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 Formation and Catalytic Roles of Compound I in the Hydrogen Peroxide-Dependent Oxidation by His64 Myoglobin Mutants

MATSUI, Toshitaka; OZAKI, Shin-ichi; WATANABE, Yoshihito

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

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 (Figure 1). The successful observation of compound I is due to at leaset 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 thaan the wild type in the one-electron oxidations and peroxygenations, erspectively. The origin of the varied activity upon the mutations are discused on the basis of the formation rate and stability of compound I.

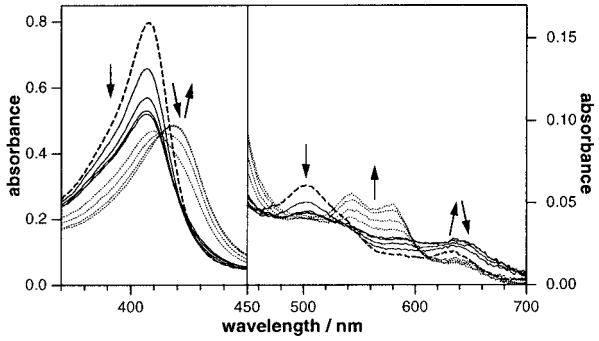

Figure 1. Spectral changes of H64D Mb in the reactions with 1 mM H_2O_2 at pH 7.0 and 20 °C. The spectra were recorded before mixing (*broken line*), at 10, 30, 60, 100, and 150 ms (*solid lines*), and at 1, 2, 4, and 6 sec (*dotted lines*) after mixing. For clarity, Soret and Visible regions are expanded and magnified, respectively.

V-A-2 The Mechanisms of *N*-Demethylation Catalyzed by Heme Enzymes: Mechanisms of Sulfoxidation Catalyzed by High-Valent Intermediates of Heme Enzymes: Electron Transfer vs Oxygen Transfer Mechanism

GOTO, Yoshio; MATSUI, Toshitaka; OZAKI, Shin-ichi; WATANABE, Yoshihito; FUKUZUMI, Shunichi[1]
([1]Osaka Univ.)

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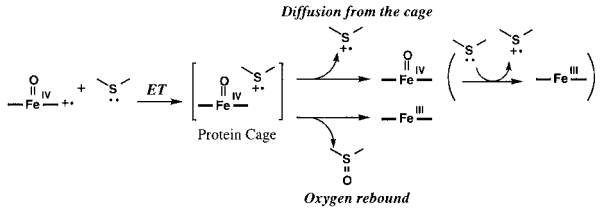
Mechanisms of sulfoxidation catalyzed by high-valent intermediates of heme enzymes have been investigated by direct observation of sulfide induced reduction of three different compound I species including HRP (horseradish peroxidase), the His64Ser myoglobin (Mb) mutant and $O=Fe^{IV}TMP^{+\bullet}$ (**1**) (TMP = 5,10,15,20-tetramesitylporphyrin dianion). The reaction of thioanisole and compound I of HRP (10 mM, pH 7.0, 298 K) 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 a 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_{obs}) 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_{ox}) of the sulfides. A comparison of these correlations with the established correlation between log k_{obs} and E^0_{ox} for the corresponding electron transfer reactions of substituted *N,N*-dimethylanilines has revealed that the sulfoxidation reactions of compound I of HRP with the sulfides proceed via electron transfer while the sulfoxidations catalyzed by **1** and compound I of His64Ser Mb occur via direct oxygen transfer.



Scheme 1. Electron Transfer *vs.* Direct Oxygen Transfer.

