RESEARCH ACTIVITIES V
Department of Applied Molecular Science

V-A Molecular Mechanisms of Oxygen Activation by Heme Enzymes

By sharing a common prosthetic group, the heme enzymes such as cytochrome P450s, peroxidases, and catalases catalyze their own unique biological functions; monooxygenation, hydrogen peroxide dependent oxidation, and dismutation of hydrogen peroxide, respectively. Our efforts have been focused on the elucidation of the structure-biological function relationship of thoses heme enzymes by employing both enzymic systems including mutants and their model systems.

V-A-1 Investigations of the Myoglobin Cavity Mutant H93G with Unnatural Imidazole Proximal Ligands as a Modular Peroxide O-O Bond Cleavage Model System

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[*Biochemistry* **39**, 1446 (2000)]

A general inability to carry out extensive variations in the electronic characteristics of proximal heme iron ligands in heme proteins has hampered efforts to obtain a clear understanding of the role of the proximal heme iron ligand in the activation of oxygen and peroxide. The disadvantage of the frequently applied site directed mutagenesis technique is that it is limited by the range of natural ligands available within the genetic code. The myoglobin cavity mutant H93G has its proximal histidine ligand replaced by glycine, a mutation which leaves an open cavity capable of accommodation of a variety of unnatural potential proximal ligands. We have carried out investigations of the effect of changing the electron donor characteristics of a variety of substituted imidazole proximal ligands on the rate of formation of myoglobin compound II and identified a correlation between the substituted imidazole N-3 pK_a (which provides a measure of the electron donor ability of N-3) and the apparent rate of formation of compound II. A similar rate dependence correlation is not observed upon binding of azide. This finding indicates that O–O bond cleavage and not the preceding peroxide binding step is being influenced by the electron donor characteristics of the substituted imidazole ligands. The proximal ligand effects are clearly visible but their overall magnitude is quite low (1.7 fold increase in O–O bond cleavage rate per pK_a unit). This appears to provide support for recent commentaries that the partial ionization of the proximal histidine ligand in typical heme peroxidases may not be enough of an influence to provide a mechanistically critical push effect. Further attempts were made to define the mechanism of the influence of N-3 pK_a on O–O bond cleavage by using peracetic acid and cumene hydroperoxide as mechanistic probes. The observation of heme destruction in these reactions indicates that displacement of the proximal imidazole ligands by peracetic acid or cumene hydroperoxide has occurred. A combination mutation; H64D/H93G was prepared with the objective of observing compound I of H64D/H93G with substituted imidazoles as proximal ligands upon reaction with H_2O_2. This double mutant was found to simultaneously bind imidazole to both axial positions, an arrangement which prevents a reaction with H_2O_2.

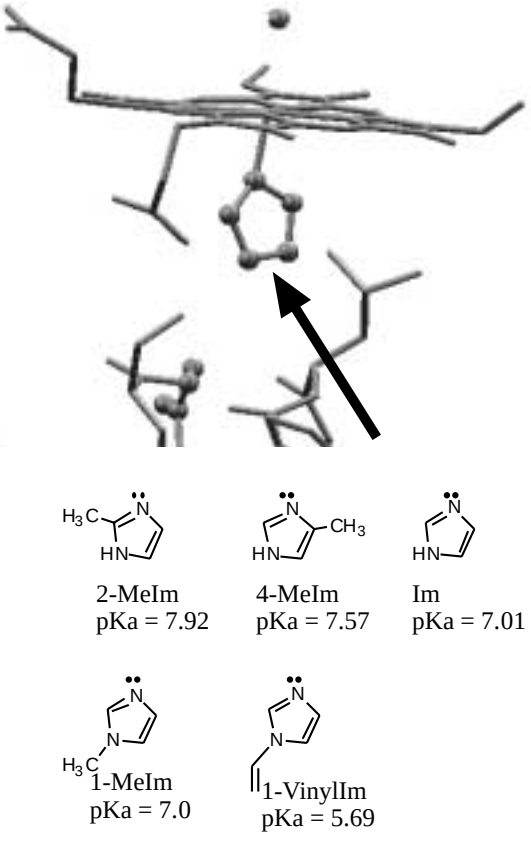

Figure 1. Active site of the ferric imidazole adduct of H93G Mb. Also shown are the five Im-X used as proximal ligands for H93G and their pK_a values

