RESEARCH ACTIVITIES IX
Center for Integrative Bioscience

IX-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 these heme enzymes by employing both enzymatic systems including mutants and their model systems.

IX-A-1 Asymmetric Sulfoxidation and Amine Binding by H64D/V68A and H64D/V68S Mb: Mechanistic Insight into the Chiral Discrimination Step

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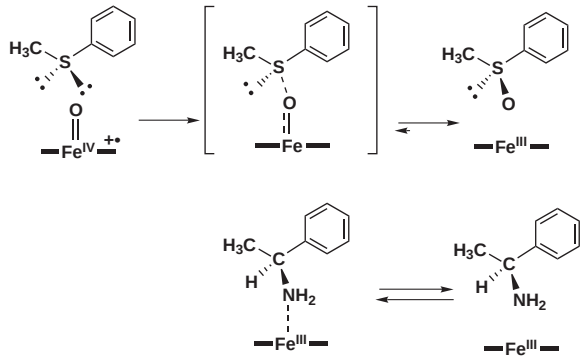
[*J. Am. Chem. Soc.* **124**, 8506 (2002)]

Myoglobin (Mb) is an oxygen transport hemoprotein that catalyzes a variety of oxidation including sulfoxidation and epoxidation in the presence of peroxides. We have recently shown that the distal histidine (His64) in sperm whale Mb is a critical residue in destabilizing a reactive intermediate, myoglobin compound I (Mb-I). The substitution of His-64 with Asp (H64D Mb) also gives Mb-I even by the reaction with H_2O_2 efficiently with the rate constant of 2.5×10^4 $M^{-1}s^{-1}$. Due to the structural similarity of α-methylbenzylamine and methylphenylsulfoxide, we have examined enantioselective ligation of (*R*)- and (*S*)-α-methylbenzylamine to H64D/V68A and H64D/V68S Mbs in comparison with the sulfoxidation of thioanisole (Scheme 1). In contrast to the *R*-selective sulfoxidation by H64D/V68A and H64D/V68S, the K values of (*S*)-α-methylbenzylamine with H64D/V68A and H64D/V68S are 27-fold and 112-fold larger than those of the corresponding (*R*)-amine, respectively. In the case of H64D Mb, which affords almost racemic sulfoxide, however, the enantioselective binding is reversed, namely the *K* value of (*R*)-amine is about 4-fold larger than that for the (*S*) isomer.



In order to determine the chiral discrimination step in the amine binding, we have measured on rate (k_1) and off rate (k_{-1}) of amine binding to the Mb mutants by stopped-flow experiments. The on rates (k_1) of (*R*)- and (*S*)-α-methylbenzylamine to H64D/V68A and H64D/V68S are almost identical, 1.3×10^4 $M^{-1}s^{-1}$ and $2.2–2.7 \times 10^4$ $M^{-1}s^{-1}$, respectively. In contrast, a tremendous difference is seen for the off rate. This indicates that the chiral discrimination of the (*S*)-amine ligation over the (*R*)-amine by H64D/V68A and H64D/V68S is exclusively caused by a very small off rate of the (*S*)-amine relative to the (*R*)-amine, 1:27 for H64D/V68A and 1:92 for H64D/V68S. These selectivities would correspond to 93 and 98% ee for the amine binding, respectively. Thus, enantioselectivity in the sulfoxidation of thioanisole by H64D/V68A and H64D/V68S Mb was concluded to be determined by the off rate of sulfoxide.

IX-A-2 Molecular Mechanism of the Catalase Reaction Studied by Myoglobin Mutants

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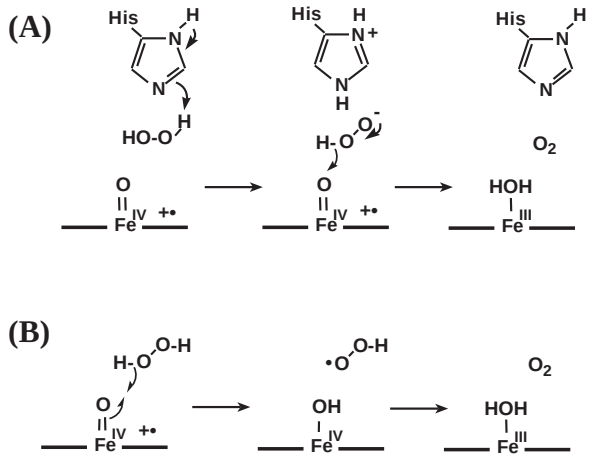
The catalase reaction has been studied in detail by using Mb mutants, whose compound I can be readily prepared by reaction with a nearly stoichiometric amount of *m*-chloroperbenzoic acid (*m*CPBA). Upon the addition of H_2O_2 to a Mb-I solution, Mb-I is reduced back to the ferric state without forming any intermediates. This reveals that Mb-I is capable of performing two-electron oxidation of H_2O_2 (catalatic reaction). GC-

MS analysis of the evolved O_2 from a 50:50 mixture of $H_2{}^{18}O_2/H_2{}^{16}O_2$ solution containing H64D or F43H/H64L shows two peaks for $^{18}O_2$ (m/e = 36) and $^{16}O_2$ (m/e = 32) but no indication of $^{16}O^{18}O$ (m/e = 34) formation. Deuterium isotope effects on rates of the catalatic reaction of Mb mutants and beef liver catalase (BLCase) suggest that the catalatic reactions of BLCase and F43H/H64L Mb proceed *via* an ionic mechanism, since the distal histidine is located at a proper position acting as a general acid-base catalyst in the ionic reaction to give a small isotope effect of less than 2.1. In contrast, other Mb mutants such as H64X (X: A, S, D) and L29H/H64L Mb oxidize H_2O_2 *via* a radical mechanism in which a hydrogen is abstracted by the ferryl species with very large isotope effects in a range of 10 to 29, due to the lack of the general acid-base catalyst. These two mechanisms are summarized in Scheme 1.



