X-D Reaction Mechanism of Metalloenzymes Related to Oxygen Activation

Oxygen is quite important molecule for most organisms. Oxygen is utilized for various physiological functions such as ATP synthesis, defense mechanism, oxidation reactions, and signal transduction. These diverse functions are realized by many metalloenzymes. In this project, we are studying molecular mechanisms of these metalloenzymes.

X-D-1 Oxidizing Intermediates from the Sterically Hindered Salen Iron Complexes Related to the Oxygen Activation by Nonheme Iron Enzymes

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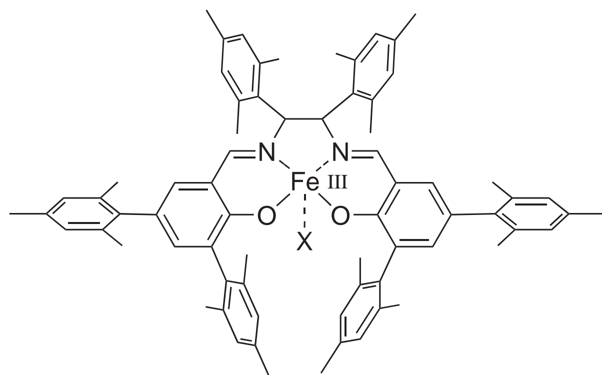
Oxidizing intermediates are generated from non-heme iron(III) complexes to investigate the electronic structure and the reactivity, in comparison with the oxoiron(IV) porphyrin π-cation radical (compound I) as a heme enzyme model. Sterically hindered salen iron complexes, bearing a fifth ligand Cl (**1**), OH_2 (**2**), OEt (**3**) and OH (**4**), are oxidized both electrochemically and chemically. Stepwise one-electron oxidation of **1** and **2** generates iron(III)–mono- and diphenoxyl radicals, as revealed by detailed spectroscopic investigations, including UV-Vis, EPR, Mössbauer, resonance Raman, and ESIMS spectroscopies. In contrast to the oxoiron(IV) formation from the hydroxoiron(III) porphyrin upon one-electron oxidation, the hydroxo complex **4** does not generate oxoiron(IV) species. Reaction of **2** with *m*CPBA also results in the formation of the iron (III)–phenoxyl radical. One-electron oxidation of **3** leads to oxidative degradation of the fifth EtO ligand to liberate acetaldehyde even at 203 K. The iron(III)–phenoxyl radical shows high reactivity for alcoxide on iron(III), but exhibits virtually no reactivity for alcohols including even benzyl alcohol without a base to remove an alcohol proton. The present study explains unique properties of mononuclear nonheme enzymes with Tyr residues, and also a poor epoxidation activity of Fe salen compared to Mn and Cr salens.



X = Cl (**1**), OH_2 (**2**), OEt (**3**), OH (**4**)

Figure 1. Structure of Sterically Hindered Salen Complexes prepared in this study.

X-D-2 Synthesis of Sterically Hidered Tris(4-imidazolyl)carbinol Ligands and their Copper(I) Complexes Related to Metalloenzymes

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In non-heme metalloenzymes, imidazole rings of histidine residues often form part of the metal-binding site. For examples, in the active sites of hemocyanin (Cu), nitrite reductase (Cu), and carbonic anhydrase (Zn), three imidazoles coordinate to one metal ion (Figure 1a). Because the coordination environment with three histidine imidazoles are generally occurred in many non-heme metalloenzymes, tripodal N-donor ligands such as hydrotris(2-pyrazolyl)borate and triazacyclononane have been used for synthetic model studies to mimic the active centers. For biomimetic studies, ligands with imidazolyl units are more desirable to understand the nature of metalloenzymes. Especially, a tripodal ligand with three 4-imidazolyl units, *e.g.*, tris(4-imidazolyl)carbinol, is better suited because the coordination enivrionment of this ligand is close to the active sites of metalloenzymes. Therefore, it is desirable the synthesis of tris(4-imidazolyl)carbinol with stable NH protecting group and sterically hidered substituent to mimic the reactive intermediates. Here, we report the synthesis of tris(4-imidazolyl)carbinol ligands having chemically stable methyl group as the NH protective group and bulky substituent (isopropyl or phenyl) for stabilizing reactive species bound to metal center. The copper complexes prepared from these ligands can reproduce the metal active site of copper enzymes and are suitable for biomimetic studies.

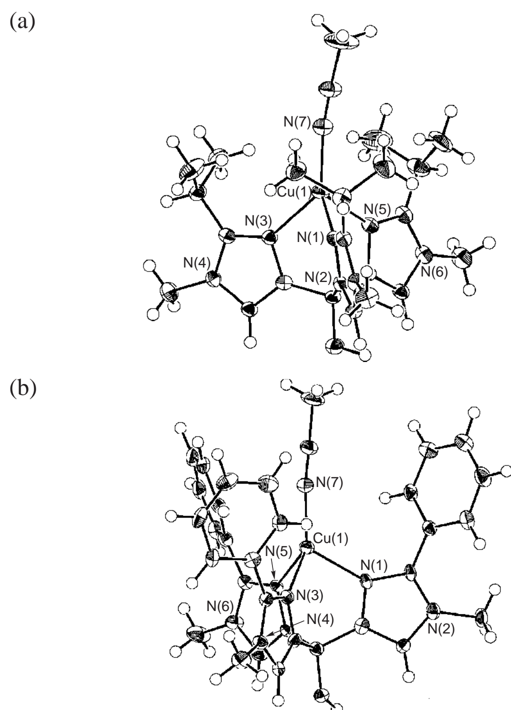

Figure 1. X-ray crystal structures of copper(I) acetonitrile complexes prepared in this study.

