Structure-Function Relationship of Metalloproteins

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

FUJII, Hiroshi	Associate Professor
KURAHASHI, Takuya	Assistant Professor
CONG, Zhiqi	IMS Fellow
NAKAGAWA, Takafumi	Post-Doctoral Fellow
WANG, Shunlan	Graduate Student
TANIZAWA, Misako	Secretary

Metalloproteins are a class of biologically important macromolecules, which have various functions such as oxygen transport, electron transfer, oxidation, and oxygenation. These diverse functions of metalloproteins have been thought to depend on the ligands from amino acid, coordination structures, and protein structures in immediate vicinity of metal ions. In this project, we are studying the relationship between the electronic structures of the metal active sites and reactivity of metalloproteins.

1. Unique Property and Reactivity of High-Valent Manganese-Oxo versus Manganese-Hydroxo in the Salen Platform[1,2)]

To gain an understanding of oxidation reactions by Mn^{III}(salen), a reaction of Mn^{III}(salen) with *m*-chloroperoxybenzoic acid in the absence of a substrate is investigated. UV-vis, perpendicular- and parallel-mode electron paramagnetic resonance and X-ray absorption spectroscopy show the resulting solution contains Mn^{IV}(salen)(O) as a major product and Mn^{IV}(salen)(OH) as a minor product. Mn^{IV}(salen)(O) readily reacts with 4-H-2,6-*tert*-$Bu_2C_6H_2OH$ (BDE_{OH} = 82.8 kcal/mol), 4-CH_3CO-2,6-*tert*-$Bu_2C_6H_2OH$ (BDE_{OH} = 83.1 kcal/mol) and 4-NC-2,6-*tert*-$Bu_2C_6H_2OH$ (BDE_{OH} = 84.2 kcal/mol) at 203 K, following second-order rate kinetics (BDE_{OH}; homolytic bond dissociation energy of an OH bond). Mn^{IV}(salen)(OH) reacts with 4-CH_3CO-2,6-*tert*-$Bu_2C_6H_2OH$ (BDE_{OH} = 83.1 kcal/mol) much more slowly under the identical conditions than Mn^{IV}(salen)(O), and does not react with 4-NC-2,6-*tert*-$Bu_2C_6H_2OH$ (BDE_{OH} = 84.2 kcal/mol), suggesting thermodynamic hydrogen-atom abstracting ability of Mn^{IV}(salen)(OH) is about 83 kcal/mol. The rate constant for reactions of Mn^{IV}(salen)(OH) with phenols are not dependent on the concentration of phenols, suggesting that Mn^{IV}(salen)(OH) might bind phenols prior to the rate-limiting oxidation reactions. Quantum chemical calculations are carried out for Mn^{IV}(salen)(O) and Mn^{IV}(salen)(OH), both of which well reproduce the EXAFS structures as well as the electronic configurations. It is also indicated that protonation of Mn^{IV}(salen)(OH) induces a drastic electronic structural change from Mn^{IV}-phenolate to Mn^{III}-phenoxyl radical, which is also consistent with the experimental observation.

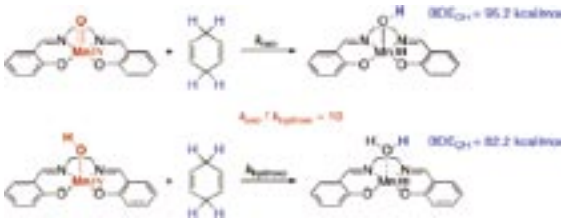

Figure 1. Hydrogen abstractions of oxo-manganese(IV) salen and hydroxy-manganese(IV) salen complxes.

2. Resonance Raman Study of a High-Valent Fe=O Porphyrin Complex as a Model for Peroxidase Compound II[3)]

Horseradish peroxidase (HRP) catalyzes the oxidation of organic substrates using H_2O_2 as a specific oxidant. Upon reaction with H_2O_2, HRP sequentially forms two reaction intermediates known as compound I and compound II, before returning to the original ferric state. Compound I and compound II are respectively 2 and 1 oxidative equivalents higher than the Fe^{III} state, and respectively correspond to the Fe^{V} and Fe^{IV} formal oxidation states. Compound II of HRP exhibits a $\nu_{Fe=O}$ resonance Raman (RR) band at 775 and 787 cm^{-1} at pH

7 and 11, respectively. The 12 cm^{-1} upshift of the $\nu_{Fe=O}$ mode of HRP compound II upon alkalization is caused by elimination of the hydrogen bond between the oxygen atom and the distal His and is regarded as a "distal effect." The $\nu_{Fe=O}$ mode of the Fe^{IV} state of myoglobin (Mb) at pH 8.5 is located at *ca.* 800 cm^{-1} (sperm whale Mb at 797 cm^{-1} and horse heart Mb at 804 cm^{-1}), where no hydrogen bond exists between the oxygen atom and the distal His. There is a 13 cm^{-1} frequency difference in the $\nu_{Fe=O}$ mode between HRP at 787 cm^{-1} and Mb at 800 cm^{-1}. This has been interpreted as a result of a "proximal effect." Both HRP and Mb have a proximal His residue. However, the proximal His of HRP has the anionic character of imidazolate (Im^-), while the proximal His of Mb is considered to be neutral.