V-A-2 Formation and Catalytic Roles of Compound I in the Hydrogen Peroxide-Dependent Oxidations by His64 Myoglobin Mutants

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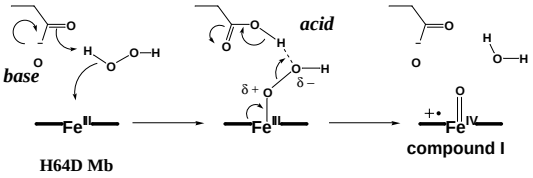
[*J. Am. Chem. Soc.* **121**, 9952 (1999)]

A His64 → Asp mutant of sperm whale myoglobin (Mb), H64D Mb, has been prepared to mimic the active site of chloroperoxidase from the marine fungus *Caldariomyces fumago*, in which distal glutamic acid is suggested to enhance the compound I formation by H_2O_2. The H64D mutant allows us to see the accumulation of compound I in the reaction of Mb with H_2O_2 for the first time. The successful observation of compound I is due to at least 50-fold improvement in the formation rate of compound I as well as its stabilization upon the His64 → Asp replacement. Catalytic activity of wild type Mb and a series of His64 Mb mutants (H64A, H64S, H64L, and H64D Mb) are examined for one-electron oxidation and oxygenation by using H_2O_2 as an oxidant. The H64D mutant is the best catalyst among the myoglobins and shows 50~70-fold and 600~800-fold higher activity than the wild type in the one-electron oxidations and peroxygenations, respectively. The origin of the varied activity upon the mutations is discussed on the basis of the formation rate and stability of compound I.



Scheme 1. Roles of distal aspartate of H64D myoglobin in the reaction with H_2O_2.

V-A-3 Proximal Ligand Control of Heme Iron Coordination Structure and Reactivity with Hydrogen Peroxide: Investigations of the Myoglobin Cavity Mutant H93G with Unnatural Oxygen Donor Proximal Ligands

ROACH, Mark P.; PUSPITA, Waheeda J.; WATANABE, Yoshihito

[*J. Inorg. Biochem.* **81**, 173 (2000)]

The role of the proximal heme iron ligand in activation of hydrogen peroxide and control of spin state and coordination number in heme proteins is not yet well understood. Although there are several examples of amino acid sidechains with oxygen atoms which can act as potential heme iron ligands, the occurrence of protein-derived oxygen donor ligation in natural protein systems is quite rare. The sperm whale myoglobin cavity mutant H93G Mb (Barrick, D. *Biochemistry* **33**, 6546 (1994)) has its proximal histidine ligand replaced by glycine, a mutation which leaves an open cavity capable of accommodation of a variety of unnatural potential proximal ligands. This provides a convenient system for studying ligand-protein interactions. Molecular modeling of the proximal cavity in the active site of H93G Mb indicates that the cavity is of a sufficient size to accommodate benzoate and phenolate in conformations that allow their oxygen atoms to come within binding distance of the heme iron. In addition, benzoate may occupy the cavity in an orientation which allows one carboxylate oxygen atom to ligate to the heme iron while the other carboxylate oxygen is within hydrogen bonding distance of serine 92. The ferric phenolate and benzoate complexes have been prepared and characterized by UV-visible and MCD spectroscopies. The benzoate adduct shows characteristics of a six-coordinate high-spin complex. To our knowledge, this is the first known example of a six-coordinate high-spin heme complex with an anionic oxygen donor proximal ligand. The benzoate ligand is displaced at alkaline pH and upon reaction with hydrogen peroxide. The phenolate adduct of H93G Mb is a five-coordinate high-spin complex whose UV-visible and MCD spectra are distinct from those of the histidine 93 to tyrosine (H93Y Mb) mutant of sperm whale myoglobin. The phenolate adduct is stable at alkaline pH and exhibits a reduced reactivity with hydrogen peroxide relative to that of both native ferric myoglobin, and the exogenous ligand free derivative of ferric H93G Mb. These observations indicate that the identity of the proximal oxygen donor ligand has an important influence on both the heme iron coordination number and the reactivity of the complex with hydrogen peroxide.