V-A-3 Effects of the Arrangement of a Distal Catalytic Residue on Regioselectivity and Reactivity in the Coupled Oxidation of Sperm Whale Myoglobin Mutants

MURAKAMI, Tatsuya[1]; MORISHIMA, Isao[1]; MATSUI, Toshitaka; OZAKI, Shin-ichi; HARA, Isao; YANG, Hui-Jun; WATANABE, Yoshihito
([1]Kyoto Univ.),

[*J. Am. Chem. Soc.* **121**, 2007 (1999)]

Heme oxygenase is a central monoxygenase of the heme catabolism and forms a stoichiometric complex with protoheme IX. The enzyme utilizes electrons and molecular oxygen for the regioselective heme degradation to afford α-biliverdin and carbon monoxide (CO) through three sequential oxygenase reactions. To understand factors which control the high regioselectivity, the regiospecific heme degradation of sperm whale myoglobin (Mb) mutants has been performed L29H/H64L myoglobin (Mb) almost exclusively gives biliverdin IXγ although H64L and wild type Mb mainly affords the a-isomer. Relocation of the distal histidine at the 43, and 107 position increases the amount of γ-isomer to 44 and 22%, respectively. Interestingly, the increase in the ratio of γ-isomer is also observed by a single replacement of either His-64 with Asp or Phe-43 with Trp. It appears that the polarity of the active site as well as hydrogen bonding between oxygen molecule bound to the heme iron and His or Trp is important in controlling the regioselectivity. The results of coupled oxidation kinetics, autooxidation kinetics, and redox potential of Fe^{3+}/Fe^{2+} couple are discussed with regards to their implications for the active site and mechanism of heme oxygenase.

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 Model for Peroxo Intermediates in Reactions Catalyzed by Non-Heme Iron Enzymes

WADA, Akira[1]; OGO, Seiji; WATANABE, Yoshihito; MUKAI, Masahiro; KITAGAWA, Teizo; JITSUKAWA, Koichiro[1]; MASUDA, Hideki[1]; EINAGA, Hisahiko[1]
([1]Nagoya Inst. Tech.)

[*Inorg. Chem.* **38**, 3592 (1999)]

Thermodynamically extremely stable alkylperoxo-iron(III) complexes have first been prepared from reaction of the ternary iron(III) complex with a tripodal pyridylamine ligand, bis(6-pivalamido-2-pyridylmethyl)(2-pyridylmethyl)amine (BPPA), and trimethylacetate with alkylperoxide (*t*BuOO or $C_6H_5C(CH_3)OO$) in MeCN. The structure of the starting complex [Fe(bppa)(tBuCOO)]$(ClO_4)_2$ (**1**) was determined by X-ray diffraction method. The electronic absorption spectra obtained by addition of aqueous solutions containing *t*BuOOH (TBHP) (69 w/w%) or $C_6H_5C(CH_3)OOH$ (CHP) (80 w/w%) to **1** showed charcteristic bands at 613 nm (ε = 2000 $M^{-1}cm^{-1}$) or 585 nm (ε = 2200 $M^{-1}cm^{-1}$) assignable to an alkylperoxide-iron(III) charge transfer transition, respectively. The resonance Raman spectra revealed strong resonance-enhanced Raman features at 873, 838, 629, and 469 cm^{-1} and 878, 838, 639, 548, and 493 cm^{-1}, respectively. The ESI-mass spectra afforded positive and negative ion peaks at *m*/*z* = 316.5 and 932 corresponding to the ions, $[Fe(bppa)(tBuOO)]^{2+}$ and $\{[Fe(bppa)(tBuOO)](ClO_4)_3\}^-$, and at *m*/*z* = 347.5 and 994 assignable to $[Fe(bppa)(C_6H_5C(CH_3)OO)]^{2+}$ and $\{[Fe(bppa)(C_6H_5C(CH_3)OO)](ClO_4)_3\}^-$, respectively. The ESR spectra at 77 K demonstrated a typical high spin iron(III) state with small rhombic distortion (g = 7.58, 5.81, 4.25, 1.82, E/D = 0.067 and g = 7.76, 5.65, 4.20, 1.78, E/D = 0.070, respectively). The cyclic voltammetry of the starting complex **1** exhibited a quasi-reversible redox wave of the $Fe^{3+/2+}$ couple at +700 mV vs NHE that is fairly agreement with that in lipoxygenase. Their alkylperoxide complexes were successfully isolated as powder precipitates, and the UV-Vis, ESR, and ESI-mass spectra of the acetone or MeCN solutions containing the precipitates were almost the same as those in solution. The above findings strongly suggest that the reaction of **1** with alkylperoxide generated the extremely stable iron(III)-alkylperoxide complexes with seven-coordinate.

