# RESEARCH ACTIVITIES X
# Okazaki Institute for Integrative Bioscience

## X-A 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-A-1 Effect of Mutation on the Dissociation and Recombination Dynamics of CO in Transcriptional Regulator CooA: A Picosecond Infrared Transient Absorption Study

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[*Biochemistry* **45**, 9246–9253 (2006)]

The CO ligation process in a mutant (H77G) of CooA, the CO-sensing transcriptional regulator in *Rhodospirillum rubrum*, is studied with femtosecond time-resolved transient absorption spectroscopy in the mid-infrared region. Following photolyzing excitation, a transient bleach in the vibrational region of bound CO due to the CO photodissociation is detected. In contrast to the spectra of the wild-type (WT) CooA, the transient bleach spectra of H77G CooA show a bimodal shape with peaks shifting to the lower frequency during spectral evolution. The CO recombination dynamics show single-exponential behavior, and the CO escaping yield is higher than that of the WT CooA. A reorientation process of CO relative to the heme plane during recombination is revealed by anisotropy measurements. These phenomena indicate changes in the protein response to the CO ligation and suggest an alteration to the CO environment caused by the mutation. On the basis of these results, the role of His77 in the CO-dependent activation of CooA and a possible activation mechanism involving collaborative movement of the heme and the amino residues at both sides of the heme plane are discussed.

### X-A-2 Evidence for Displacements of the C-Helix by CO Ligation and DNA Binding to CooA Revealed by UV Resonance Raman Spectroscopy

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[*J. Biol. Chem.* **281**, 11271–11278 (2006)]

The UV and visible resonance Raman spectra are reported for CooA from Rhodospirillum rubrum, which is a transcriptional regulator activated by growth in a CO atmosphere. CO binding to heme in its sensor domain causes rearrangement of its DNA-binding domain, allowing binding of DNA with a specific sequence. The sensor and DNA-binding domains are linked by a hinge region that follows a long C-helix. UV resonance Raman bands arising from Trp-110 in the C-helix revealed local movement around Trp-110 upon CO binding. The indole side chain of Trp-110, which is exposed to solvent in the CO-free ferrous state, becomes buried in the CO-bound state with a slight change in its orientation but maintains a hydrogen bond with a water molecule at the indole nitrogen. This is the first experimental data supporting a previously proposed model involving displacement of the C-helix and heme sliding. The UV resonance Raman spectra for the CooA-DNA complex indicated that binding of DNA to CooA induces a further displacement of the C-helix in the same direction during transition to the complete active conformation. The Fe–CO and C–O stretching bands showed frequency shifts upon DNA binding, but the Fe–His stretching band did not. Moreover, CO-geminate recombination was more efficient in the DNA-bound state. These results suggest that the C-helix displacement in the DNA-bound form causes the CO binding pocket to narrow and become more negative.

### X-A-3 Crystallization and Preliminary X-Ray Analysis of CooA from *Carboxydothermus hydrogenoformans*

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[*Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.* **62**, 471–473 (2006)]

CooA, a homodimeric haem-containing protein, is responsible for transcriptional regulation in response to carbon monoxide (CO). It has a b-type haem as a CO sensor. Upon binding CO to the haem, CooA binds promoter DNA and activates expression of genes for CO metabolism. CooA from *Carboxydothermus hydrogeno-*

*formans* has been overexpressed in *Escherichia coli*, purified and crystallized by the vapour-diffusion method. The crystal belongs to space group $P2_1$, with unit-cell parameters $a$ = 61.8, $b$ = 94.7, $c$ = 92.8 angstroms, β = 104.8 degrees. The native and anomalous difference Patterson maps indicated that two CooA dimers are contained in the asymmetric unit and are related by a translational symmetry almost parallel to the *c* axis.