V-A-4 Mechanisms of Sulfoxidation Catalyzed by High-Valent Intermediates of Heme Enzymes: Electron Transfer *vs.* Oxygen Transfer Mechanism

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[*J. Am. Chem. Soc.* **121**, 9497 (1999)]

The mechanism of sulfoxidation catalyzed by high-valent intermediates of heme enzymes has been studied by direct observation of the reduction of compounds I of HRP (horseradish peroxidase) and His64Ser myoglobin (Mb) mutant as well as $O=Fe^{IV}TMP^{+\bullet}$ (**1**) (TMP = 5,10,15,20-tetramesitylporphyrin dianion) by sulfides. The reaction of thioanisole and compound I of HRP (10 μM, pH 7.0, 298K) gives the resting state of HRP with accumulation of compound II as an intermediate. The yield of sulfoxide by a stoichiometric reaction of HRP compound I with thioanisole was only 25 ± 5%. On the other hand, the same sulfoxidation by both **1** and His64Ser Mb compound I exclusively exhibited two-electron process resulting in quantitative formation of sulfoxide. When 1,5-dithiacyclooctane (DTCO) is employed as a substrate, the reaction of His64Ser Mb compound I with DTCO exhibits rapid formation of compound II which decays to the ferric state due to the low oxidation potential of DTCO. The observed rate constants (log k) of the reactions of **1** and compounds I of HRP and His64Ser Mb with a series of p-substituted thioanisoles correlate with the one-electron oxidation potentials (E^0) of the sulfides. A comparison of these correlations with the established correlation between log k and E^0 for the corresponding electron transfer reactions of substituted *N,N*-dimethylanilines has revealed that the reactions of compound I of HRP with the sulfides proceed via electron transfer while that the sulfoxidation of sulfides by **1** and compound I of His64Ser Mb occurs via direct oxygen transfer rather

than electron transfer.

V-B Model Studies of Non-Heme Proteins

Non-heme proteins play important roles in biological redox processes. Many reactions catalyzed by the non-heme enzymes are quite similar to those by hemoproteins. We are interested in the active intermediates responsible for oxidation and oxygenation by non-heme enzyme, especially the similarity and differences.

V-B-1 A Bis(μ-oxo)dicopper(III) Complex with Aromatic Nitrogen Donors: Structural Characterization and Reversible Conversion between Copper(I) and Bis(μ-oxo)dicopper(III) Species

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[*J. Am. Chem. Soc.* **122**, 2124 (2000)]

It is important to explore how the nature of donor atoms and the stereochemistry of ligands influence the structures and properties of bis(μ-oxo)dicopper(III) complexes. In this context, we have synthesized a bis(μ-oxo)dicopper(III) complex, $[Cu_2(O)_2(Me_2\text{-tpa})_2tpa](PF_6)_2\cdot 2(CH_3)_2CO$ (**1b**), having a tetradentate tripodal ligand containing aromatic nitrogen donors. The most striking feature of **1b** is the reversible conversion with a precursor copper (I) complex $[Cu(Me_2\text{-tpa})]^+$ (**1a**) in CH_2Cl_2 at –80 °C by bubbling N_2 gas. Such reversible behavior has not been observed for the bis(μ-oxo)-dicopper(III) complexes. Thus reactivity patterns in copper–dioxygen chemistry significantly vary with ligand system. Me_2-tpa has a unique ability to stabilize both copper(I) and copper(III) oxidation states: it can take not only a square planar structure having weak ligation from the axial positions suitable for formation of copper (III) oxidation state but also a pyramidal structure sitable for formation of copper(I) oxidation state.