V-B-2 An Unusual Conversion of a $Ni(III)_2(\mu\text{-}O)_2$ Core into a $Ni(II)_2(\mu\text{-}OO)_2$ Core by H_2O_2 and Oxygenation of Ligand

SHIREN, Kazushi[1]; OGO, Seiji; FUJINAMI, Shuhei[1]; HAYASHI, Hideki[1]; SUZUKI, Masatatsu[1]; UEHARA, Akira[1]; WATANABE, Yoshihito; MORO-OKA, Yoshihiko[2]
([1]Kanazawa Univ.; [2]Tokyo Inst. Tech.)

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

A six-coordinate bis(μ-oxo)nickel(III) complex, $[Ni_2(\mu\text{-}O)_2(Me_3\text{-}tpa)_2]^{2+}$ (**1**), was synthesized by

reaction of $[Ni_2(\mu\text{-}OH)_2(Me_3\text{-}tpa)_2]^{2+}$ (**2**) with 1 equiv. of hydrogen peroxide in methanol at –90 °C, where Me_3-tpa = tris(6-methyl-2-pyridylmethyl)amine. The 6-methyl groups of Me_3-tpa have a significant influence on the formation and stabilization of the high-valent bis(μ-oxo)dinickel(III) species. Reaction of **2** with a large excess of hydrogen peroxide (> 10 equiv.) afforded a novel bis(μ-superoxo)dinickel(II) complex, $[Ni_2(\mu\text{-}O_2)_2(Me_3\text{-}tpa)_2]^{2+}$ (**3**). The reaction demonstrates an unique conversion of a $Ni^{III}(\mu\text{-}O)_2Ni^{III}$ core into a $Ni^{II}(\mu\text{-}OO)_2Ni^{II}$ core upon exposure to hydrogen peroxide. Complexes **1**, **2**, and **3** were characterized by X-ray crystallography and various physicochemical techniques. Complex **1** has an edge-shared bioctahedral structure with a $Ni(\mu\text{-}O)_2Ni$ core. Each nickel atom is coordinated by Me_3-tpa to complete a distorted octahedral coordination sphere. The average Ni–O and Ni–N bond distances of **1** (1.871 and 2.143 Å, respectively) are significantly shorter than those of **2**, (2.018 and 2.185 Å, respectively), suggesting that **1** is a bis(μ-oxo)dinickel(III) complex. The crystal structure of **3** consists of a centrosymmetric $Ni(\mu\text{-}OO)_2Ni$ core with Me_3-tpa nitrogens. The nickel centers are in a distorted octahedral structure and linked by two μ-1,2-O–O bridges to form a six-membered ring with a chair conformation. The O–O bond distance is 1.345(6) Å, which is intermediate between those of the typical peroxo and superoxo complexes. The resonance Raman spectrum of a powdered sample of **3** measured at 110 K showed an isotope-sensitive band at 1096 cm^{-1} (1044 cm^{-1} for an ^{18}O labeled sample), indicating that **3** is a bis(μ-superoxo)Ni_2(II) complex. Thermal decomposition of both **1** and **3** in acetone at –20 °C under N_2 atmosphere resulted in a partial hydroxylation of a methyl group of Me_3-tpa in yield of 21–29% for both complexes. A carboxylate complex, $[Ni(Me_2\text{-}tpaCOO)](OH_2)]^+$ (**4**), where one of the three methyl groups of Me_3-tpa is oxidized to carboxylate, was isolated from the decomposition under N_2 atmosphere. During the decomposition process, dioxygen evolution was simultaneously observed in yield of 35 ± 4%. Thermal decomposition of **1** under O_2 atmosphere also gave **4**. The Electrospray ionization mass spectrometry (ESIMS) of **3** revealed the formation of **1** during the decomposition process. These results suggest that one possible decomposition pathway of **3** is a disproportionation of two coordinated superoxides to dioxygen and peroxide followed by the O–O bond scission of peroxide to regenerate **1**, which is responsible to the hydroxylation and the oxidation of the 6-methyl group of Me_3-tpa.

