oxygen) control.

HemAT is well known as a MCP that senses molecular oxygen for aerotaxis control, which contains a *b*-type heme as the active site for sensing molecular oxygen. Though both of HemAT and Aer2 use a heme as the active site for sensing molecular oxygen, the sensor domains of HemAT and Aer2 are different. HemAT and Aer2 adopt a globin domain and PAS domain as their sensor domains, respectively. Though the globin domain of HemAT shows a structural homology to myoglobin, it has a different heme environmental structure in the distal heme pocket from myoglobin. In the case of myoglobin, a distal His forms a hydrogen bond with the heme-bound oxygen to stabilize the heme-oxygen complex. However, there is no distal His in HemAT, in which a Thr is involved in the formation of a hydrogen bonding network upon oxygen binding to HemAT. We have studied conformational changes induced by oxygen binding by means of resonance Raman spectroscopy.

Aer2 is a new MCP responsible for aerotaxis of *Pseudomonas aeruginosa*, which adopts a PAS domain as a sensor domain. We have constructed an expression system of Aer2 in *E. coli* and purified the recombinant protein. The purified Aer2 shows the Soret, α and β peaks at 419, 578, and 543 nm, respectively, as shown in Figure 1. When the purified Aer2 is

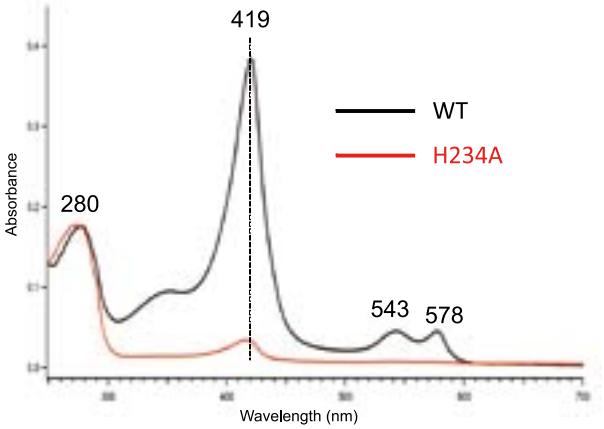

Figure 1. Electronic absorption spectra of wild-type and H234A mutant Aer2.

reacted with CO, CO-bound Aer2 is formed. The purified Aer2 shows a typical Resonance Raman spectrum of oxygen-bound hemeproteins. These results indicate that Aer2 is purified as an oxygen-bound form.

Domain analysis by using SMART program (http://smart.embl-heidelberg.de/) reveals that Aer2 consists of a PAS domain in its N-terminal region. PAS domain is widely used as a sensor domain sensing various chemical and physical signals. PAS domains show a similar 3D structure, though their amino acid sequences do not necessarily show a high homology. Figure 2 shows X-ray crystal structures of the heme-containing PAS domain of FixL that is an oxygen sensor protein in FixL/FisJ two-component system. The PAS domain of Aer2 would

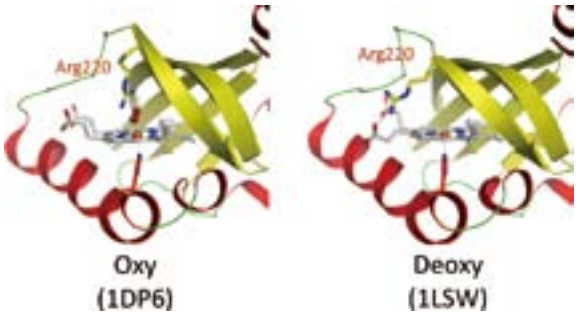

Figure 2. X-ray crystal structure of the PAS domain of FixL in oxy- and deoxy-forms. PDB accession numbers are shown in parenthesis.

be similar to that of FixL. Amino acid sequence alignments among heme-containing PAS domains including Aer2 show that a His residue is conserved at the position of 234 in Aer2, at which the proximal ligand of the heme is located in other heme-containing PAS domains. Site-directed mutagenesis reveals that His234 is indeed the proximal ligand of the heme in Aer2. Mutation of His234 to Ala results in loss of heme (Figure 1). Further characterization of Aer2 is now in progress.

2. The Role of the Fe-S Cluster in the Sensory Domain of Nitrogenase Transcriptional Activator VnfA from *Azotobacter vinelandii*[2)]

Transcriptional activator VnfA is required for the expression of the second nitrogenase system encoded in the *vnfH* and *vnfDGK* operons in *Azotobacter vinelandii*. VnfA belongs to a σ^N-dependent regulatory protein generally consisting of three major domains, and contains a GAF domain in its N-terminal region as a sensory domain. VnfA has a characteristic Cys-rich motif, Cys-X-Cys-XXXX-Cys, in its GAF domain. Although this motif is supposed to be a metal ions/cluster binding motif, metal ions/cluster in VnfA is not characterized yet. In the present study, we have purified full-length VnfA produced in *E. coli* as recombinant proteins (*Strep*-tag attached and tag-less proteins), enabling detailed characterization of VnfA for the first time. The EPR spectra of whole cells producing tag-less VnfA (VnfA) show distinctive signals assignable to a 3Fe-4S cluster in the oxidized form ($[Fe_3S_4]^+$). Although aerobically purified VnfA shows no vestiges of any Fe-S clusters, enzymatic reconstitution under anaerobic conditions reproduced $[Fe_3S_4]^+$ dominantly in the protein. Additional spectroscopic evidence of $[Fe_3S_4]^+$ *in vitro* is provided by anaerobically purified *Strep*-tag attached VnfA. Thus, spectroscopic studies both *in vivo* and *in vitro* indicate the involvement of $[Fe_3S_4]^+$ as a prosthetic group in VnfA. Molecular mass analyses reveal that VnfA is a tetramer both in the presence and absence of the Fe-S cluster. Quantitative data of iron and acid-labile sulfur in reconstituted VnfA are fitted with four 3Fe-4S clusters per a tetramer, suggesting that one subunit bears one cluster. *In vivo* β-gal assays reveal that the Fe-S cluster which is presumably anchored in the GAF domain by the N-terminal cysteine residues is essential for VnfA to exert its transcription activity on the target nitrogenase genes. Unlike the NifAL system of *A. vinelandii*, O_2 shows no effect on the transcriptional activity of VnfA but reactive oxygen species is reactive to cause disassembly of the Fe-S cluster and turns active VnfA inactive.

References

1) S. Aono, *Dalton Trans.* 3125–3248 (2008).
2) H. Nakajima, N. Takatani, K. Yoshimitsu, M. Itoh, S. Aono, Y. Takahashi and Y. Watanabe, *FEBS J.* **277**. 817–832 (2010).

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