### X-A-4 Specific Hydrogen-Bonding Networks Responsible for Selective $O_2$ Sensing of the Oxygen Sensor Protein HemAT from *Bacillus subtilis*

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[*Biochemistry* **45**, 8301–8307 (2006)]

HemAT from *Bacillus subtilis* (HemAT-Bs) is a heme-based $O_2$ sensor protein that acts as a signal transducer responsible for aerotaxis. HemAT-Bs discriminates its physiological effector, $O_2$, from other gas molecules to generate the aerotactic signal, but the detailed mechanism of the selective $O_2$ sensing is not obvious. In this study, we measured electronic absorption, electron paramagnetic resonance (EPR), and resonance Raman spectra of HemAT-Bs to elucidate the mechanism of selective $O_2$ sensing by HemAT-Bs. Resonance Raman spectroscopy revealed the presence of a hydrogen bond between His86 and the heme propionate only in the $O_2$-bound form, in addition to that between Thr95 and the heme-bound $O_2$. The disruption of this hydrogen bond by the mutation of His86 caused the disappearance of a conformer with a direct hydrogen bond between Thr95 and the heme-bound $O_2$ that is present in WT HemAT-Bs. On the basis of these results, we propose a model for selective $O_2$ sensing by HemAT-Bs as follows. The formation of the hydrogen bond between His86 and the heme propionate induces a conformational change of the CE-loop and the E-helix by which Thr95 is located at the proper position to form the hydrogen bond with the heme-bound $O_2$. This stepwise conformational change would be essential to selective $O_2$ sensing and signal transduction by HemAT-Bs.

### X-A-5 Recognition and Discrimination of Gases by the Oxygen-Sensing Signal Transducer Protein HemAT as Revealed by FTIR Spectroscopy

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[*Biochemistry* **45**, 7763–7766 (2006)]

The determination of ligand binding properties is a key step in our understanding of gas sensing and discrimination by gas sensory proteins. HemAT is a newly discovered signal transducer heme protein that recognizes $O_2$ and discriminates against other gases such as CO and NO. We have used FTIR spectroscopy on CO- and NO-bound sensor domain HemAT and sensor domain distal mutants Y70F, T95A, R91A, and L92A to gain insight into the structure of the iron-bound ligand at ambient temperature. These mutations were designed to perturb the electrostatic field near the iron-bound gaseous ligand and also allow us to investigate the communication pathway between the distal residues of the protein and the heme. We show the formation of both H-bonded and non-H-bonded conformations in the CO-bound forms. In addition, we report the presence of multiple conformations in the NO-bound forms. Such distal H-bonding is crucial for ligand binding and activation by the heme. The comparison of the $O_2$, NO, and CO data demonstrates that Thr95 and Tyr70 are crucial for ligand recognition and discrimination and, thus, for specific sensing of gases, and L92 is crucial for controlling the conformational changes of the Thr95 and Tyr70 residues upon NO binding.

### X-A-6 Biophysical Properties of a *c*-Type Heme in Chemotaxis Signal Transducer Protein DcrA

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[*Biochemistry* **44**, 15406–15413 (2005)]

Chemotaxis signal transducer protein DcrA from a sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough was previously shown to contain a *c*-type heme in its periplasmic domain (DcrA-N) for sensing redox and/or oxygen, 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 spectroscopies indicates that 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). Our study supports the initial idea by Fu *et al*. that DcrA would act as a redox and/or oxygen sensor, in which the ligand exchange between water and an endogenous amino acid is a trigger for signal transduction. While the affinity of CO to DcrA-N ($K_d$ = 138 μM) is significantly weak compared to those of other heme proteins, we suggest that CO might be another physiological effector molecule.

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

Aldoxime dehydratases from various bacteria catalyze the dehydration reaction of aldoxime to produce nitrile under mild conditions. Aldoxime dehydratase from *Bacillus* sp. OxB-1 (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 and OxdRE (aldoxime dehydratase from *Rhodococcus* sp. N-771) to elucidate the structure-function relationships of these novel heme enzymes.

## X-B-1 Spectroscopic and Substrate Binding Properties of Heme-Containing Aldoxime Dehydratases, OxdB and OxdRE

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[*J. Inorg. Biochem.* **100**, 1069–1074 (2006)]

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, , and 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.