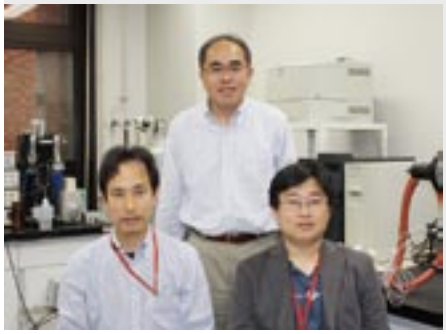
Structure-Function Relationship of Metalloproteins

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



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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. Critical Role of External Axial Ligands in Chirality Amplification of *trans*-Cyclohexane-1,2-diamine in Salen Complexes[1)]

A series of $Mn^{IV}(salen)(L)_2$ complexes bearing different external axial ligands (L = Cl, NO_3, N_3 and OCH_2CF_3) from chiral salen ligands with *trans*-cyclohexane-1,2-diamine as a chiral scaffold are synthesized, in order to gain insight into conformational properties of metal salen complexes. X-ray crystal structures show that $Mn^{IV}(salen)(OCH_2CF_3)_2$ and $Mn^{IV}(salen)(N_3)_2$ adopt a stepped conformation with one of two salicylidene rings pointing upward and the other pointing downward due to the bias from the *trans*-cyclohexane-1,2-diamine moiety, which is in clear contrast to a relatively planar solid-state conformation for $Mn^{IV}(salen)(Cl)_2$. The CH_2Cl_2 solution of $Mn^{IV}(salen)(L)_2$ shows circular dichroism of increasing intensity in the order of L = Cl < NO_3 « N_3 < OCH_2CF_3, which indicates $Mn^{IV}(salen)(L)_2$ adopts a solution conformation of an increasing chiral distortion in this order. Quantum-chemical calculations with symmetry adapted cluster–configuration interaction method indicate that a stepped conformation exhibits more intense circular dichroism than a planar conformation. The present study clarifies an unexpected new finding that the external axial ligands (L) play a critical role in amplifying the chirality in *trans*-cyclohexane-1,2-diamine in $Mn^{IV}(salen)(L)_2$ to facilitate the formation of a chirally-distorted conformation, possibly a stepped conformation.

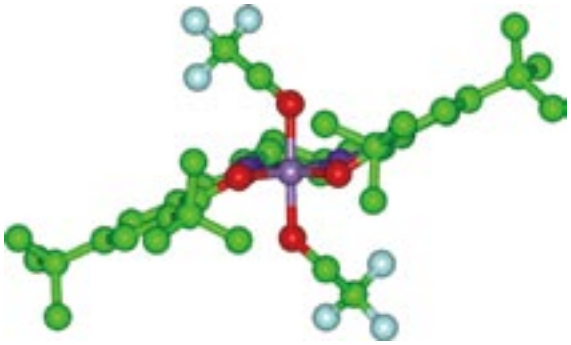

Figure 1. Chiral stepped conformation of $Mn^{IV}(salen)(OCH_2CF_3)_2$.

2. Catalytic Reactivity of a *Meso*-N-Substituted Corrole and Evidence for a High-Valent Iron–Oxo Species[2)]

It is shown that an iron(III) *meso*-N-substituted corrole ($TBP_8Cz)Fe^{III}$ (**1**) (TBP_8Cz) octakis(4-*tert*-butylphenyl)corrol azinato), is a potent catalyst for the oxidation of alkenes in the presence of pentaflouroiodosylbenzene (C_6F_5IO) as oxidant. In the case of cyclohexene, complex **1** performs on a par with one of the best porphyrin catalysts (($TPPF_{20}$)FeCl), exhibiting rapid turnover and a high selectivity for epoxide ($CzFe^{III}$/C_6F_5IO/cyclohexene (1:100:1000) in CH_2Cl_2/CH_3OH (3:1 v:v) gives 33 turnovers of epoxide in <2 min). Reaction rates for **1** are greatly enhanced compared to other Fe or Mn corroles

under similar catalytic conditions, consistent with an increase in the electrophilicity of a high-valent iron–oxo intermediate induced by *meso*-N substitution. Reaction of dark-green **1** (λ_{max}) 440, 611, 747 nm) under single-turnoverlike conditions at –78 °C leads to the formation of a new dark-brown species (**2**) (λ_{max}) 396, 732, 843 nm). The Fe^{III} complex **1** is restored upon the addition of 2 equiv of ferrocene to **2**, or by the addition of 1 equiv of PPh_3, which concomitantly yields $OPPh_3$. In addition, complex **2** reacts with excess cyclohexene at –42 °C to give **1**. Complex **2** was also characterized by EPR spectroscopy, and all of the data are consistent with **2** being an antiferromagnetically coupled iron(IV)-oxo π-cation-radical complex. Rapidmixing stopped-flow UV–vis studies show that the low-temperature complex **2** is generated as a shortlived intermediate at room temperature.

