IX-E Bioinorganic Chemistry of Heme-Based Sensor Proteins

Heme-based sensor proteins are a newly recognized class of heme proteins, in which the heme acts as a sensor of gaseous effector molecules such as O_2, NO, and CO. Our research interests focus on the CO-sensing transcriptional activator CooA and the O_2-sensing signal transducer HemAT. We have elucidated the structure and function relationships of CooA and HemAT by mutagenesis and some spectroscopic studies.

IX-E-1 Ligand-Switching Intermediates for the CO-Sensing Transcriptional Activator CooA Measured by Pulse Radiolysis

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CooA is a heme-containing and CO-sensing transcriptional activator whose activity is regulated by CO. The protoheme that acts as a CO sensor in CooA shows unique properties for its coordination structure. The Cys^{75} axial ligand of the ferric heme is replaced by His^{77} upon the reduction of the heme iron, and *vice versa*. In this work, the ligand-switching process induced by the reduction of the heme was investigated by the technique of pulse radiolysis. Hydrated electron reduced the heme iron in ferric CooA within 1 μs to form the first intermediate with the Soret peak at 440 nm, suggesting that a six-coordinated ferrous heme with a thiolate axial ligand was formed initially. The first intermediate was converted into the second intermediate with the time constant of 40 μs ($k = 2.5 \times 10^4$ s^{-1}). In the second intermediate, the thiolate from Cys^{75} was thought to be protonated and/or the Fe–S bond was thought to be elongated. The second intermediate was converted into the final reduced form with the time constant of 2.9 ms ($k = 3.5 \times 10^2$ s^{-1}) for wild-type CooA. The ligand exchange between Cys^{75} and His^{77} took place during the conversion of the second intermediate into the final reduced form.

IX-E-2 Conformational Dynamics of the Transcriptional Regulator CooA Protein Studied by Subpicosecond Mid-Infrared Vibrational Spectroscopy

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CooA, which is a transcriptional regulator heme protein allosterically triggered by CO, is studied by femtosecond visible-pump mid-IR-probe spectroscopy. Transient bleaching upon excitation of the heme in the Soret band is detected at approximately 1979 cm^{-1}, which is the absorption region of the CO bound to the heme. The bleach signal shows a nonexponential decay with time constants of 56 and 290 ps, caused by the rebinding of the CO to the heme. About 98% of dissociated CO recombines geminately. The geminate recombination rate in CooA is significantly faster than those in myoglobin and hemoglobin. The angle of the bound CO with respect to the porphyrin plane is calculated to be about 78 degrees on the basis of the anisotropy measurements. A shift of the bleached mid-IR spectrum of the bound CO is detected and has a characteristic time of 160 ps. It is suggested that the spectral shift is caused by a difference in the frequency of the bound CO in different protein conformations, particularly in an active conformation and in an intermediate one, which is on the way toward an inactive conformation. Thus, the biologically relevant conformation change in CooA was traced. Possible assignment of the observed conformation change is discussed.

IX-E-3 Resonance Raman and Ligand Binding Studies of the Oxygen Sensing Signal Transducer Protein HemAT from *Bacillus subtilis*

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HemAT-Bs is a heme-containing signal transducer protein responsible for aerotaxis of *Bacillus subtilis*. The recombinant HemAT-Bs expressed in *E. coli* was purified as the oxy form in which oxygen was bound to the ferrous heme. Oxygen binding and dissociation rate constants were determined to be $k_{on} = 32$ $\mu M^{-1}s^{-1}$ and $k_{off} = 23$ s^{-1}, respectively, revealing that HemAT-Bs has a moderate oxygen affinity similar to that of sperm whale Mb. The rate constant for autoxidation at 37 °C was 0.06 h^{-1}, which is also close to that of Mb. Although the electronic absorption spectra of HemAT-Bs were similar to those of Mb, HemAT-Bs showed some unique characteristics in its resonance Raman spectra. Oxygen-bound HemAT-Bs gave the $\nu(Fe–O_2)$ band at a noticeably low frequency (560 cm^{-1}), which suggests a unique hydrogen bonding between a distal amino acid residue and the proximal atom of the bound oxygen molecule. Deoxy HemAT-Bs gave the $\nu_{Fe–His}$ band at a higher frequency (225 cm^{-1}) than those of ordinary His-coordinated deoxy heme proteins. CO-bound HemAT-

Bs gave the ν(Fe–CO) and ν(C–O) bands at 494 and 1964 cm^{-1}, respectively, which fall on the same ν(C–O) *vs* ν(Fe–CO) correlation line as that of Mb. Based on these results, the structural and functional properties of HemAT-Bs are discussed.