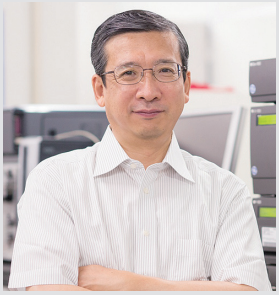
Bioinorganic Chemistry of Metalloproteins Responsible for Metal Homeostasis and Signal Sensing

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Transition metal ions and metalloproteins play crucial roles in meeting the energy demands of the cell by playing roles in intermediary metabolism and in signal transduction processes. Although they are essential for biological function, metal ion bioavailability must be maintained within a certain range in cells due to the inherent toxicity of all metals above a threshold. This threshold varies for individual metal ions. Homeostasis of metal ions requires a balance between the processes of uptake, utilization, storage, and efflux and is achieved by the coordinated activities of a variety of proteins including extracytoplasmic metal carriers, ion channels/pumps/transporters, metal-regulated transcription and translation proteins, and enzymes involved in the biogenesis of metal-containing cofactors/metalloproteins. In order to understand the processes underlying this complex metal homeostasis network, the study of the molecular processes that determine the protein-metal ion recognition, as well as how this event is transduced into a functional output, is required. My research interests are focused on the elucidation of the structure and function relationships of metalloproteins responsible for the regulation of biological homeostasis.

I am also working on gas sensor proteins. 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 signaling 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 transition metal-based gas-sensor proteins and the signaling systems working with them.

Selected Publications

- N. Muraki, C. Kitatsuji, Y. Okamoto, T. Uchida, K. Ishimori and S. Aono, "Structural Basis for Heme Transfer Reaction in Heme Uptake Machinery from Corynebacteria," *Chem. Commun.* **55**, 13864–13867 (2019).
- N. Muraki, K. Ishii, S. Uchiyama, S. G. Itoh, H. Okumura and S. Aono, "Structural Characterization of HypX Responsible for CO Biosynthesis in the Maturation of NiFe-Hydrogenase," *Commun. Biol.* **2**, 385 (12 pages) (2019).
- 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).

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

Regulatory hydrogenase (RH) that acts as a H_2 sensor 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, as shown in Figure1. In 2019, we have determined the crystal structure of HypX, which catalyzes CO biosynthesis for the ligand of the Ni-Fe complex in RH, to find that HypX adopt coenzyme A (CoA) as a cofactor for CO biosynthesis using formyl-tetrahydrofolate as a substrate to form formyl-CoA, and that formyl-CoA is the reaction intermediate to form CO.

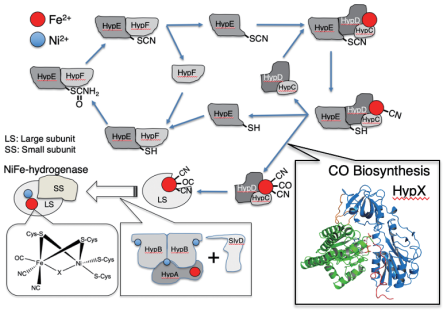

Figure 1. Reaction scheme of the biosynthesis and maturation of the Ni-Fe dinuclear complex for the active site in RH.

CO produced by HypX is used as a ligand of the iron in the $NiFe(CN)_2(CO)$ center of NiFe hydrogenases. The $Fe(CN)_2(CO)$ unit of the NiFe dinuclear center is assembled in the HypC/HypD complex as a scaffold. The binding site of the $Fe(CN)_2(CO)$ unit is proposed to be located at the bottom of a tunnel ca. 20 Å deep inside from the protein surface in the HypC/HypD complex, to which Fe is initially bound and then CN^- and CO ligands bind to the Fe. If CO produced by HypX is diffused into solvent, it will be inefficient for the assembly of the $Fe(CN)_2(CO)$ unit in the HypC/HypD. It may be a solution to utilize CO produced by HypX effectively is that HypX and HypC/HypD form a complex. The SEC analyses reveal the formation of HypC/HypD and HypC/HypD/HypX complexes as described below.

HypC (10.4 kDa in monomer) and HypD (45.0 kDa in monomer) are eluted from a Superdex75 column with an apparent mass of 21.6 kDa and 35.5 kDa, respectively, indicating that HypC and HypD exist as a homo-dimer and monomer in solution, respectively. The mixture of HypC and HypD is eluted with an apparent mass of 76.4 kDa. Though this result indicates the formation of the complex between HypC and HypD (probably $(HypC)_2HypD$ complex), its quaternary structure is not clear at present. The mixture of HypC, HypD, and HypX was eluted from a Superdex200 column with an apparent mass of 119.7 kDa, suggesting the formation of the 1:1:1 complex of HypC, HypD, and HypX. The structural characterization of this complex is now in progress.

2. Structural Basis for Heme Transfer Reaction in Heme Uptake Machinery from Corynebacteria

Corynebacteria including *Corynebacterium diphtheriae* and *Corynebacterium glutamicum*, which are classified as a high GC content Gram-positive bacteria (Actinomycetes), adopt a different heme uptake machinery (Hta/Hmu system). It consists of the membrane-bound heme binding/transport proteins (HtaA and HtaB) and the ABC-type heme transporter system (HmuTUV). HtaA and HtaB consist of two and one CR (Conserved Region) domains, respectively, which are responsible for heme binding/transport. In this study, we have determined the crystal structures of HtaA and HtaB from *C. glutamicum* to understand the structural basis of the heme-uptake in Corynebacteria.

Though these interactions and the overall structure are conserved among HtaA and HtaB, the orientation of heme is different from one another. Good fitting of the model into the electron density of heme was obtained with the single orientation of heme for HtaA. On the other hand, assuming a 1:1 mixture of two orientations of heme was needed to obtain good fitting for the electron density of heme in HtaB. Thus, heme is accommodated with a mixture of two different orientations in HtaB unlike HtaA. The difference of the heme orientation suggests that heme transfer reaction between HtaA and HtaB proceeds through the HtaA/HtaB complex formation.

We also determined the crystal structure of the apo-form of H434A-HtaA at a resolution of 2.0 Å. Though the holo-form of H434A-HtaA is a monomer, the apo-form of this variant was dimer. The N-terminal region (Ser364-Gly391) including the β1 strand and the α1 helix in each protomer are separated from the core region and each β1 strand is swapped between two protomers to form a domain-swapped dimer (Figure 2).

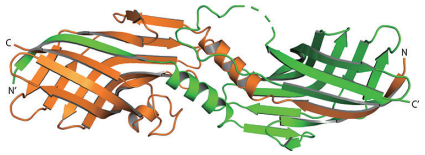

Figure 2. The structure of the apo-form of H343A-HtaA. The chains A and B are shown in orange and light green, respectively. A dotted line in the chain B is a disordered region including the α2 helix.

The structure of the domain-swapped dimer of apo-HtaA would be a model of a reaction intermediate for the heme transfer. In the domain-swapped dimer, the chain A would be a model of the holo-HtaA because it is superimposable to the structure of the holo-HtaA. The chain B in the domain-swapped dimer would be a model of the apo-HtaB in the holo-HtaA/apo-HtaB complex.