IX-C Electronic Structure and Reactivity of Active Sites of Metalloproteins

Metalloproteins are a class of biologically important macromolecules that 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 structures of the metal active sites and functions of metalloproteins.

IX-C-1 Trigonal Bipyramidal Ferric Aqua Complex with Sterically Hindered Salen Ligand as a Model for Active Site of Protocatechuate 3,4-Dioxygenase

FUJII, Hiroshi; FUNAHASHI, Yasuhiro

[*Angew. Chem., Int. Ed.* **41**, 3638–3641 (2002)]

Protocatechurate 3,4-dioxygenase (3,4-PCD) has been found in soil bacteria and is known to play a role in degrading aromatic molecules in nature. The enzyme is classified as an intradiol dioxygenase and cleaves catechol analogues bound to the iron(III) site into aliphatic products with incorporation of both atoms of molecular oxygen. It has been proposed that the enzyme does not activate an iron-bound oxygen molecule, but rather induces an iron-bound catecholate to react with O_2. Therefore, knowledge of the structure and electronic state of the iron site is essential to understanding the unique reaction of 3,4-PCD. A previous crystalstructu re analysis of 3,4-PCD from Pseudomonas putida revealed a distorted trigonal-bipyramidal ferric iron center with four endogenous protein ligands (Tyr 408, Tyr 447, His 460, and His 462) and a solvent-derived water molecule (see Figure 1). To understand the structure±function relationship of 3,4-PCD, attempts have been made over several decades to prepare inorganic modelcompl exes of 3,4-PCD. However, no iron(III) complex that reproduces the active site of 3,4-PCD has been characterized. We report here the first example of a distorted trigonal-bipyramidal ferric aqua complex with a sterically hindered salen ligand that not only duplicates the active site but also mimics the spectral characteristics of 3,4-PCD.

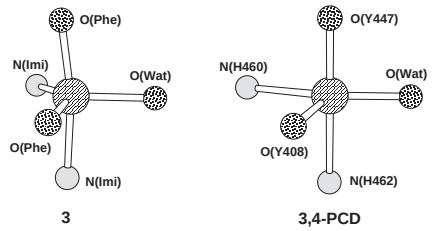

Figure 1. Comparison of Active Site Structures of Our Model Complex and 3,4-PCD.

IX-C-2 An Oxidizing Intermediate Generated from a Salen Iron Complex, Related to the Oxygen Activation by Mononuclear Nonheme Iron Enzymes

KURAHASHI, Takuya; KOBAYASHI, Yoshio[1]; NAGATOMO, Shigenori; KITAGAWA, Teizo; FUJII, Hiroshi
([1]RIKEN)

High-valent iron-oxo species are proposed to be the key reactive intermediate in oxidation reactions catalyzed by the oxygen-activating iron enzyme. In the case of heme enzymes, porphyrin model complexes have been extensively investigated. However, an insight into the nonheme oxidizing intermediate is still limited. Very recently, Wieghardt *et al.* reported the first example of the high-valent iron(IV) species from a nonheme model complex, utilizing the cyclam iron complex. In 2003, Que Jr. *et al.* succeeded in the X-ray crystallographic analysis of the iron(IV) μ-oxo species, using the similar nonheme iron complex. On the other hand, we have been attempting to prepare the nonheme Compound I analog from a salen iron complex. Herein we report the detailed electronic structure and the reactivity of the oxidizing intermediate generated from a salen iron complex and *m*CPBA. To model the monomeric iron center of enzymes, bulky mesityl substituents are introduced to the salen iron complex. Indeed, the aquo complex **1** is found in a monomeric form, due to the steric repulsion by the mesityl group, as already reported. Addition of *m*CPBA to the purple solution of the salen iron complex **1** in dichloromethane at –80 °C produced a blue-green solution (Figure 1). This solution gave a distinct UV-Vis spectrum (λ_{max} = 815 nm) from the starting solution. The intensity of the signal at 815 nm was totally attenuated at room temperature. These results suggest that oxidation of **1** with *m*CPBA generates a transient intermediate having a distinct electronic structure from **1**. The detailed structure of the blue-green intermediate is now being investigated by means of Mössbauer, EPR, NMR and resonance Raman spectroscopy.

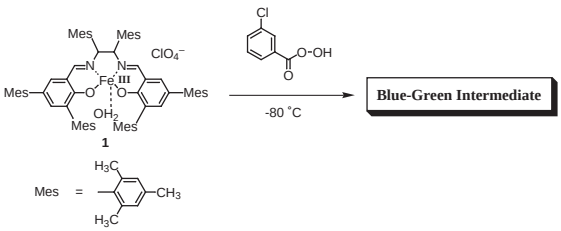

Figure 1. Formation of Blue-Green Intermediate from **1**.

