

# Structure-Function Relationship of Metalloproteins

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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. Effect of a Tridentate Ligand on the Structure, Electronic Structure, and Reactivity of the Copper(I) Nitrite Complex: Role of the Conserved Three-Histidine Ligand Environment of the Type-2 Copper Site in Copper-Containing Nitrite Reductases<sup>1)</sup>

It is postulated that the copper(I) nitrite complex is a key reaction intermediate of copper containing nitrite reductases (Cu-NiRs), which catalyze the reduction of nitrite to nitric oxide (NO) gas in bacterial denitrification. To investigate the structure-function relationship of Cu-NiR, we prepared five new copper(I) nitrite complexes with sterically hindered tris(4-imidazolyl)carbinols [Et-TIC: tris(1-methyl-2-ethyl-4-imidazolyl)carbinol and *i*Pr-TIC: tris(1-methyl-2-isopropyl-4-imidazolyl)carbinol] or tris(1-pyrazolyl)methanes [Me-TPM: tris(3,5-dimethyl-1-pyrazolyl)methane, Et-TPM: tris(3,5-diethyl-1-pyrazolyl)methane, and *i*Pr-TPM: tris(3,5-diisopropyl-1-pyrazolyl)methane]. The X-ray crystal structures of all of these copper(I) nitrite complexes were mononuclear  $\eta^1$ -

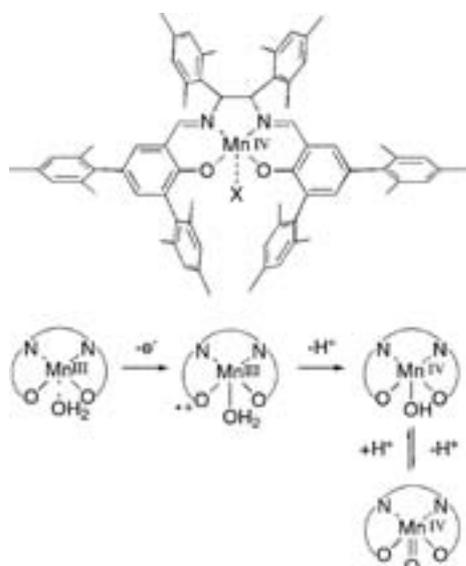


**Figure 1.** Structure of Active Site Model Complex of Copper Nitrite Reductase (left) and its Electron Density of the HOMO (right).

N-bound nitrite complexes with a distorted tetrahedral geometry. The electronic structures of the complexes were investigated by absorption, MCD, NMR and vibrational spectroscopy. All of these complexes are good functional models of Cu-NiR that form NO and copper(II) acetate complexes well from reactions with acetic acid under anaerobic conditions. A comparison of the reactivity of these complexes, including previously reported (*i*Pr-TACN)Cu(NO<sub>2</sub>), *i*Pr-TACN: 1,4,7-triisopropyl-1,4,7-triazacyclononane, clearly shows the drastic effects of the tridentate ligand on Cu-NiR activity. The copper(I) nitrite complex with the Et-TIC ligand, which is similar to the highly conserved three-histidine ((His)<sub>3</sub>) ligand environment in the catalytic site of Cu-NiR, had the highest Cu-NiR activity. This result suggests that the (His)<sub>3</sub> ligand environment is essential for acceleration of the Cu-NiR reaction. The highest Cu-NiR activity for the Et-TIC complex can be explained by the structural and spectroscopic characterizations and the molecular orbital calculations presented in this paper. Based on these results, the functional role of the (His)<sub>3</sub> ligand environment in Cu-NiR is discussed.

