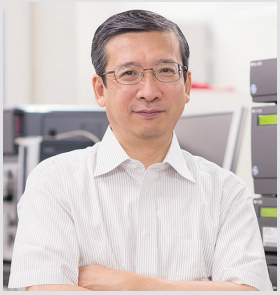
Bioinorganic Chemistry of Metalloproteins Responsible for Metal Homeostasis and Signal Sensing

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



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Keywords Bioinorganic Chemistry, Metalloproteins, Sensor Protein

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

- D. Matsui, N. Muraki, K. Chen, T. Mori, A. A. Ingram, K. Oike, H. Gröger, S. Aono and Y. Asano, "Crystal Structural Analysis of Aldoxime Dehydratase from *Bacillus sp.* OxB-1: Importance of Surface Residues in the Optimization for Crystallization," *J. Inorg. Biochem.* **230**, 111770–111779 (2022).
- Y. Ikenoue, Y. Tahara, M. Miyata, T. Nishioka, S. Aono and H. Nakajima, "Use of a Ferritin L134P Mutant for the Facile Conjugation of Prussian Blue in the Apoferritin Cavity," *Inorg. Chem.* **60**, 4693–4704 (2021).
- M. Nishinaga, H. Sugimoto, Y. Nishitani, S. Nagai, S. Nagatoishi, N. Muraki, T. Tosha, K. Tsumoto, S. Aono, Y. Shiro and H. Sawai, "Heme Controls the Structural Rearrangement of Its Sensor Protein Mediating Bacterial Survival," *Commun. Biol.* **4**, 467 (12 pages) (2021).
- N. Muraki, K. Takeda, D. Nam, M. Muraki and S. Aono, "Structural Characterization of Thermoglobin from a Hyperthermophilic Bacterium *Aquifex aeolicus*," *Chem. Lett.* **50**, 603–606 (2021).
- 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).

1. Complex Formation between [NiFe] Hydrogenase Maturation Factors Responsible for $Fe(CN)_2CO$ Biosynthesis

[NiFe] hydrogenase is a metalloenzyme that catalyzes the oxidation of hydrogen and the reduction of protons reversibly. As its name implies, the metal cluster of [NiFe] hydrogenase is composed of nickel and iron. In the active center of [NiFe] hydrogenase, nickel is ligated by the three cysteine side chains of the protein, while iron is coordinated with two cyanide ions and one carbon monoxide in addition to the cysteine side chains. This intricate metal complex is not spontaneously formed, but is biosynthesized step-by-step in coordination with multiple proteins. The cyanide ions and carbon monoxide are also biosynthesized to form $Fe(CN)_2CO$ complex, which is then incorporated into hydrogenase. We have reported the crystallographic analysis of HypX, which is an enzyme responsible for carbon monoxide biosynthesis during [NiFe] hydrogenase maturation. Additionally, we have showed that the HypC-HypD complex, which acts as a scaffold protein for $Fe(CN)_2CO$ biosynthesis, forms a complex with HypX.

Recently, we have determined the crystal structures of *A. aeolicus* HypC, HypD, and HypE, which are involved in cyanide ion transport (Figure 1 (A), (B), (C)). The crystal structures of these maturation factors show high structural similarity to previously reported structures of corresponding proteins from Archaea. Based on these structure, we propose that the HypCDXE complex will be transiently formed to assemble $Fe(CN)_2CO$ unit (Figure 1 (D)).

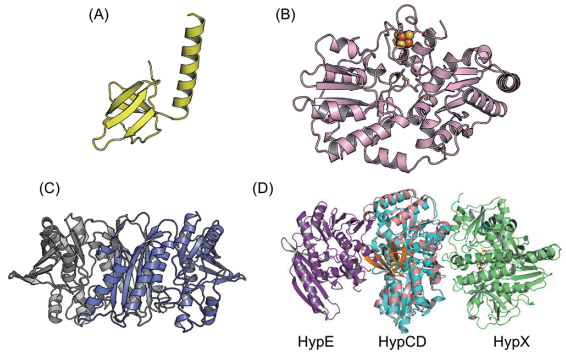

Figure 1. X-ray crystal structures of (A) HypC, (B) HypD, and (C) HypE from *Aquifex aeolicus*, and (D) proposed model of HypCDEX complex.

