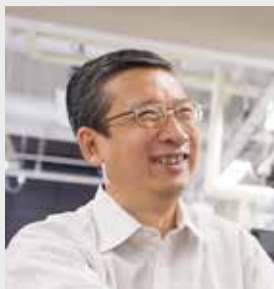# Bioinorganic Chemistry of Metalloproteins Responsible for Signal Sensing

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1982 B.S. Tokyo Institute of Technology
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Gas molecules such as O_2, NO, CO and ethylene are present in the environment and are endogenously (enzymatically) produced to act as signaling molecules in biological systems. Sensing these gas molecules is the first step in their acting as signalling molecules. Sensor proteins are usually required. Input signals generated by gas sensing have to transduce to output signals that regulate biological functions. This is achieved by biological signal-transduction systems. Recognition of the cognate gas molecules is a general mechanism of functional regulation for gas-sensor proteins. This induces conformational changes in proteins that controls their activities for following signal transductions. Interaction between gas molecules and sensor proteins is essential for recognition of gas molecules. Metal-containing prosthetic groups are widely used. In my research group, our research focuses on heme-based gas-sensor proteins and the signalling systems working with them.

The prosthetic group heme acts as the active center of hemeproteins that show a variety of functions, including O_2 or NO storage/transport, electron transfer, redox catalysis of various substrate, and dehydration of aldoxime. In the present context, it acts as the active site for sensing of diatomic gas molecules such as NO, O_2, and CO. These gas molecules are able to bind to heme iron as an axial ligand, which is a reason why heme can be adapted as the active center for sensing gas molecules. Heme-based gas-sensor proteins constitute a major group in the gas-sensor proteins. Binding of a cognate gas molecule to heme is the initial step for gas sensing, which is followed by the signalling processes. The binding affinities of gas molecules, that measures of the sensitivities of the sensor proteins, can be controlled by heme environmental structures. Differences in the heme coordination structure of the axial ligand(s) and/or of interaction(s) between the heme-bound gas molecule and surrounding amino acid residue(s) in a heme pocket play important roles. They not only regulate the binding affinities of gas molecules but also discriminate one cognate effector gas molecule from others, allowing the sensor to respond with the proper signal transductions. We have been elucidating the relationships of structures and functions of heme-based sensor proteins by crystallographic, biochemical, biophysical, and molecular biological studies.

Selected Publications

• A. Pavlou, H. Yoshimura, S. Aono and E. Pinakoulaki, "Protein Dynamics of the Sensor Protein HemAT as Probed by Time-Resolved Step-Scan FTIR Spectroscopy," *Biophys. J.* **114**, 584–591 (2018).

• A. Pavlou, A. Loullis, H. Yoshimura, S. Aono and E. Pinakoulaki, "Probing the Role of the Heme Distal and Proximal Environment in Ligand Dynamics in the Signal Transducer Protein HemAT by Time-Resolved Step-Scan FTIR and Resonance Raman Spectroscopy," *Biochemistry* **56**, 5309–5317 (2017).

• N. Muraki, C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Structural Characterization of Heme Environmental Mutants of CgHmuT that Shuttles Heme Molecules to Heme Transporters," *Int. J. Mol. Sci.* **17**, 829 (2016).

• N. Muraki and S. Aono, "Structural Basis for Heme Recognition by HmuT Responsible for Heme Transport to the Heme Transporter in *Corynebacterium glutamicum*," *Chem. Lett.* **45**, 24–26 (2015).

• C. Kitatsuji, M. Ogura, T. Uchida, K. Ishimori and S. Aono, "Molecular Mechanism for Heme-Mediated Inhibition of 5-Aminolevulinic Acid Synthase 1," *Bull. Chem. Soc. Jpn.* **87**, 997–1004 (2014).

• Y. Okamoto, H. Sawai, M. Ogura, T. Uchida, K. Ishimori, T. Hayashi and S. Aono, "Heme-Binding Properties of HupD Functioning as a Substrate-Binding Protein in a Heme-Uptake ABC-Transporter System in *Listeria monocytogenes*," *Bull. Chem. Soc. Jpn.* **87**, 1140–1146 (2014).