IX-C-3 A Superoxo-Ferrous State in a Reduced Oxy-Ferrous Hemoprotein and Model Compounds

DAVYDOV, Roman[1]; SATTERLEE, James[2]; FUJII, Hiroshi; SAUER-MASARWA, Alexandra[3]; BUSCH, Daryle H.[3]; HOFFMAN, Brian M.[1]
([1]Northwestern Univ.; [2]Washington State Univ.; [3]Univ. Kansas)

[*J. Am. Chem. Soc.* in press]

Cryoreduction of the $[FeO_2]^6$ (n = 6 is the number of electrons in 3*d* orbitals on Fe and π* orbitals on O_2) dioxygen-bound ferroheme through (irradiation at 77 K generates an $[FeO_2]^7$ reduced oxy-heme. Numerous investigations have examined $[FeO_2]^7$ centers that have been characterized as peroxo-ferric centers, denoted $[FeO_2]^7_{per}$, in which a ferriheme binds a dianionic peroxo-ligand. The generation of such an intermediate can be understood heuristically if the $[FeO_2]^6$ parent is viewed as a superoxo-ferric center and the injected electron localizes on the O–O moiety. We here report EPR/ENDOR experiments which show quite different properties for the $[FeO_2]^7$ centers produced by cryoreduction of monomeric oxy-hemoglobin (oxy-GMH3) from *Glycera dibranchiata*, which is unlike mammalian globins in having a leucine in place of the distal histidine, and of frozen aprotic solutions of oxy-ferrous octaethyl porphyrin and of the oxy-ferrous complex of the heme model, cyclidene. These $[FeO_2]^7$ centers are characterized as superoxo-ferrous centers, ($[FeO_2]^7_{sup}$), with nearly unit spin density localized on a superoxo moiety which is end-on coordinated to a low-spin ferrous ion. This assignment is based on their *g* tensors and ^{17}O hyperfine couplings, which are characteristic of the superoxide ion coordinated to a diamagnetic metal ion, and on the absence of detectable endor signals either from the in-plane ^{14}N ligands or from an exchangeable H-bond proton. Such a center would arise if the electron that adds to the $[FeO_2]^6$ superoxo-ferric parent localizes on the Fe ion, to make a superoxo-ferrous moiety. Upon annealing to T > 150 K the $[FeO_2]^7_{sup}$ species recruit a proton and converts to peroxo/hydroperoxo-ferric ($[FeO_2H]^7$) intermediates. These experiments suggest that the primary reduction product is $[FeO_2]^7_{sup}$, and that the internal redox transition to $[FeO_2]^7_{per}/[FeO_2H]^7$ states is driven at least in part by Hbonding/ proton donation by the environment.

IX-C-4 Preparation of Artificial Metalloenzymes by Insertion of Chromium(III) Schiff Base Complexes into Apomyoglobin Mutants

OHASHI, Masataka[1]; KOSHIYAMA, Tomomi[1]; UENO, Takafumi[1]; YAMASE, Manabu[1]; FUJII, Hiroshi; WATANABE, Yoshihito[1]
([1]Nagoya Univ.)

[*Angew. Chem., Int. Ed.* **42**, 1005–1008 (2003)]

Construction of artificial metalloenzymes is one of the most important subjects in bioinorganic chemistry, because metalloenzymes catalyze chemical transformations with high selectivity and reactivity under mild conditions. There are several reports on protein design: introduction of metal binding sites, design of substrate binding cavities, chemical modification of prosthetic groups, and covalent attachment of metal cofactors. In particular, the covalent modification of proteins is a powerful tool for the generation of new metalloenzymes, while the efficiency of the modification is very much dependent on the position and reactivity of the cysteinyl thiol functional group. Herein, we describe a novel strategy for the preparation of artificial metalloenzymes by noncovalent insertion of metal-complex catalysts into protein cavities. The resulting semisynthetic metalloenzymes, apo-myoglobin (apo-Mb) reconstituted with Cr(III) Schiff base complexes, are able to catalyze enantioselective sulfoxidation.

IX-C-5 ^{63}Cu Study of Copper(I) Carbonyl Complexes with Various Tridentate Ligands

