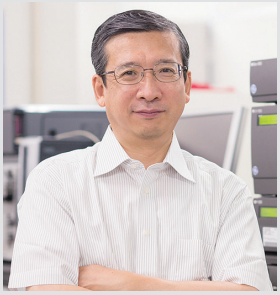
Bioinorganic Chemistry of Metalloproteins Responsible for Metal Homeostasis and Signal Sensing

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

- 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).
- 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).

1. Structural Characterization of Thermoglobin from a Hyperthermophilic Bacterium *Aquifex aeolicus*

Globins are heme-binding proteins, which show a variety of biological functions such as oxygen transport, oxygen storage, redox catalysis and gas sensing. In bacteria, four distinct globins are identified; single domain hemoglobin (sdHb), truncated hemoglobin (tHb), flavohemoglobin (fHb) and globin-coupled sensor (GCS). Truncated hemoglobins (tHbs) are shorter than the canonical vertebrate hemoglobins by 20–40 residues. Whereas the canonical hemoglobins, sdHb, fHb and GCS are composed of eight α-helices (A–H), that fold into a 3-on-3 α-helical sandwich structure, tHbs form a 2-on-2 α-helical sandwich in which helices B and E lie over helices G and H. The physiological function of some sdHb, tHb, and fHb is proposed to provide resistance to nitrosative stress such as reactive nitrogen species. In this work, the structural characterization of AaTgb was carried out by X-ray crystallography.

We have determined the crystal structure of Y29F-AaTgb in the imidazole-bound form. Y29F-AaTgb shares the structural similarity to known bacterial sdHb structures; *Campylobacter jejuni* Hb (CjHb, 44% sequence identity), *Methylacidiphilum infernorum* Hb known as hell's gate globin I (HGbI, 32% sequence identity) and *Vitreoscilla stercoraria* Hb (VsHb, 43% sequence identity). sdHb was discovered from *Vitreoscilla stercoraria*, which shares approximately 30% amino acid sequence identity with the globin domain of fHb that functions as nitric oxide dioxygenase. Based on the amino acid sequence homology, it is assumed that sdHb also acts as nitric oxide dioxygenase. Indeed, *Campylobacter jejuni* Hb and *Helicobacter pullorum* Hb have been reported to contribute to remove nitric oxide.

The superposition between the Cα atoms of Y29F-AaTgb and CjHb (PDB ID: 2wy4), HGbI (PDB ID: 3s1i) and VsHb (PDB ID: 3vhb) shows a root mean square deviation of 0.89 Å, 1.04 Å and 1.18 Å, respectively. By using a structural homology search in Structure Navigator in PDBj, Y29F-AaTgb has the highest structural homology to the globin domain of nitric oxide dioxygenase from *Rhodothermus marinus* (RmaNOD, UniProt ID: D0MGT2). The superposition between the Cα atoms of Y29F-AaTgb and the globin domain of RmaNOD (PDB ID: 6wk3) shows a root mean square deviation of 0.60 Å with 48% sequence identity. The structural similarity of AaTgb to sdHb and RmaNOD suggests a possibility that AaTgb is also responsible for NO detoxification, though further studies must be required to confirm this hypothesis.

The heme environmental structure of Y29F-AaTgb is shown in Figure 1. In Y29F-AaTgb, the heme iron is coordinated by His82 and imidazole in the proximal and distal side, respectively. The distances between iron and nitrogen atom are 2.17 and 2.13 Å for His82 and imidazole, respectively. ND1 of His82 forms hydrogen bonds with OH of Tyr92 and OE2 of Glu132. These hydrogen bonds will fix the orientation of imidazole ring of His82. These amino acids and hydrogen bonds network in the proximal site are conserved in sdHbs except to HGbI.

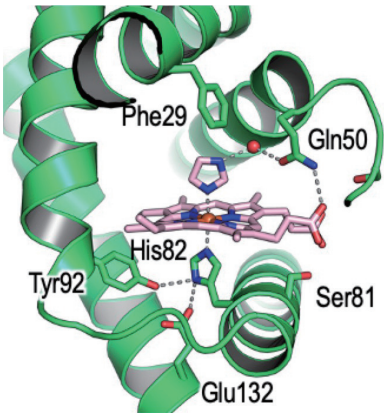

Figure 1. Heme environmental structure of Y29F-AaTgb in the imdazole-bound form. Hydrogen bonds are shown in dashed lines. Nitrogen and oxygen atoms are shown in blue and red, respectively. Red sphere in the heme pocket shows the water molecule W.

Imidazole bound to heme participate in a hydrogen bonding network in the distal heme pocket. A well-defined water molecule (W) present in the distal heme pocket forms hydrogen bonds with imidazole and Gln50. Gln50 forms a hydrogen bond with a propionate group of heme. This hydrogen bonding network will stabilize and fix the orientation of imidazole ligand. On the other hand, Phe29 was 3.41 Å from nitrogen of imidazole and 3.69 Å from the water molecule W.

The binding affinity of imidazole (K_d) to the ferric form of AaTgb was 4.1 and 5.7 μM for the wild type and Y29F variant, respectively, which were determined by measuring absorbance changes upon imidazole titration. Similar binding affinity of imidazole will be achieved as a loss of the hydrogen bond between Tyr29 and imidazole in Y29F variant is compensated by the hydrogen bond between the water molecule W and imidazole.

2. Structural and Functional Analyses of Heme Sensing Transcriptional Regulator PefR

Hemes (iron-porphyrins) are critical for biological processes in all organisms. In this work, structural, functional and spectroscopic analyses of the heme-responsive sensor protein PefR from *Streptococcus agalactiae*, were carried out to elucidate the molecular mechanisms of how heme molecule regulates the functional acitivity of PefR. The crystal structures of apo-PefR, apo-PefR/DNA complex, and heme-bound (holo-) PefR were determined at 2.6, 2.5 Å, and 1.7 Å resolutions, respectively. Structural comparison of the apo-PefR/DNA complex and holo-PefR reveals that conformational change occur around the heme-binding site, which is induced by the coordination of His114 of one subunit to heme followed by the coordination of the N-terminal amino group of the other subunit. Rigid-body motion of the α1 helix in association with heme accommodation alters the relative orientation of the DNA-binding domain in holo-PefR from the apo form, resulting in a conformational change in the DNA-binding domain.