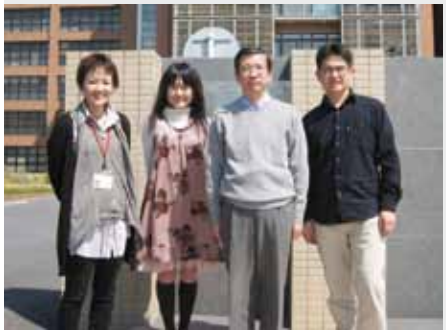
Bioinorganic Chemistry of Metal-Containing Sensor Proteins

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. 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)] 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. A new hemeprotein, aldoxime dehydratase (Oxd), also shows a novel biological function of heme, which catalyzes dehydration reaction of organic substrates. Oxd is the first example of hemeproteins that act as a hydro-lyase. Our research interests are focused on the elucidation of the structure-function relationships of these hemeproteins.

1. Bacterial Gas Sensor Proteins Using Transition Metal-Containing Prosthetic Groups as Active Sites[2,3)]

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.

HemAT from *Bacillus subtilis* (HemAT-*Bs*) is a heme-containing O_2 sensor protein that acts as a chemotactic signal transducer. Binding of O_2 to the heme in the sensor domain of HemAT-*Bs* induces a conformational change in the protein matrix, and this is transmitted to a signaling domain. To characterize the specific mechanism of O_2-dependent conformational changes in HemAT-*Bs*, we investigated time-resolved resonance Raman spectra of the truncated sensor domain and the full-length HemAT-*Bs* upon O_2 and CO dissociation.

A comparison between the O_2 and CO complexes provides insights on O_2/CO discrimination in HemAT-*Bs*. While no spectral changes upon CO dissociation were observed in our experimental time window between 10 ns and 100 μs, the band position of the stretching mode between the heme iron and the proximal histidine, ν(Fe–His), for the O_2-dissociated HemAT-*Bs* was lower than that for the deoxy form on time-resolved resonance Raman spectra. This spectral change specific to O_2 dissociation would be associated with the O_2/CO discrimination in HemAT-*Bs*. We also compared the results obtained for the truncated sensor domain and the full-length HemAT-*Bs*, which showed that the structural dynamics related to O_2 dissociation for the full-length HemAT-*Bs* are faster than those for the sensor domain HemAT-*Bs*. This indicates that the heme proximal structural dynamics upon O_2 dissociation are coupled with signal transduction in HemAT-*Bs*.

We have also studied the protein dynamics of HemAT-*Bs* following CO photodissociation by time-resolved ultraviolet resonance Raman spectroscopy (UVRR). The UVRR spectra indicated two phases of intensity changes for Trp, Tyr, and Phe bands of both full-length and sensor domain proteins. The

W16 and W3 Raman bands of Trp, the F8a band of Phe, and the Y8a band of Tyr increased in intensity at hundreds of nanoseconds after CO photodissociation, and this was followed by recovery in ~50 μs. These changes were assigned to Trp-132 (G-helix), Tyr-70 (B-helix), and Phe-69 (B-helix) and/or Phe-137 (G-helix), suggesting that the change in the heme structure drives the displacement of B- and G-helices. The UVRR difference spectra of the sensor domain displayed a positive peak for amide I in hundreds of nanoseconds after photolysis, which was followed by recovery in ~50 μs. This difference band was absent in the spectra of the full-length protein, suggesting that the isolated sensor domain undergoes conformational changes of the protein backbone upon CO photolysis and that the changes are restrained by the signaling domain. The time-resolved difference spectrum at 200 μs exhibited a pattern similar to that of the static (reduced–CO) difference spectrum, although the peak intensities were much weaker. Thus, the rearrangements of the protein moiety toward the equilibrium ligand-free structure occur in a time range of hundreds of microseconds.