V-B-2 Characterization of Imidazolate-Bridged Cu(II)-Zn(II) Heterodinuclear and Cu(II)-Cu(II) Homodinuclear Hydroperoxo Complexes as Reaction Intermediate Models of Cu, Zn-SOD

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[*Chem. Commun.* 1051 (2000)]

Copper-zinc superoxide dismutase (Cu, Zn-SOD) contains an imidazolate-bridged Cu(II)-Zn(II) heterodinuclear metal center in its active site. This enzyme catalyses a very rapid two-step dismutation of superoxide to dioxygen and hydrogen peroxide through an alternate reduction and oxidation of the active-site copper ion. An outer-sphere electron transfer from superoxide to Cu(II) center occurs to produce O_2 and Cu(I) center which may be oxidized by another molecule of superoxide in the presence of proton to produce H_2O_2 via a hydroperoxo-Cu(II) species. The hydroperoxo-copper(II) species is a key intermediate in biological oxidations catalyzed by copper enzymes including SOD. We report herein the first characterization of SOD model hydroperoxo-Cu(II) intermediates generated by the reactions of hydrogen peroxide with the imidazolate-bridged Cu(II)-Zn(II) heterodinuclear and Cu(II)-Cu(II) homodinuclear complexes.

V-B-3 Synthesis and X-ray Crystal Structure of a Novel Mn(II)-Semiquinonate Complex $[Mn^{II}(TPA)(DTBSQ)]BPh_4$, and Its Dioxygenase-like Activity: Relevance to Manganese(II)-Dependent Catechol Dioxygenases

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Catechol dioxygenases play key roles in the metabolism of various aromatic compounds, converting aromatics to aliphatics with insertion of molecular oxygen between a C–C bond of a benzene ring, and have been studied extensively in recent years from both sides of enzymes and models. We here report synthesis of a novel Mn(II)-semiquinonate complex, $[Mn^{II}(TPA)(DTBSQ)]X$ (**1**, DTBSQ: 3,5-di-tert-butyl-1,2-benzosemiquinonate; TPA: tris(2-pyridylmethyl)amine; X: Cl or BPh_4), that is oxygenated with molecular oxygen in the intradiol cleavage fashion. The complex is a new type of mononuclear mono(3,5-di-tert-butyl-1,2-benzosemiquinonate)manganese(II) complex, and highly peculiar not only for the electronic configuration keeping the Mn(II) state even after coordination of a semiquinonate radical anion, but also for the geometric configuration that is not in the usual octahedral geometry.

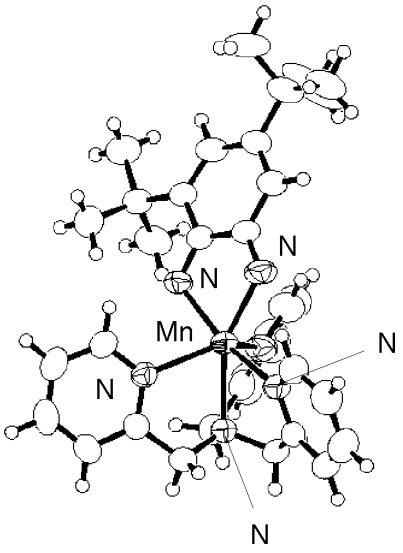

Figure 1. ORTEP drawing of **1**.

V-B-4 Infrared Spectroscopic Features of the Cyclic Hydrogen-Bonded *cis*(Hydroxo)–Fe^{III}–(Carboxylato) Unit of Lipoxygenase Active Site Models

AKI, Michihiko; OGURA, Takashi[2]; KITAGAWA, Teizo; MASUDA, Hideki[1]; WATANABE, Yoshihito
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[submitted for publication]