V-B-3 Structural and Functional Model Complexes for the Catechol-Bound Intermediate of Intradiol-Cleaving Catechol Dioxygenases

YAMAHARA, Ryo[1]; OGO, Seiji; WATANABE, Yoshihito; FUNABIKI,Takuzo[2]; JITSUKAWA, Koichiro[1]; MASUDA, Hideki[1]; EINAGA, Hisahiko[1]
([1]Nagoya Inst. Tech.; [2]Kyoto Univ.)

[*Inorg. Chim. Acta* in press]

This paper reports the synthesis and structures of (catecholato)iron(III) complexes with tetradentate tripodal ligands ($L_{R',R''}$ = {2-hydroxy-3-R'-5-R''-phenyl-bis(2-pyridylmethyl)amine}) containing substituted phenol and pyridine units: $[Fe^{III}(L_{R',R''})(DBC)]$ (**1a**: R',R'' = H,H, **1b**: R',R'' = Me,Me, and **1c**: R',R'' = H,Cl, DBC = 3,5-di-*tert*-butylcatechol). X-ray structure analysis has revealed that the coordination arrangement around the iron atom of **1a** is very similar to that proposed for the active site of the catechol-bound intermediate of protocatechuate 3,4-dioxygenase (3,4-PCD). The series of complex **1** derivatives has been synthesized by two different methods: (i) reaction of $[Fe^{III}(L_{R',R''})(acac)]^+$ (**2a**: R',R'' = H,H, **2b**: R',R'' = Me,Me, and **2c**: R',R'' = H,Cl, acac = acetylacetonate) with 1 equiv. of DBC and 1 equiv. of Et_3N in *N*,*N*-dimethylformamide, and (ii) reaction of $[Fe^{III}(L_{R',R''})Cl_2]$ (**3a**: R',R'' = H,H, **3b**: R',R'' = Me,Me, and **3c**: R',R'' = H,Cl) with 2 equiv. of AgOTf (OTf = $O_3SCF_3^-$), 1 equiv. of the catechols, and 2 equiv. of Et_3N in DMF. The exogenous acac ligand of **2** acts as a Lewis-base like the Tyr447 ligand in the active site of 3,4-PCD in the formation of the catechol-bound intermediate. Complexes **1**, **2**, and **3** have been characterized by X-ray analysis, visible and EPR spectroscopies, and cyclic voltammetry. Oxygenation of the bound DBC on **1** in the presence of O_2 has also been investigated and is discussed based on the Lewis basicity of the tripodal ligand containing the substituted phenolato group which is introduced to mimic the Tyr408 ligand of 3,4-PCD.

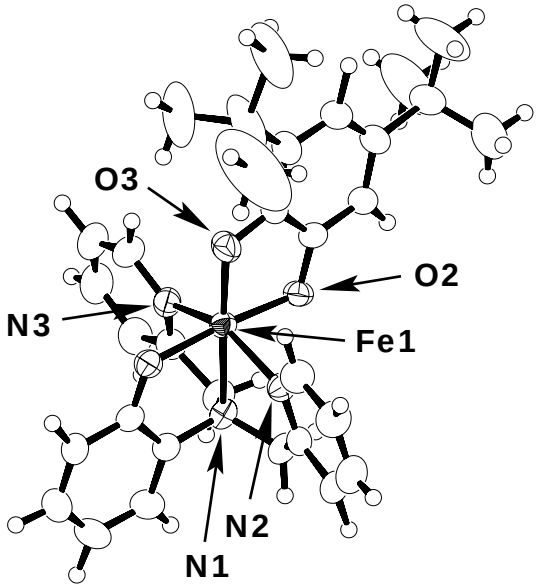


Figure 1. ORTEP drawing of **1a**.

V-C Transition Metal Oxide Clusters

Organometallic oxide clusters with cubic and incomplete cubic frameworks have useful applications as homogeneous and heterogeneous catalysts for reactions such as the oxidation and metathesis of propene. Understanding of the formation mechanisms of such oxide clusters may lead to the further development of synthetic methodologies for the construction of desired clusters having efficient catalytic ability for hydrocarbon transformations.