Many Fe=O porphyrin model complexes with 1-methylimidazole (1MeIm) or a solvent molecule acting as the axial ligand have been prepared and characterized by RR spectroscopy, in order to obtain insights into the electronic structures and reactivities of hemoproteins. However, there have been no reports of model complexes with Im^- as a *trans* axial ligand. In the present study, we have prepared an Fe=O porphyrin model complex with Im^- as the axial ligand and identified the $\nu_{Fe=O}$ mode at 792 cm^{-1}, which is significantly lower than that of an analogous complex with 1MeIm as the axial ligand (815 cm^{-1}). Thus, the imidazolate complex could be regarded as a model for compound II of HRP. The experimental details are described in Supporting Information.

3. Direct Probing of Spin State Dynamics Coupled with Electronic and Structural Modifications by Picosecond Time-Resolved XAFS[4)]

Molecular magnetic systems such as nanomagnets and biological systems have attracted much interest in resent years. In disordered magnetic systems, where the spin system does not have macroscopic magnetization, it is crucial to directly observe the transient spin states to aid in the understanding and controlling of the dynamic magnetic properties. In studies of ultrafast spin dynamics, pico- and femtosecond time scales are now accessible with advanced optical pump–probe measurement using two ultrafast lasers. However, it is not trivial to deconvoluting the dynamics of the spin state from transient optical signal. Although the magneto-optical effect has been applied to macroscopic magnetization, it is difficult to apply in disordered magnetic systems. To overcome these difficulties, a pulsed hard X-ray has been utilized as a probe for the dynamics of the inner-atomic transitions.

The first direct observation of the transient spin-state in a disordered magnetic system with time-resolved XAFS is reported. By observing the evolution of the Fe^{II} 1s–3d transition, the spin crossover transition from the $^1A_1$ low spin state to $^5T_2$ high spin state has been directly observed on a picosecond time scale. Moreover, observation of the transient spin state with time-resolved XAFS allows for the investigation of the variations in the electronic state and molecular structure. This unique experimental technique probes the excited states involved in the ultrafast photoinduced reactions in disordered magnetic systems.

4. Paramagnetic ^{13}C and ^{15}N NMR Analyses of the Push- and Pull-Effects in Cytochrome *c* Peroxidase and *Coprinus cinereus* Peroxidase Variants: Functional Roles of Highly-Conserved Amino Acids around Heme[5)]

Paramagnetic ^{13}C and ^{15}N nuclear magnetic resonance (NMR) spectroscopy of heme-bound cyanide ($^{13}C^{15}N$) was applied to 11 cytochrome *c* peroxidase (CcP) and *Coprinus cinereus* peroxidase (CIP) mutants to investigate contributions to the push- and pull-effects of conserved amino acids around heme. The ^{13}C and ^{15}N NMR data for the distal His and Arg mutants indicated that distal His is the key amino acid residue creating the strong pull-effect and that distal Arg assists. The mutation of distal Trp of CcP to Phe, the amino acid at this position in CIP, changed the push- and pull-effects close to those of CIP, whereas the mutation of distal Phe of CIP to Trp changed this mutant to become CcP-like. The ^{13}C NMR shifts for the proximal Asp mutants clearly showed that the proximal Asp-His hydrogen-bonding increase the push-effect. However, even in absence of a hydrogen-bond the push-effect of proximal His in peroxidase is significantly stronger than in globins. Comparison of the present NMR data with the compound I formation rate constants and crystal structures of these mutants showed that (1) the base catalysis of the distal His is more critical for rapid compound I formation than its acid catalysis, (2) the primary function of the distal Arg is to maintain the distal heme pocket in favor of rapid compound I formation via hydrogen-bonding, and (3) the push-effect is the major contributor to the differential rates of compound I formation in wild-type peroxidases.

References

1) T. Kurahashi, M. Hada and H. Fujii, *J. Am. Chem. Soc.* **131**, 12394–12405 (2009).
2) T. Kurahashi, A. Kikuchi, Y. Shiro, M. Hada and H. Fujii, *Inorg. Chem.* **49**, 6664–6672 (2010).
3) H. Ishimaru, H. Fujii and T. Ogura, *Chem. Lett.* **39**, 332–333 (2010).
4) S. Nozawa, T. Sato, M. Chollet, K. Ichiyanagi, A. Tomita, H. Fujii, S. Adachi and S. Koshihara, *J. Am. Chem. Soc.* **132**, 61–63 (2010).
5) D. Nonaka, H. Wariishi and H. Fujii, *Biochemistry* **49**, 49–57 (2010).