X-B Bioinorganic Chemistry of Heme-Based Sensor Proteins

Studies of heme-containing gas sensor proteins have revealed a novel function for heme, which acts as an active site for sensing the corresponding gas molecule of a physiological effector. 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) and O_2 sensor protein (HemAT).

X-B-1 Activation Mechanisms of Transcriptinal Regulator CooA Revealed by Small-Angle X-Ray Scattering

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CooA, a heme-containing transcriptional activator, binds CO to the heme moiety and then undergoes a structural change that promotes the specific binding to the target DNA. To elucidate the activation mechanism coupled to CO binding, we investigated the CO-dependent structural transition of CooA with small-angle X-ray scattering (SAXS). In the absence of CO, the radius of gyration R_g and the second virial coefficient (A_2) were 25.3(±0.5) Å and –0.39(±0.25) × 10^{-4} ml mol g^{-2}, respectively. CO binding caused a slight increase in R_g (by 0.5 Å) and a marked decrease in A_2 (by 5.09 × 10^{-4} ml mol g^{-2}). The observed decrease in A_2 points to higher attractive interactions between CO-bound CooA molecules in solution compared with CO-free CooA. Although the minor alternation of R_g rules out changes in the overall structure, the marked change in the surface properties points to a CO-induced conformational transition. The experimental R_g and SAXS curves of the two states did not agree with the crystal structure of CO-free CooA. We thus simulated the solution structures of CooA based on the experimental data using rigid-body refinements as well as low-resolution model reconstructions. Both results demonstrate that the hinge region connecting the N-terminal heme domain and C-terminal DNA-binding domain is kinked in CO-free CooA, so that the two domains are positioned close to each other. The CO-dependent structural change observed by SAXS corresponds to a slight swing of the DNA-binding domains away from the heme domains coupled with their rotation by about 8 degrees around the axis of 2-fold symmetry.

X-B-2 Structure and Function of the CO-Sensor Protein CooA

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CooA is a heme-containing and CO-sensing transcriptional activator whose activity is regulated by CO, which is the first example of a transcriptional regulator containing a heme as a prosthetic group and of a heme protein in which CO plays a physiological role. A protoheme acts as a CO sensor in CooA. Mutagenesis and spectroscopic studies on CooA from *R. rubrum* (Rr-CooA) have revealed that the heme in Rr-CooA shows several unique features as described in the following: (1) The exchange of the axial ligand of the heme takes place during the change in the oxidation state of the heme iron. Cys^{75}, one of the axial ligands of the ferric heme, is replaced by His^{77} when the heme in CooA is reduced, and vice versa. (2) Hysteresis is observed in electrochemical redox titration, *i.e.*, the observed reduction and oxidation midpoint potentials are –320 mV and –260 mV, respectively. (3) Although the ferrous heme is 6-coordinate with two endogenous axial ligands, CO reacts with the ferrous heme to form the CO-bound heme under physiological conditions. Only CO-bound Rr-CooA is active as the transcriptional activator.

A CooA homologue from a thermophilic CO oxidizing bacterium, *C. hydrogenoformans*, (Ch-CooA) has also been studies. Ch-CooA shows different properties from Rr-CooA for the coordination structure and redox properties. Some information of what is essential for CooA function have been elucidated by comparing the properties between Ch-CooA and Rr-CooA.

X-B-3 Structure and Function 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*, 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.

Oxygen binding and dissociation rate constants are determined to be k_{on} = 32 μM^{-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 is 0.06 h^{-1}, which is also close to that of Mb. Although the electronic absorption spectra of HemAT-Bs are similar to those of Mb, HemAT-Bs shows some unique characteristics in its resonance Raman spectra. Oxygen-bound HemAT-Bs gives the ν(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 gives the ν(Fe–His) band at a higher fre-

quency (225 cm^{-1}) than those of ordinary His-coordinated deoxy heme proteins.

HemAT-Bs consists of two domains, a N-terminal sensor domain and a C-terminal signaling domain. We have also prepared a truncated mutant consisting of the only N-terminal sensor domain. The heme environmental structure is perturbed by truncating the C-terminal domain. The resonance Raman spectroscopy reveals that a hydrogen bonding pattern toward the heme-bound oxygen is different from each other between wild-type and sensor domain mutant. The rate constant for autoxidation is 0.6 h^{-1} for the sensor domain mutant. The oxygen binding kinetics are also changed for the sensor domain mutant to be k_{on} = 69 μM^{-1} s^{-1} and k_{off} = 1.2 s^{-1}, indicating the binding affinity of oxygen increases in this mutant compared with wild-type HemAT-Bs.