## 2. Transient Intermediates from Mn(salen) with Sterically-Hindered Mesityl Groups: Interconversion between Mn<sup>IV</sup>-Phenolate and Mn<sup>III</sup>-Phenoxy Radical as an Origin for Unique Reactivity<sup>2)</sup>

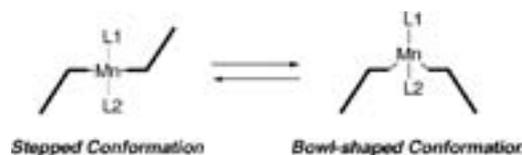
In order to reveal structure-reactivity relationships for the high catalytic activity of the epoxidation catalyst Mn(salen), transient intermediates are investigated. Steric hindrance incorporated to the salen ligand enables highly selective generation of three related intermediates, O=Mn<sup>IV</sup>(salen), HO-Mn<sup>IV</sup>(salen) and H<sub>2</sub>O-Mn<sup>III</sup>(salen<sup>+</sup>), each of which is thoroughly characterized using various spectroscopic techniques including UV-vis, EPR, resonance Raman, ESI-MS, <sup>2</sup>H-NMR and X-ray absorption spectroscopy. These intermediates are all one-electron oxidized from the starting Mn<sup>III</sup>(salen) precursor, but differ only in the degree of protonation. However, structural and electronic features are strikingly different: The Mn–O bond length of HO-Mn<sup>IV</sup>(salen) (1.83 Å) is considerably longer than that of O=Mn<sup>IV</sup>(salen) (1.58 Å); the electronic configuration of H<sub>2</sub>O-Mn<sup>III</sup>(salen<sup>+</sup>) is Mn<sup>III</sup>-phenoxy radical, while those of O=Mn<sup>IV</sup>(salen) and HO-Mn<sup>IV</sup>(salen) are Mn<sup>IV</sup>-phenolate. Among O=Mn<sup>IV</sup>(salen), HO-Mn<sup>IV</sup>(salen) and H<sub>2</sub>O-Mn<sup>III</sup>(salen<sup>+</sup>), only the O=Mn<sup>IV</sup>(salen) can transfer oxygen to phosphine and sulfide substrates, as well as abstract hydrogen from weak C–H bonds, although the oxidizing power is not enough to epoxidize olefins. The high activity of Mn(salen) is a direct consequence of the favored formation of the reactive O=Mn<sup>IV</sup>(salen) state.



**Figure 2.** Structure of Manganese(IV) Salen Complex synthesized in this study (upper) and protonation and deprotonation of Mn(IV)=O(Salen) complex (bottom).

## 3. Chiral Distortion in a Mn<sup>IV</sup>(salen)(N<sub>3</sub>)<sub>2</sub> Derived from Jacobsen's Catalyst as a Possible Conformation Model for its Enantioselective Reactions<sup>3)</sup>

The Mn<sup>IV</sup>(salen)(N<sub>3</sub>)<sub>2</sub> complex (**3**) from Jacobsen's catalyst is synthesized, and the X-ray crystal structures of **3** as well as the starting Mn<sup>III</sup>(salen)(N<sub>3</sub>)(CH<sub>3</sub>OH) complex (**2**) are determined in order to investigate the conformation of the high-valent Mn<sup>IV</sup>(salen) molecule in comparison with that of Mn<sup>III</sup>(salen). The asymmetric unit of the crystal of **3** contains four complexes, all of which adopt a nonplanar stepped conformation effectively distorted by the chirality of the diimine bridge. The asymmetric unit of **2** also contains four complexes. Two of them show a stepped conformation of a lesser degree, but the other two adopt a bowl-shaped conformation. Comparison of the structural parameters shows that the Mn center in **3** is coordinated from both sides by two external axial N<sub>3</sub> ligands with significantly shorter bond length, which could induce greater preference for the stepped conformation in **3**. The CH<sub>3</sub>CN solution of **3** shows circular dichroism with a significantly strong band at 275 nm as compared to **2**, suggesting that **3** may adopt a more chirally-distorted conformation also in solution. The circular dichroism spectrum of **3** is slightly altered with isodichroic points from 298 to 253 K, and shows no further change at temperatures lower than 253 K, suggesting that the solution of **3** contains equilibrium between two conformers, where a low-energy conformer with more chiral distortion is predominantly favored even at room temperature. **2** and **3** are thoroughly characterized using various techniques including cyclic voltammetry, magnetic susceptibility, UV-vis, electron paramagnetic resonance, <sup>1</sup>H NMR, infrared spectroscopy and electrospray ionization mass spectrometry.



**Figure 3.** Equilibrium between stepped and bowl-shaped conformations of Mn(salen), which is directed by the position of the Mn center from the basal N<sub>2</sub>O<sub>2</sub> ligand plane.

### References

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