2. Spectroscopic Analyses of the Heme Environmental Structure of Aldoxime Dehydratase[4)]

Aldoxime dehydratase (Oxd) is a new heme-containing enzyme that works as a hydro-lyase catalyzing dehydration of aldoximes to nitriles. The enzymatic activity of Oxd is dependent on the oxidation state of the heme iron, though the reaction catalyzed by Oxd is not a redox reaction. Ferrous Oxd containing a Fe^{2+}-heme shows the enzymatic activity, but ferric Oxd containing a Fe^{3+}-heme does not. Previous spectroscopic analyses reveal a novel mechanism, where the change in the coordination mode of the substrate plays a crucial role for the regulation of the enzymatic activity. Unlike other heme enzymes, the organic substrate is directly bound to the heme iron in OxdRE. While the oxygen atom of aldoxime is coordinated to the ferric heme, the nitrogen atom of aldoxime is coordinated to the ferrous heme. The dehydration reaction proceeds only via N-coordinated substrate in the ferrous heme.

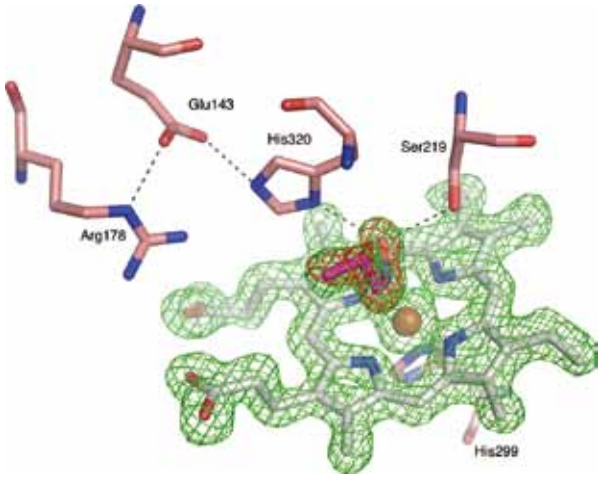

Figure 1. Heme environmental structure of the substrate-bound OxdRE.

In this study, we have elucidated the heme environmental structure of Oxd from *Rhodococcus sp.* N-771 (OxdRE) by analyzing Fourier transform infrared (FTIR) spectra and time-resolved step-scan FTIR spectra of CO-bound Oxd. Two C–O modes of heme at 1945 and 1964 cm^{-1} have been identified and remained unchanged in H_2O/D_2O exchange and in the pH 5.6–8.5 range, suggesting the presence of two conformations at the active site. The "light" minus "dark" difference FTIR spectra indicate that the heme propionates are in both the protonated and deprotonated forms, and the photolyzed CO becomes trapped within a ligand docking site (ν(CO) = 2138 cm^{-1}). The time-resolved step-scan FTIR spectra show that the rate of recombination of CO to the heme is $k_{1945\ cm^{-1}}$ = 126 ± 20 s^{-1} and $k_{1964\ cm^{-1}}$ = 122 ± 20 s^{-1} at pH 5.6, and $k_{1945\ cm^{-1}}$ = 148 ± 30 s^{-1} and $k_{1964\ cm^{-1}}$ = 158 ± 32 s^{-1} at pH 8.5. The rate of decay of the heme propionate vibrations is on a time scale coincident with the rate of rebinding, suggesting that there is a coupling between ligation dynamics in the distal heme environment and the environment sensed by the heme propionates.

The vibrational properties of the CO adduct of OxdRE indicate the formation of a photolabile species in which the proximal histidine and the H-bonding interactions of the negatively charged heme propionates are dominant factors in controlling the strength of the Fe–CO bond. The latter observation indicates that other factors beyond the well-known proximal and distal back-bonding contributions are effective in Oxd. Taken together, these results indicate that the distal residues control the proper orientation of the bound aldoxime and, thus, modulate the heme conformation from inactive to active. Obviously, there is communication linkage between the distal and proximal sites through bond networks suggesting that there is a coupling between ligation dynamics and the environment sensed by the heme propionates. The complexity of the structural implications involved in the transition from oxidized to reduced state in the presence of aldoxime should serve as a basis for uncovering the dynamic processes involved in the reaction mechanism of the enzyme.

References

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