2. Structural Analysis of Heme-Based Oxygen Sensor Protein HemAT

Recently, it has been reported that gas molecules, including oxygen and nitric oxide, have a role as "signal molecules," and they regulate various physiological functions. In these systems, the regulation of physiological functions is performed when some signal transduction proteins that selectively recognize gas molecules sense external signals. Therefore, signal sensing and signal transduction proteins are essential for regulating physiological functions in response to gas molecules.

O_2 acts as a signal molecule in the bacterial chemotaxis regulating system, in which the heme-containing signal transducer protein HemAT (Heme Aerotaxis Transducer protein) works as an oxygen sensor protein. HemAT is mainly composed of two domains, sensor and siganaling domain. Sensor domain and signaling domain of HemAT is homologous to globin structures and the chemotaxis receptor Methyl-accepting Chemotaxis Protein (MCP), respectively.

HemAT forms a complex with the histidine kinase CheA and the conjugation protein CheW. Signal transduction proceeds in the HemAT/CheA/CheW complex upon O_2 sensing by HemAT, which results in the activation of CheA kinase activity. However, the molecular mechanisms of O_2-dependent signal transduction in the HemAT/CheA/CheW complex remain to be elucidated because of a lack of the structural information of HemAT and the HemAT/CheA/CheW complex. To understand the sensing mechanism and signaling mechanism of the HemAT and HemAT/CheA/CheW complex, we tried to solve the structures of HemAT and HemAT/CheA/CheW complex by X-ray crystallography and cryo-electron microscopy (Cryo-EM), respectively. In this year, we have determined the crystal structure of the sensor domain of HemAT from *Bacillus smithii* (Figure 2).

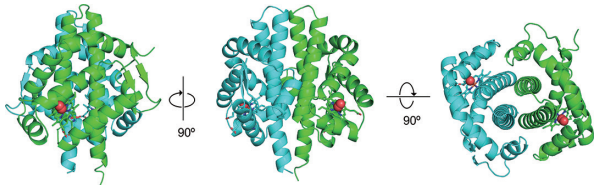

Figure 2. X-ray crystal structure of the sensor domain of HemAT from *Bacillus smithii*.

3. Iron Sensing by Sensor Kinase, VgrS, Responsible for Intracellular Iron Homeostasis

Iron is an essential trace element for all organisms. While it is essential, excess intracellular iron can generate reactive oxygen species, leading to oxidative stress and cellular damage. Therefore, iron homeostasis is essential for cells. In *Xanthomonas campestris*, the two-component system, VgrS/VgrR, plays an important role for the regulation of iron homeostasis. The periplasmic sensor domain of histidine kinase VgrS senses extracellular iron ions. However, detailed mechanism for regulating iron homeostasis by VgrS/VgrR has not yet been elucidated. In this work, we examined the structure-function relationships of VgrS.

To determine the stoichiometry of metal ion binding to VgrS sensor domain, ICP analyses was carried out, which revealed that VgrS sensor domain bound 2.5 equivalents Fe(III) or 1 equivalents Mn(II) or Co(II), respectively. The ExxE motif in VgrS seems to be a metal binding site at which Fe(III) binds. To determine the structure of VgrS, we prepared three constructs of the sensor domain of VgrS composed of Met1-Thr100, Met1-Met87, and Met27-Met87, respectively. The single crystal was obtained for the truncated sensor domain composed of Met27-Met87 while two other samples were not crystalized. X-ray crystallographic analysis of this construct is now in progress.