Lipoxygenases (LOs) are mononuclear non-heme iron enzyme which are widely distributed among plants and mammals. LOs catalyze the peroxidation of polyunsaturated fatty acids containing the *cis,cis*-1,4-diene moiety to the corresponding 1-hydroperoxy-*trans,cis*-2,4-diene. This paper reports infrared (IR) spectroscopic features of the structurally characteristic *cis*(hydroxo)–Fe^{III}–(carboxylato) unit of lipoxygenase active site model complexes, $[Fe^{III}(tnpa)(OH)(RCO_2)]ClO_4$ (**1a**: R = CH_3 and **1b**: R = H). The vibrational modes were unequivocally assigned by isotopic substitution of the hydroxo ($^{18}OH^-$, $^{16}OD^-$, and $^{18}OD^-$ for $^{16}OH^-$) and the carboxylato (**1a**: $^{12}CH_3{}^{12}C^{18}O_2^-$, $^{13}CH_3{}^{12}C^{16}O_2^-$, $^{12}CH_3{}^{13}C^{16}O_2^-$, and $^{13}CH3{}^{13}C^{16}O_2^-$ for $^{12}CH_3{}^{12}C^{16}O_2^-$ and **1b**: $H^{13}C^{16}O_2^-$ for $H^{12}C^{16}O_2^-$) ligands in the solid state (in mineral oil) and in the liquid state (in acetonitrile). The crystal structure of **1a** was determined by X-ray analysis. It was confirmed by electrospray ionization mass spectrometry (ESI-MS) that the structures of the complexes are preserved in acetonitrile.

V-C Aqueous Organometallic Chemistry

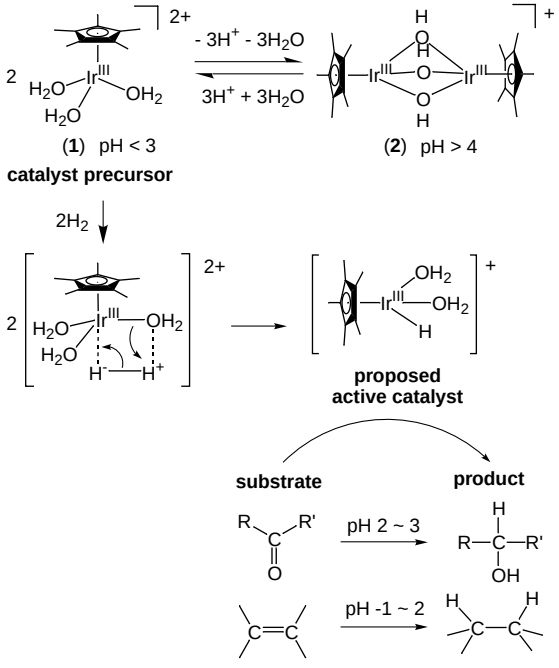
In recent years, aqueous organometallic chemistry has been widely studied because of industrial advantages and environmental concerns. Few organometallic aqua complexes have been, until now, isolated and used as water-soluble reagents in aqueous media. We have investigated a homogeneous hydrogenation in aqueous media using organometallic aqua complexes whose structures and properties drastically change as a function of pH because of deprotonation of the aqua ligands.

V-C-1 pH-Selective Hydrogenation with an Organometallic Aqua Complex as a Catalyst Precursor in Very Acidic Media

MAKIHARA, Nobuyuki; OGO, Seiji; WATANABE, Yoshihito

[*Organometallics* in press]

An organometallic aqua complex $[Cp^*Ir^{III}(H_2O)_3]^{2+}$ (**1**) serves as a catalyst precursor for aqueous hydrogenation of water-soluble compounds with C=O or C=C bonds under very acidic conditions. The hydrogenation shows unique pH-selectivity depending upon substrates. The rates of hydrogenation of carbonyl compounds show a maximum in a pH range of about 2 to 3. The rates of hydrogenation of water-soluble alkenes show a maximum in a pH range of about –1 to 2. Above pH 4, complex **1** is deprotonated to form a catalytically inactive dinuclear complex $[(Cp^*Ir^{III})_2(\mu\text{-}OH)_3]^+$ (**2**). This is the first example of a pH-selective hydrogenation using an organometallic aqua complex as the catalyst precursor under very acidic conditions.



Scheme 1. pH-Selective hydrogenation with an organometallic aqua complex.