KUJIME, Masato; FUJII, Hiroshi

Copper is an essential trace element that plays an important role in a variety of biological functions. We have been studying coordination chemistry of copper complexes ligated by various tridentate ligands as synthetic models for the active site of copper protein which has three imidazolyl groups involved in the histidine residues. Previously we found that the copper(I) carbonyl complexes bearing hydrotris-(pyrazolyl)borates (TPB) exhibit the sharp ^{63}Cu NMR signal in contrast to the other CuL_3L' type complexes, and these chemical shifts were found to be related to the electron donating or withdrawing capabilities of TPB as supported by the dependence of the C≡O stretching vibration. The other copper(I) carbonyl complexes with a series of triazacyclononanes (TACN), tris(4-imidazolyl)carbinols (TIC), trispyrazolylmethanes (TPM), and tris(2-pyridyl)carbomethoxide (TPC) also have shown the good correlations between the δ(^{63}Cu) and C≡O stretching vibration as observed in the TPB system (Figure 1). On the other hand, no meaningful differences were observed for δ(^{13}C) of coordinating carbon monoxide for each complexes, which appears in narrow range around 175 ppm from TMS. These results shows that δ(^{63}Cu) is the sensitive sensor of the extent of back-donation of the Cu-*d* electrons to the antibonding C≡O orbitals, *i.e.*, electron density at the metal center affected by the tridentate ligands. This study also shows the possibility that ^{63}Cu NMR can be the useful tool for the investigation of copper proteins.

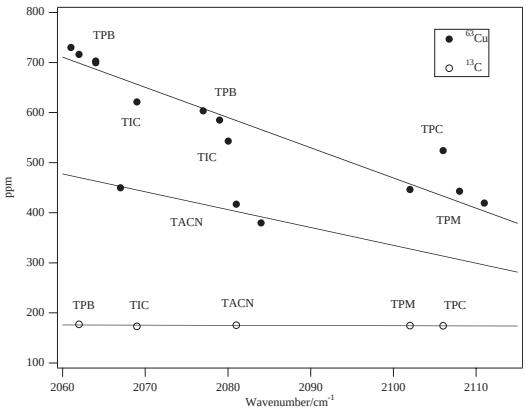

Figure 1. Electronic Effect of Tridentate Ligand on ^{63}Cu- and ^{13}C-NMR Signals of Copper(I) Carbonyl Complexes.

IX-D Molecular Mechanism of Heme Degradation and Oxygen Activation by Heme Oxygenase

Heme oxygenase (HO), an amphipathic microsomal proteins, catalyzes the regiospecific oxidative degradation of iron protoporphyrinIX (heme) to biliverdinIXa, carbon monoxide, and iron in the presence of NADPH-cytochrome P-450 reductase, which functions as an electron donor. Heme oxygenase reaction is the biosynthesis processes of bile pigments and CO, which is a possible physiological messenger. Recent development in the bacterial expression of a soluble form of heme oxygenase has made it possible to prepare in the large quantities for structural studies. In this project, we are studying the molecular mechanism of heme degradation and the oxygen activation by heme oxygenase using various spectroscopic methods.

IX-D-1 Regiospecificity of Each of the Three Steps of Heme Oxygenase Reaction from Hemin to *meso*-Hydroxyhemin, from *meso*-Hydroxyhemin to Verdoheme, and from Verdoheme to Biliverdin

ZHANG, Xuhong[1]; FUJII, Hiroshi; MANSFIELD-MATERA, Kathryn[2]; MIGITA, Catharina Taiko[2]; SUN, Danyu[1]; SATO, Michihiko[1]; IKEDA-SAITO, Masao[2]; YOSHIDA, Tadashi[1]
([1]Yamagata Univ.; [2]Tohoku Univ.)

[*Biochemistry* **42**, 7418–7426 (2003)]

Heme oxygenase catalyzes the regiospecific oxidation of hemin to biliverdin IXα with concomitant liberation of CO and iron by three sequential monooxygenase reactions. The α-regioselectivity of heme oxygenase has been thought to result from the regioselective oxygenation of the heme α-*meso* position at the first step, which leads to the reaction pathway *via meso*-hydroxyheme IXα and verdoheme IXα intermediates. However, recent reports concerning heme oxygenase forming biliverdin isomers other than biliverdin IXα raise a question whether heme oxygenase can degrade *meso*-hydroxyhemin and verdoheme isomers other than the α-isomers. In this paper, we investigated the stereoselectivity of each of the two reaction steps from *meso*-hydroxyhemin to verdoheme and verdoheme to biliverdin, by using a truncated form of rat heme oxygenase-1 and the chemically synthesized four isomers of *meso*-hydroxyhemin and verdoheme. Heme oxygenase-1 converted all four isomers of *meso*-hydroxyhemin to the corresponding isomers of verdoheme. In contrast, only verdoheme IXα was converted to the corresponding biliverdin IXα. We conclude that the third step, but not the second, is stereoselective for the α-isomer substrate. The present findings on regioselectivities of the second and the third steps have been discussed on the basis of the oxygen activation mechanisms of these steps.

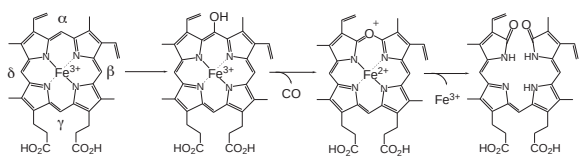

Figure 1. α-Regioselective Heme Catabolism by Heme oxygenase.