X-B Bioinorganic Chemistry of Heme-Based Sensor Proteins

Heme-based sensor proteins show a novel function of the heme prosthetic group, in which the heme acts as an active site for sensing the external environmental signal such as diatomic gas molecules and redox change. Heme-based O_2, NO, and CO sensor proteins have now been found in which these gas molecules act as a signaling factor that regulates the functional activity of the sensor proteins. Our research interest focuses on the elucidation of structure-function relationships of CO sensor protein (CooA), O_2 sensor protein (HemAT), and redox sensor protein (DcrA).

X-B-1 Spectroscopic and Redox Properties of a CooA Homologue from *Carboxydothermus hydrogenoformans*

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CooA is a CO-sensing transcriptional activator that contains a b-type heme as the active site for sensing its physiological effector, CO. In this study, the spectroscopic and redox properties of a new CooA homologue from *Carboxydothermus hydrogenoformans* (Ch-CooA) were studied. Spectroscopic and mutagenesis studies revealed that His-82 and the N-terminal -amino group were the axial ligands of the Fe(III) and Fe(II) hemes in Ch-CooA and that the N-terminal -amino group was replaced by CO upon CO binding. Two neutral ligands, His-82 and the N-terminal -amino group, are coordinated to the Fe(III) heme in Ch-CooA, whereas two negatively charged ligands, a thiolate from Cys-75 and the nitrogen atom of the N-terminal Pro, are the axial ligands of the Fe(III) heme in Rr-CooA. The difference in the coordination structure of the Fe(III) heme resulted in a large positive shift of redox potentials of Ch-CooA compared with Rr-CooA. Comparing the properties of Ch-CooA and Rr-CooA demonstrates that the essential elements for CooA function will be: (i) the heme is six-coordinate in the Fe(III), Fe(II), and Fe(II)–CO forms; (ii) the N-terminal is coordinated to the heme as an axial ligand, and (iii) CO replaces the N-terminal bound to the heme upon CO binding.

X-B-2 Oxygen Sensing Mechanism of HemAT from *B. subtilis*: A Resonance Raman Spectroscopic Study

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HemAT-Bs is a heme-containing signal transducer protein responsible for aerotaxis of *Bacillus subtilis*, where the heme acts as an oxygen sensor. We have characterized the recombinant *HemAT-Bs* to elucidate the mechanisms of oxygen-sensing and signal transduction by *HemAT-Bs*. *HemAT-Bs* shows similar uv/vis spectra to those of myoglobin (Mb). Site-directed mutagenesis reveals that His123 is the proximal ligand of the heme in *HemAT-Bs*.

Resonance Raman (RR) evidence for structural linkage between the distal side of heme pocket and the signaling domain of an oxygen sensing hemoprotein, *HemAT-Bs*, is reported. The band-fitting analyses of the RR spectra in the Fe–O_2 stretching (ν(Fe–O_2)) region revealed the presence of three conformers with ν(Fe–O_2) at 554, 566, and 572 cm^{-1}, which reflect different H-bond strengths on the bound O_2 molecule. While recent X-ray analysis for CN^--bound *HemAT-Bs* suggested the importance of Thr95 and Tyr70, the species with the strongest H-bond (554 cm^{-1}) was deleted in the T95A mutant and also by removal of the linker and signal domains; however, the Y70F mutant maintained the same three conformers. A scheme for specific O_2 sensing and signaling mechanism is discussed.

X-B-3 Structure and Function of a Novel Redox Sensor DcrA Containing a *C*-Type Heme

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Chemotaxis signal transducer protein DcrA from a sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough contains a *c*-type heme in its periplasmic domain (DcrA-N), which is the first example of a heme-based sensor protein containing a *c*-type heme as a prosthetic group. Optical absorption and resonance Raman spectroscopy indicates that the heme *c* in DcrA-N shows a redox-dependent ligand exchange. Upon reduction, a water molecule that may be the sixth ligand of the ferric heme *c* is replaced by an endogenous amino acid. Although the reduced heme in DcrA-N is six-coordinated with two endogenous axial ligands, CO can easily bind to the reduced heme to form CO-bound DcrA-N. Reaction of the reduced DcrA-N with molecular oxygen results in autoxidation to form a ferric state without forming any stable oxygen-bound form, probably due to the extremely low redox potential of DcrA-N (–250 mV). DcrA will act as a redox sensor, where the ligand exchange between water and an endogenous amino acid would be a trigger for signal transduction.

X-C Bioinorganic Chemistry of a Novel Heme Enzyme that Catalyzes the Dehydration Reaction

Phenylacetaldoxime dehydratase from *Bacillus* sp. Oxd-1 (OxdB) catalyzes the dehydration reaction of Z-phenylacetaldoxime (PAOx) to produce phenylacetonitrile under mild conditions. OxdB exists in a monomer of a 40-kDa polypeptide containing a protoheme. The heme in OxdB is thought to be the active site for the dehydration reaction. OxdB is the first example of a hemeprotein catalyzing the dehydration reaction physiologically, although many functions of hemeproteins have been elucidated, including oxygen storage/transport, electron transfer, gas molecule sensor, and redox catalysis of various substrates. We are working on OxdB to elucidate the structure-function relationships of this novel heme enzyme.

X-C-1 Regulation of Aldoxime Dehydratase Activity by Redox-Dependent Change in the Coordination Structure of the Aldoxime-Heme Complex

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Phenylacetaldoxime dehydratase from *Bacillus* sp. strain OxB-1 (OxdB) catalyzes the dehydration of Z-phenylacetaldoxime (PAOx) to produce phenylacetonitrile. OxdB contains a protoheme that works as the active center of the dehydration reaction. The enzymatic activity of ferrous OxdB was 1150-fold higher than that of ferric OxdB, indicating that the ferrous heme was the active state in OxdB catalysis. Although ferric OxdB was inactive, the substrate was bound to the ferric heme iron. Electron paramagnetic resonance spectroscopy revealed that the oxygen atom of PAOx was bound to the ferric heme, whereas PAOx was bound to the ferrous heme in OxdB *via* the nitrogen atom of PAOx. These results show a novel mechanism by which the activity of a heme enzyme is regulated; that is, the oxidation state of the heme controls the coordination structure of a substrate-heme complex, which regulates enzymatic activity. Rapid scanning spectroscopy using stopped-flow apparatus revealed that a reaction intermediate (the PAOx-ferrous OxdB complex) showed Soret, α and β bands at 415, 555, and 524 nm, respectively. The formation of this intermediate complex was very fast, finishing within the dead time of the stopped-flow mixer (3 ms). Site-directed mutagenesis revealed that His-306 was the catalytic residue responsible for assisting the elimination of the hydrogen atom of PAOx. The pH dependence of OxdB activity suggested that another amino acid residue that assists the elimination of the OH group of PAOx would work as a catalytic residue along with His-306.