V-C-1 Direct Observation by Electrospray Ionization Mass Spectrometry of a Key Intermediate in the Formation of a Double Bookshelf-Type Oxide Cluster

TAKARA, Satoshi[1]; OGO, Seiji; NISHIKAWA, Koji[1]; KINOSHITA, Isamu[1]; ISOBE, Kiyoshi[1]; WATANABE, Yoshihito
([1]Osaka City Univ.)

[*Angew. Chem., Int. Ed. Engl.* in press]

We have found that a 1:4 reaction of [{Cp*Rh(*m*-Cl)Cl}$_2$] (**1**) and [(*n*Bu)$_4$N]$_2$[Mo$_2$O$_7$] (**2**) in MeOH quantitatively yields a double bookshelf-type oxide cluster [(*n*Bu)$_4$N]$_2$[(Cp*Rh)$_2$Mo$_6$O$_{20}$(OMe)$_2$] (**3**) with a multi-incomplete cubic framework. We have investigated the formation mechanism of **3** by electrospray ionization mass spectrometry (ESI-MS) which allows us to detect unstable species generated in solution. Herein, we report a direct observation by ESI-MS of [Cp*RhMo$_3$O$_8$(OMe)$_5$]$^-$ (**3$_{im}$**: *m*/*z* 809), a key intermediate in the formation of **3** from the reaction of **1** and **2** in MeOH at –78 °C. Time dependent-behavior of selected ions during the reaction of **1** and **2** was monitored by rapid scanning of ESI-MS. The existence of the methoxo ligands in **3$_{im}$** was confirmed by isotopic labeling experiments.

V-D 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-D-1 A Unique pH-Dependent Transfer Hydrogenation of Water-Soluble Carbonyl Compounds with an Organometallic Aqua Complex as a Catalyst Precursor in Water

OGO, Seiji; MAKIHARA, Nobuyuki; WATANABE, Yoshihito

[*Organometallics* in press]

This paper reports a unique pH-dependent hydrogen transfer from HCOONa to water-soluble carbonyl compounds with an organometallic aqua complex [Cp*Ir^{III}(H$_2$O)$_3$]$^{2+}$ (**1**, Cp* = η^5-C$_5$Me$_5$) as a catalyst precursor in water. The structure of **1** was unequivocally determined by X-ray analysis. Complex **1** is deprotonated to form a dinuclear complex [(Cp*Ir^{III})$_2$(μ-OH)$_3$]$^+$ (**2**) at pH 3.2. ^{1}H NMR, IR, and electrospray ionization mass spectrometry (ESI-MS) experiments show that the active species in this catalytic reaction is a dinuclear μ-hydride complex [(Cp*Ir^{III})$_2$(μ-H)(μ-OH)(μ-HCOO)]$^+$ (**3**). The rate of this transfer hydrogenation shows a sharp maximum at pH 3.2. The series of carbonyl compounds used in this catalytic reaction are a straight chain aldehyde (*n*-butyraldehyde), a cyclic aldehyde (cyclopropanecarboxaldehyde), a ketone (2-butanone), an aldehyde-acid (glyoxylic acid), and a keto-acid (pyruvic acid).

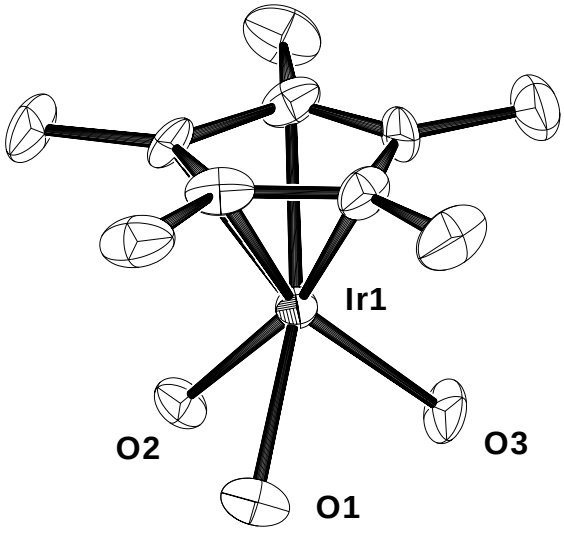


Figure 1. ORTEP drawing of **1**.