X-D-3 O_2- and H_2O_2-Dependent Verdoheme Degradation by Heme Oxygenase: Reaction Mechanisms and Potential Physiological Roles of the Dual Pathway Degradation

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Heme oxygenase (HO) catalyzes catabolism of heme to biliverdin, CO and a free iron through three successive oxygenation steps. The third oxygenation, oxidative degradation of verdoheme to biliverdin, has been the least understood step in spite of its importance to regulate the HO activity. We have thoroughly examined degradation of a synthetic verdoheme IXa complexed with rat HO-1. Our major findings include: (1) HO degrades verdoheme through a dual pathway using either O_2 or H_2O_2; (2) the newly found H_2O_2 pathway is approximately 40-fold faster than the O_2-dependent degradation; (3) both reactions are initiated by the binding of O_2 or H_2O_2 to allow the first direct observation of degradation intermediates of verdoheme; and (4) Asp140 in HO-1 is critical for the verdoheme degradation regardless of the oxygen source. On the basis of these findings, we propose that the HO enzyme activates O_2 and H_2O_2 on the verdoheme iron with the aid of a nearby water molecule linked with Asp140. These mechanisms are similar to a well-established mechanism of the first oxygenation, *meso*-hydroxylation of heme, and thus, HO can utilize a common architecture to promote the first and third oxygenation steps of the heme catabolism. We also point out a possible involvement of the H_2O_2-dependent verdoheme degradation *in vivo*, and propose potential roles of the dual pathway reaction of HO against oxidative stress.

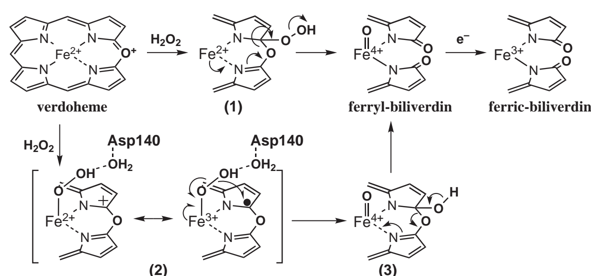

Figure 1. Proposed reaction mechanism of verdoheme in this study.

X-E Reaction Mechanism of Metalloenzymes related to Global Nitrogen Cycle

For all organisms, organic nitrogen and ammonia are required as a constituent part of the cell. In order to keep the environment of the earth constant, the organic nitrogen, fixed nitrogen, must be completely reconverted into dinitrogen gas. The reverse process of the nitrogen fixing is called denitrification process. In this process, nitrate or nitrite ion is reduced to nitrogen gas via nitric oxide and nitrous oxide by many metalloenzymes, nitrate reducatse, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. In this project, we are studying the molecular mechanism of these metalloenzymes relating to the denitrification process.

X-E-1 Spectroscopic Characterization of Reaction Intermediates in a Model for Copper Nitrite Reductase

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Reduction of nitrite (NO_2^-) to gaseous nitric oxide (NO) is one of key processes in the global nitrogen cycle and carried out by bacterial copper-containing nitrite reductases (NiR). The enzymes contain two copper ion centers: the type 1 copper site for electron transfer and the type 2 copper site for the catalytic nitrite reduction. Crystallographic and spectroscopic

studies of NiR have proposed the mechanism of the nitrite reduction at the type 2 copper site. The enzyme reaction is initiated by the binding of the nitrite to the reduced form of the type 2 copper site to yield a copper(I) nitrite complex. Subsequently, the copper bound nitrite is reduced to NO and water with intramolecular one electron transfer from the type 2 copper(I) ion and two protons from a conserved aspartic acid placed near the type 2 site. During the course of synthetic study of the copper(I) nitrite complexes, we found that the rapid mixing of copper(I) nitrite complex with trifluoroacetic acid (TFA) with stopped flow at low temperature allows to detect new reaction intermediates in the reduction process. Here, we report detection and characterization of new reaction intermediates in the nitrite reduction. This study shows a new reaction mechanism, in which two protons required for the reaction are not provided to the copper bound nitrite simultaneously but stepwise and that the intramolecular electron transfer from the copper(I) ion to the copper bound nitrite occurs in the second protonation step (Figure 1).

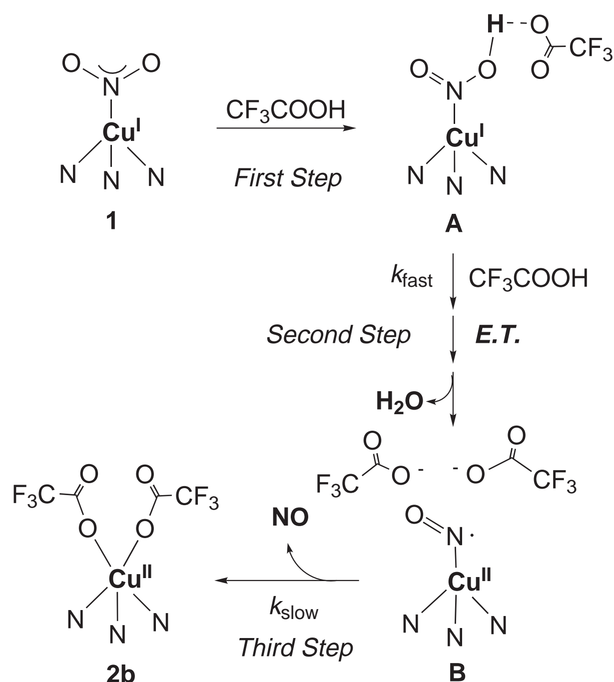

Figure 1. A proposed reaction mechanism of NiR in this study.