Bioinorganic Chemistry of Novel Hemeproteins

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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. Crystal Structure of CO-Sensing Transcription Activator CooA Bound to Exogenous Ligand Imidazole[1)]

CooA is a CO-dependent transcriptional activator and transmits a CO sensing signal to a DNA promoter that controls the expression of the genes responsible for CO metabolism. CooA contains a b-type heme as the active site for sensing CO. CO binding to the heme induces a conformational change that switches CooA from an inactive to an active DNA-binding form. Here, we report the crystal structure of an imidazole-bound form of CooA from *Carboxydothermus hydrogenoformans* (Ch-CooA). In the resting form, Ch-CooA has a six-coordinate ferrous heme with two endogenous axial ligands, the α-amino group of the N-terminal amino acid and a histidine residue. The N-terminal amino group, which is coordinated to the heme in CooA, is replaced by CO. This substitution presumably triggers a structural change leading to the active form. The crystal structure of Ch-CooA reveals that imidazole binds to the heme, which replaces the N terminus, as does CO. The dissociated N terminus is positioned approximately 16 Å from the heme iron in the imidazole-bound form. In addition, the heme plane is rotated by 30° about the normal of the porphyrin ring compared to that found in the inactive form of *Rhodospirillum rubrum* CooA. Even though the ligand exchange takes place, imidazole-bound Ch-CooA remains in the inactive form for DNA binding. These results indicate that the release of the N terminus resulting from imidazole binding is not sufficient to activate CooA. The structure provides new insights into the structural changes required to achieve activation.

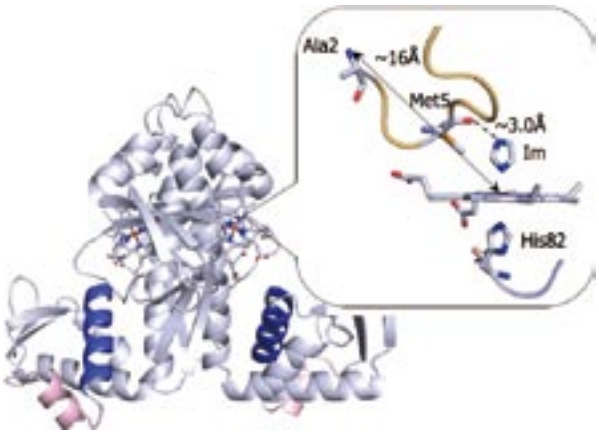

Figure 1. Structure of imidazole-bound Ch-CooA and the close-up view around the heme.

2. The Formation of Hydrogen Bond in the Proximal Heme Pocket of HemAT-Bs upon Ligand Binding[2)]

HemAT-Bs is the heme-based O_2 sensor responsible for aerotaxis control in *Bacillus subtilis*. In this study, we measured the time-resolved resonance Raman spectra of full-length HemAT-Bs wild-type (WT) and Y133F in the deoxy form and the photoproduct after photolysis of CO-bound form.

In WT, the ν_{Fe-His} band for the 10 ps photoproduct was observed at higher frequency by about 2 cm^{-1} compared with that of the deoxy form. This frequency difference is relaxed in hundreds of picoseconds. This time-dependent frequency shift would reflect the conformational change of the protein matrix. On the other hand, Y133F mutant does not show such a substantial ν_{Fe-His} frequency shift after photolysis. Since a hydrogen bond to the proximal His induces an up-shift of the ν_{Fe-His} frequency, these results indicate that Tyr133 forms a hydrogen bond to the proximal His residue upon the ligand binding. We discuss a functional role of this hydrogen bond formation for the signal transduction in HemAT-Bs.

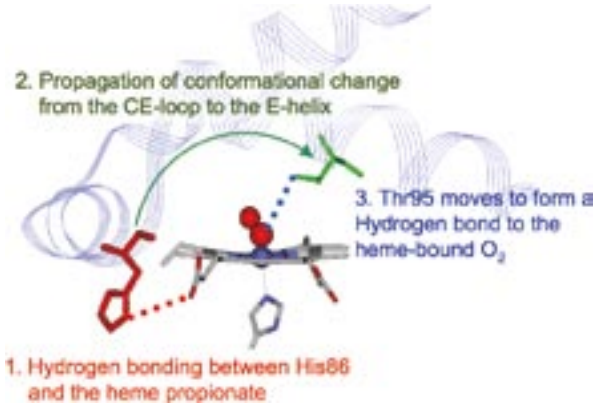

Figure 2. Signal transduction pathway of HemAT-Bs.

3. Two Ligand Binding Sites in the O_2-Sensing Signal Transducer HemAT: Implication for Ligand Recognition/ Discrimination and Signaling[3)]

We have identified a ligand (CO) accommodation cavity in the signal transducer sensor protein HemAT (heme-based aerotactic transducer) that allows us to gain single-molecule insights into the mechanism of gas sensor proteins. Specific mutations that are distal and proximal to the heme were designed to perturb the electrostatic field near the ligand that is bound to the heme and near the accommodated ligand in the cavity. We report the detection of a second site in heme proteins in which the exogenous ligand is accommodated in an internal cavity. The conformational gate that directs the ligand-migration pathway from the distal to the proximal site of the heme, where the ligand is trapped, has been identified. The data provide evidence that the heme pocket is the specific ligand trap and suggest that the regulatory mechanism may be tackled starting from more than one position in the protein. Based on the results, we propose a dynamic coupling between the two distinct binding sites as the underlying allosteric mechanism for gas recognition discrimination that triggers a conformational switch for signaling by the oxygen sensor protein HemAT.

4. Systematic Regulation of the Enzymatic Activity of Phenylacetaldoxime Dehydratase by Exogenous Ligand[4)]

Phenylacetaldoxime dehydratase from *Bacillus* sp. OxB-1 (OxdB) contains a heme that acts as the active site for the dehydration reaction of aldoxime. Ferrous heme is the active form, in which the heme is 5-coordinated with His282 as the proximal ligand. In this work, we evaluated the functional role of the proximal ligand for the catalytic properties of the enzyme by "the cavity mutant technique." H282G mutant of OxdB lost the enzymatic activity, though the heme, which was 5-coordinated with a water (or OH^-) as an axial ligand, existed in the protein matrix. The enzymatic activity was rescued by imidazole or pyridine derivatives that acted as the exogenous proximal ligand. By changing electron donation ability of these exogenous ligands with different substituents, the enzymatic activity could be regulated systematically. The stronger electron donation ability of the exogenous ligand, the higher restored enzymatic activity. Interestingly, H282G OxdB with 2-methyl imidazole showed a higher activity than wild type enzyme. Kinetic analyses revealed that the proximal His regulated not only the affinity of the substrate binding to the heme but the elimination of the OH group from the substrate.

References

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