IX-B 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-B-1 Characterization of the Heme Environmental Structure of Cytoglobin, a Fourth Globin in Humans

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Cytoglobin (Cgb) represents a fourth member of the globin superfamily in mammals, but its function is unknown. Site-directed mutagenesis, in which six histidine residues were replaced with alanine, was carried out, and the results indicate that the imidazoles of His81 (E7) and His113 (F8) bind to the heme iron as axial ligands in the hexacoordinate and the low-spin state. The optical absorption, resonance Raman, and IR spectral results are consistent with this conclusion. The redox potential measurements revealed an *E*' of 20 mV (*vs* NHE) in the ferric/ferrous couple, indicating that the imidazole ligands of His81 and His113 are electronically neutral. On the basis of the ν(Fe–CO) and ν(C–O) values in the resonance Raman and infrared spectra of the ferrous–CO complexes of Cgb and its mutants, it was found that CO binds to the ferrous iron after the His81 imidazole is dissociated, and three conformers are present in the resultant CO coordination structure. Two are in closed conformations of the heme pocket, in which the bound CO ligand interacts with the dissociated His81 imidazole, while the third is in an open conformation. The ν(Fe–O_2) in the resonance Raman spectra of oxy Cgb can be observed at 572 cm^{-1}, suggesting a polar heme environment. These structural properties of the heme pocket of Cgb are discussed with respect to its proposed *in vivo* oxygen storage function.

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

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CooA from a photosynthetic bacterium, *R. rubrum*, is the only example of CO-sensor protein so far. We have found a CooA homologue in a thermophilic CO oxidizing bacterium, *Carboxydothermus hydrogenoformans*, and constructed an expression system of CooA from *C. hydrogenoformans* (Ch-CooA) as described below. The gene encoding Ch-CooA was prepared by PCR with a chromosomal DNA of *C. hydrogenoformans* as the template and the two primers (5'-AGG AGA GGA CTA TGG CCA CCC AAA TGA GAT TAA CCG AC-3' and 5'- TTA CTA AAC GCC TGA GGA AAA CTC-3'). The DNA fragment containing *Ch-cooA* was subcloned in pCR4-TOPO vector and then inserted at the EcoRI-site in pKK223-3 vector to construct an expression vector. Ch-CooA was expressed in the soluble fraction of *E. coli* and purified to a homogeneous state by using Q-Sepharose, Hitrap Heparin, and Sephacryl S-100 column chromatography. The purified Ch-CooA showed typical uv/vis spectra for 6-coordinate, low-spin heme proteins. Characterization of the biochemical and biophysical properties of Ch-CooA is now in progress.

IX-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*. The recombinant HemAT-Bs expressed in *E. coli* is purified as the oxy form in which oxygen is bound to the ferrous heme. HemAT-Bs as isolated gives the Soret, α and β peaks at 414, 578, and 543 nm, respectively. This spectrum is typical of 6-coordinate, low-spin hemoproteins and resembles that of the oxy form of Mb. On deoxygenation with sodium dithionite, HemAT-Bs shows a spectrum with the Soret peak at 431 nm and a single peak at 563 nm in the Q-band region. This spectrum is typical of 5-coordinate, high-spin ferrous hemoproteins, which shows the formation of deoxy HemAT-Bs. CO-bound HemAT-Bs is formed upon the reaction of dithionite reduced HemAT-Bs with CO, which shows the Soret, α and β peaks at 422, 567, and 543 nm, respectively.

HemAT-Bs consists of two domains, *i.e.*, the N-terminal sensor domain that contains a heme and the C-terminal signaling domain. When the C-terminal signaling domain is truncated, the resulting mutants show a different oxygen affinity and a heme environmental structure from those of wild-type HemAT-Bs. These results suggest that a conformational change around the heme pocket is responsible for the intramolecular signal transduction from the sensor domain to the signaling domain.