3. Effect of Imidazole and Phenolate Axial Ligands on the Electronic Structure and Reactivity of Oxoiron(IV) Porphyrin π-Cation Radical Complexes: Drastic Increase in Oxo-Transfer and Hydrogen Abstraction Reactivities[3)]

To study the effect of axial ligands on the electronic structure and reactivity of compound I of peroxidases and catalases, oxoiron(IV) porphyrin π-cation radical complexes with imidazole, 2-methylimidazole, 4(5)-methylimidazole, and 3-fluoro-4-nitrophenolate as the axial ligands were prepared by ozone oxidation of iron(III) complexes of 5, 10, 15, 20-tetramesitylporphyrin (TMP) and 2, 7, 12, 17-tetramethyl-3, 8, 13, 18-tetramesitylporphyrin (TMTMP). These complexes were fully characterized by absorption, ^1H, ^2H, and ^{19}F NMR, EPR, and ESI-MS spectroscopy. The characteristic absorption peak of compound I at approximately 650 nm was found to be a good marker for estimation of the electron donor effect from the axial ligand. The axial ligand effect did not change the porphyrin π-cation radical state, the a_{2u} state of the TMP complexes, or the a_{1u} radical state of both the TMTMP complexes and compound I. The ferryl iron and porphyrin π-cation radical spins were effectively transferred into the axial ligands for the a_{2u} complexes, but not for the a_{1u} complexes. Most importantly, the reactivity of the oxoiron(IV) porphyrin π-cation radical complex was drastically increased by the imidazole and phenolate axial ligands. The reaction rate for cyclooctene epoxidation was increased 100 ~ 400-fold with axial coordination of imidazoles and phenolate. A similar increase was also observed for oxidation of 1,4-cyclohexadiene, *N*,*N*-dimethyl-*p*-nitroaniline, and hydrogen peroxide. These results suggest extreme enhancement of the reactivity of compound I by the axial ligand in heme enzymes. The functional role of axial ligands on the compound I in heme enzymes is discussed.

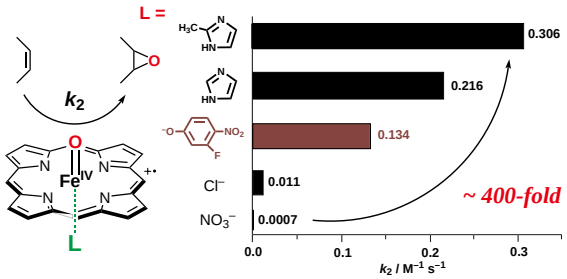

Figure 2. Axial ligand effect on the epoxidation reactivity.

4. Paramagnetic ^{13}C and ^{15}N NMR Analyses of Cyanide ($^{13}C^{15}N$)-Ligated Ferric Peroxidases: The Push-Effect, not Pull-Effect, Modulates the Compound I Formation Rate[4)]

Paramagnetic ^{13}C and ^{15}N NMR spectroscopy of heme-bound cyanide ($^{13}C^{15}N$) was utilized to quantitatively distinguish the electron donor effect (the push-effect) from the proximal histidine and hydrogen bonding effect (the pull-effect) from the distal amino acid residues in cytochrome *c* peroxidase (CcP), ascorbate peroxidase (APX), lignin peroxidase (LiP) and manganese peroxidase (MnP). Paramagnetic ^{13}C NMR signals of heme-bound $^{13}C^{15}N$ of these peroxidases were observed in a wide range: –3501 ppm (CcP), –3563 ppm (APX), –3823 ppm (MnP), and –3826 ppm (LiP), while paramagnetic ^{15}N NMR signals of those were detected in a narrow range: 574 ppm (ARP), 605 ppm (CcP), 626 ppm (LiP), and 654 ppm (MnP). Detailed analysis, combined with the previous results for horseradish peroxidase and *Arthromyces ramosus* peroxidase, indicated that the push-effect is quite different among these peroxidases while the pull-effect is similar. More importantly, a strong correlation between the ^{13}C NMR shift (the push-effect) and the compound I formation rate was observed, indicating that the push-effect causes a variation in the compound I formation rate. Comparison of the ^{13}C and ^{15}N NMR results of these peroxidases with their crystal structures suggests that the orientation of the proximal imidazole plane to the heme N–Fe–N axis controls the push-effect and the compound I formation rate of peroxidase.

References

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