Bioinorganic Chemistry of Metalloproteins Responsible for Signal Sensing

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

Hydrogenases are metalloenzymes that catalyze the oxidation of H_2 into electrons and protons and the reduction of protons into H_2 reversibly, which are expected as biocatalysts for fuel cells and H_2 production for clean and sustainable energy. Based on the differences of metal content and the structure of the active site, they are classified into three groups: FeFe-, NiFe-, and Fe-hydrogenases containing a dinuclear Fe unit linked to a [4Fe-4S] cluster, a hetero dinuclear Ni-Fe cluster, and a mononuclear Fe center, respectively. In addition to the enzymatic function of hydrogenases, some hydrogenase that is classified as a regulatory hydrogenase (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 and maturation of the Ni-Fe dinuclear complex are not clear yet.

CO and CN^- ligands are coordinated to the Fe in the Ni-Fe dinuclear complexe in RH. These CO and CN^- are biosynthesized and assembled into the metal clusters, for which several accessory and chaperone proteins are required. It is reported recentrly that HypX protein is responsible for CO biosynthesis for the maturation of Ni-Fe hydrogenases including RH, but the detailed molecular mechanism of CO biosynthesis by HypX is not elucidated. 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 dinulear complex in RH. We have obtained single crystals of HypX and determined the crystal structure.

HypX consists of the N-terminal (residues 1-270) and the C-terminal (residues 289-542) domains with the C-terminal tail (residues 543-562) as shown in Figure 1. The N- and C-terminal domains are linked by a loop (residues 271-288). The N-terminal domain is composed of two subdomains, subdomains A (residues 1-151) and B (residues 182-270), which are linked by a long loop (residues 152-181). The subdomain A consists of six β-strands and five α-helices. It forms a Rossmann-fold with a mixed parallel β-sheet, which is constructed by six β-strands that is sandwiched by two sets of two α-helices. The subdomain B has a barrel-helix framework as an oligonucleotide/oligosaccharide-binding (OB) fold consisting of six β-strands and one α-helix, in which six β-strands form an open barrel-like structure.

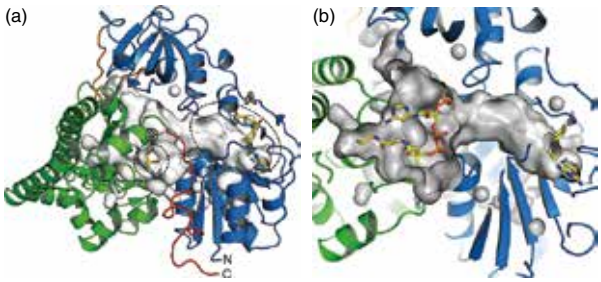

Figure 1. (a) Overall structure of HypX, in which the N-terminal domain (blue) and the C-terminal domain (green) are linked by a loop (orange). The cavity is shown in gray. (b) A sectional view of the cavity. CoA and THF are shown in the stick model.

A continuous cavity connecting the N- and C-terminal domains is present in the interior of HypX. Coenzyme A (CoA) is bound in the C-terminal region of the cavity with a "folded conformation" in which adenine and pantetheine groups are stacked in parallel. The HypX-THF complex has been obtained by soaking HypX crystals with THF. THF is bound at the N-terminal region inside the cavity.

The crystallographic analyses reveal that HypX consists of the N- and C-terminal domains that are structurally homologous to the hydrolase domain of N^{10}-formyl-tetrahydrofolate (N^{10}-formyl-THF) dehydrogenase (FDH) and enoyl-CoA hydratase/isomerase (ECH/ECI), respectively. The comparison of amino acid sequences and crystal structures between HypX and FDH reveals that His and Asp among the catalytic triad are conserved at the corresponding positions (His74 and Asp109 in HypX). Though Ser among the catalytic triad is not conserved in HypX, Asp80 forms a hydrogen bond with τN of His74, which fixes the orientation of His74 as does Ser106 in FDH. Asp80 not only sustain a functional role for the fixation of the orientation of His74 but may enhance the catalytic activity of Asp109 through the hydrogen bonding network among His74, Asp80, and Asp109. Thus, HypX adopts a slightly modified catalytic triad for formyl-group transfer reaction. While 4-phosphopantetheine in ACP accepts formyl group from N^{10}-formyl-THF in the case of FDH, CoA will do so for HypX because it has the phosphopantetheine moiety identical to ACP.

While CoA adopts the folded conformation in HypX, an "extended conformation" of CoA, in which the ADP and pantetheine moieties are extended in a linear fashion, is observed in some CoA-dependent enzymes. We examined whether the extended conformation of CoA was also available in HypX and found that CoA is able to adopt the extended conformation in the A392F-I419F variant. Ala392 and Ile419 are located near the pantetheine moiety of the folded form of CoA, whose positions correspond to "a neck of a bottle" accommodating the pantetheine moiety of CoA in the folded form. Replacing Ala392 and Ile419 with Phe will narrow "the neck of a bottle," which will destabilize the folded conformation of CoA by a steric hindrance to make the extended conformation of CoA more favorable because the residues 392 and 419 are no longer interacting with the pantetheine moiety of CoA in the extended conformation. In fact, we have found that CoA in the A392F-I419F variant adopts the extended conformation. Taken together, we propose the following reaction scheme of CO biosynthesis by HypX. HypX will catalyze two consecutive reactions, the formyl-group transfer from N^{10}-formyl-THF to CoA and decarbonylation of formyl-CoA, in the N- and C-terminal domains, respectively, to produce CO.

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