1. Protein Dynamics and Signal Transduction of Heme-Based Oxygen Sensor Protein HemAT-Bs

HemAT from *Bacillus subtilis* (HemAT-*Bs*) is a heme-based O_2 sensor protein responsible for aerotaxis (chemotaxis toward molecular oxygen) control in this bacterium. It consists of the N-terminal sensor domain in which heme acts as an oxygen sensing site and C-terminal signaling domain. The binding of O_2 to the heme induces a conformational change in the sensor domain of HemAT-*Bs*, triggering intramolecular signal transductions to result in the regulation of the chemotactic signaling in *B. subtilis*. An important issue to understand the signal transduction mechanism of HemAT-*Bs* is to reveal the pathway to transmit the conformational changes induced upon ligand binding/dissociation to/from the heme. In this study, we have elucidated conformational changes upon CO ligand dissociation from the heme for full-length wild-type HemAT, and the Y70F (B-helix), L92A (E-helix), T95A (E-helix), and Y133F (G-helix) HemAT mutants by time-resolved step-scan FTIR spectroscopy.

These mutations perturb hydrogen bonding and electrostatic interactions between the heme-bound ligand and the surrounding amino acid residues. While Tyr70 and Thr95 in the distal heme pocket form hydrogen bonds to the heme-bound O_2, a reversible hydrogen bond formation/cleavage takes place between Tyr133 and His123 upon ligand binding/dissociation to/from the heme in the proximal heme pocket. Rebinding of CO to the heme is biphasic in the sensor domain and full-length HemAT as well as in the mutants, with the exception of the Y133F mutant protein. The monophasic rebinding of CO in Y133F suggests that the ligand rebinding process is significantly affected in the absence of the hydrogen bond between Tyr133 and His123 residue in the proximal heme pocket.

Time-resolved step-scan FTIR studies reveal the spectral components to discrete substructures, which originate from a helical structure that is solvated ($1638\ cm^{-1}$) and a native helix that is protected from solvation by interhelix tertiary interactions ($1654\ cm^{-1}$). The full-length protein is characterized by an additional amide I absorbance at $1661\ cm^{-1}$, which is attributed to disordered structure suggesting that further protein conformational changes occur in the presence of the signaling domain in the full-length protein. The kinetics monitored within the amide I absorbance of the polypeptide backbone in the sensor domain exhibit two distinct relaxation phases ($t_1 = 24$ and $t_2 = 694\ \mu s$), whereas that of the full-length protein exhibits monophasic behavior for all substructures in a time range of $t = 1253$–$2090\ \mu s$. These observations can be instrumental in monitoring helix motion and the role of specific mutants in controlling the dynamics in the communication pathway from the sensor to the signaling domain. The kinetics observed for the amide I relaxation for the full-length protein indicate that the discrete substructures within full-length HemAT, unlike those of the sensor domain, relax independently.
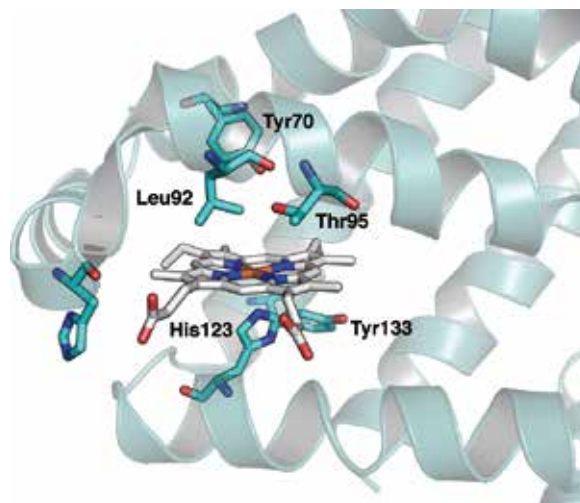

Figure 1. Heme environmental structure of HemAT-*Bs*.

2. Molecular Mechanisms for Biosynthesis and Maturation of Hydrogen Sensing Regulatory Hydrogenase

Regulatory hydrogenase (RH), HoxJ, and HoxA proteins consist a H_2-dependent regulatory system of gene expression for proteins involved in hydrogen metabolism, in which RH acts as a molecular hydrogen sensor. RH consists of two subunits, a large subunit containing the Ni-Fe dinuclear complex and a small subunit containing iron-sulfur clusters. Though the Ni-Fe dinuclear complex in the large subunit is assumed to be the active site for H_2 sensing by RH, the molecular mechanisms of biosynthesis maturation of the Ni-Fe dinuclear complex and RH protein remain elusive. Several accessory proteins are involved in the formation of the Ni-Fe complex and its insertion into the large subunit to mature RH. We are now elucidating the structural and functional relationships of the accessory protein HypX responsible for the construction of the Fe(CO) unit in the Ni-Fe dinuclear complex in RH. We have obtained single crystals of HypX and the crystallographic analyses of HypX are now in progress.