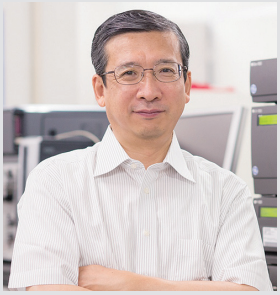
Bioinorganic Chemistry of Metalloproteins Responsible for Metal Homeostasis and Signal Sensing

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

- H. Matsuura, N. Sakai, S. Toma-Fukai, N. Muraki, K. Hayama, H. Kamikubo, S. Aono, Y. Kawano, M. Yamamoto and K. Hirata, "Elucidating Polymorphs of Crystal Structures with Intensity-Based Hierarchical Clustering Analysis on Multiple Diffraction Datasets," *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **79**, 909–924 (2023).
- 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, 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. Structural and Functional Analysis of Heme-Based Oxygen Sensor Protein HemAT

Aerotaxis is a typical biological signal transduction system that consists of a signal transducer protein (MCP), CheA, CheY, and other Che proteins. Signal transducer proteins, sometimes called as MCPs (methyl-accepting chemotoxis proteins), bind a repellant or attractant in their sensor domain. Many chemical and physical stimuli act as a repellant or attractant, among which molecular oxygen is a typical gaseous signaling molecule. HemAT is a MCP responsible for aerotaxis control, which consists of two domains, the sensor domain and the signaling domain. Though the sensor domain of HemAT shows structural homology to myoglobin, it has a different heme environmental structure in the distal heme pocket from myoglobin. In the case of myoglobin, a distal His forms a hydrogen bond with the heme-bound oxygen to stabilize the heme-oxygen complex. However, there is no distal His in HemAT, in which a Thr is involved in the formation of a hydrogen bonding network upon oxygen binding to HemAT.

In this work, we have studied the molecular mechanisms of O_2 sensing and signal transduction of HemAT and HemAT/CheA/CheW complex based on the results of X-ray crystallography and cryo-electron microscopy (cyro-EM). We have determined the crystal structures of ferric-, ferrous (deoxy)-, and O_2-bound (oxy)-forms of the sensor domain of HemAT from Bacillus smithii (BsmHemAT) (Figure 1). We will discuss the molecular mechanisms of O_2 sensing of HemAT by comparing these structures. We have also carried out cryo-EM single particle analysis to determine the structure of HemAT/CheA/CheW complex, which revealed that BsmHemAT, CheA, and CheW formed the complex in 2:1:1 ratio.

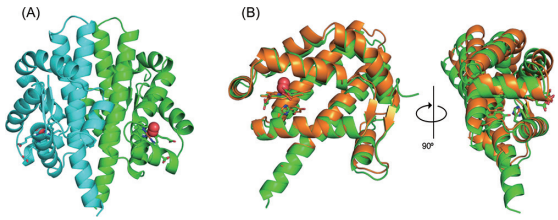

Figure 1. (A) The overall structure of the sensor domain in O_2-bound form. (B) The structural comparison of O_2-bound (green) and reduced (orange) forms of HemAT.

2. CO Biosynthesis for the Construction of the Active Site in [NiFe]-Hydrogease

Hydrogenase, an enzyme that catalyzes the oxidation of hydrogen gas and the reduction of protons, plays a central role in hydrogen metabolism in bacteria and other microorganisms. Recently, it is also expected to be utilized as a catalyst for fuel cells. There are three types of hydrogenases classified based on the structure of their active centers: [NiFe]-, [FeFe]-, and [Fe]-hydrogenases. In all cases, carbon monoxide (CO) is coordinated to the Fe in the active center. While it is known that CO is biosynthesized through enzyme reactions, the molecular mechanism of CO generation has been unclear. In this work, the crystal structure of the enzyme HypX involved in CO biosynthesis used by [NiFe]-hydrogenase was determined. It was revealed that HypX synthesizes CO through a completely novel reaction. HypX consists of two domains (N-terminal domain and C-terminal domain), and within the molecule, there is a cavity spanning across these two domains. It was also found that coenzyme A (CoA) binds to the cavity on the C-terminal domain side. Based on the obtained crystal structure, the following CO biosynthesis reaction mechanism was proposed: Two different chemical reactions occur in the N-terminal domain and the C-terminal domain of HypX. In the N-terminal domain, a formyl transfer reaction from formyl-tetrahydrofolate, which is bound in the cavity of the N-terminal domain, to CoA takes place. During this process, CoA in the cavity adopts an extended linear conformation, and the -SH group at the end of CoA is positioned adjacent to the formyl group in formyl-tetrahydrofolate. As a result of the formyl transfer reaction, formyl-CoA is generated as an intermediate. The generated formyl-CoA undergoes a significant conformational change within the cavity to position the formyl group at the end of the CoA molecule towards the enzyme active site in the C-terminal domain of HypX. In the C-terminal domain, the CO release reaction from formyl-CoA occurs, resulting in the production of CO and CoA.

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

Iron is an essential trace element for all organisms, which is used as active sites in iron proteins for electron transfer, chemical reactions, and gene regulation, *etc*. 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. Transcriptional regulators and/or iron uptake/export systems are responsible for regulating iron concentrations in cells. It is reported that several two-component systems are responsible for intracellular iron concentration in response to iron repletion/deficiency. 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 the two-component system has not yet been elucidated. In this work, we examined the structure-function relationships of *X. campestris* VgrS in detail.

To elucidate iron sensing mechanism of VgrS based on the structure, we prepared three constructs of the sensor domain of VgrS composed of Met1-Thr100, Met1-Met87, and Met27-Met87, respectively, to determine their crystal structures. The single crystal of apo and holo forms was obtained for the truncated sensor domain composed of Met27-Met87 while two other samples were not crystalized. To determine the stoichiometry of metal ion binding to VgrS M27-M87, ICP analyses was carried out, which revealed that VgrS M27-M87 bound 2 equivalents Fe(III) or 1 equivalent Mn(II), respectively. The ExxE motif in VgrS seems to be a metal binding site at which Fe(III) binds.