Scheme 1. Proposed mechanisms for the catalatic reaction. (A): ionic mechanism by utilizing a general acid-base catalyst. (B): Radical mechanism.

IX-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.

IX-B-1 Reactivity of Hydrogenperoxide Bound to a Mononuclear Non-Heme Iron Site

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[*Inorg. Chem.* **41**, 616 (2002)]

The first isolation and spectroscopic characterization of the mononuclear hydroperoxo-iron(III) complex $[Fe(H_2bppa)(OOH)]^{2+}$ (**1**) and the stoichiometric oxidation of substrates by the mononuclear iron-oxo intermediate generated by its decomposition have been described. The purple species (**1**) obtained from reaction of $[Fe(H_2bppa)(HCOO)](ClO_4)_2$ with H_2O_2 in acetone at –50 °C gave characteristic UV-vis (λ_{max} = 568 nm, ε = 1200 $M^{-1}cm^{-1}$), ESR (g = 7.54, 5.78, and 4.25, S = 5/2), and ESI mass spectra (m/z 288.5 corresponding to the iron, $[Fe(bppa)(OOH)^{2+}$), which revealed that **1** is a high-spin mononuclear iron(III) complex with a hydroperoxide in an end-on fashion. The resonance Raman spectrum of **1** in d_6-acetone revealed two intense bands at 621 and 830 cm^{-1}, which shifted to 599 and 813 cm^{-1}, respectively, when reacted with ^{18}O-laeled H_2O_2. Reactions of the isolated $(bppa)Fe^{III}$–OOH (**1**) with various substrate (single turnover oxidations) exhibited that the iron-oxo intermediate generated by decomposition of **1** is a nucleophilic species formulated as $[(H_2bppa)Fe^{III}–O\cdot]$.

IX-B-2 Synthesis, Structure, and Properties of A Novel Mononuclear Iron(III) Complex Containing Peroxocarbonate Ligand

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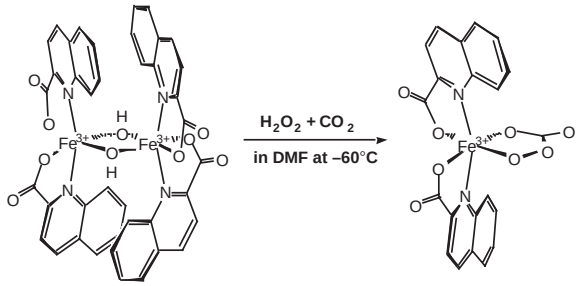
[*Angew. Chem. Int. Ed. Engl*. **41**, 1202 (2002)]

Mononuclaer Peroxo iron(III) complexes have been proposed as a key intermediate in various oxidation reactions catalyzed by mononuclear non-heme iron enzymes and their functional model complexes. Various types of synthetic mononuclear iron(III) complexes having η^2-peroxo, η^1-hydroperoxo, and alkylperoxo ligand have been characterized by various spectroscopic studies. It has been shown that the structure, electronic structure, and reactivity of the peroxo complexes can be modified by the coordination environment around iron(III) center. Most of those peroxo-iron(III) complexes reported so far have nitrogen-rich coordination environments except for an edta complex. Thus it is of interest to investigate how the nature of the donor atoms and the stereochemistry of supporting ligands influence the formation, structure, and properties of such peroxo-iron(III) complexes. In this study, we have succeeded in synthesis of a mononuclear peroxocarbonate iron(III) complex with a carboxylate-rich coordination environment, $Ph_4P[Fe(qn)_2\text{-}(O_2C(O)O)]\cdot 1.5CH_3OH\cdot 0.5(CH_3)_2$-

NCHO (**1a**), derived from the reaction of a bis(μ-hydroxo)diiron(III) complex, $[Fe_2(qn)_4(OH)_2]\cdot 2H_2O$ (**2**) with H_2O_2 and CO_2, where Hqn = quinaldic acid, which was characterized by X-ray, ESI-MS, EPR, UV-vis, and resonance Raman spectroscopic measurements (Scheme 1). This is the first example of a structurally characterized transition metal complex with a peroxocarbonate ligand and a mononuclear iron(III) complex having a peroxo group. We believe that the findings in this study provide an important basis for developing and expanding mononuclear iron(III) complexes having a peroxo group and are of interest to wide audience.



Scheme 1.

IX-B-3 Structural and Spectroscopic Features of a *cis* (Hydroxo)–Fe^{III}–(Carboxylato) Configuration as an Active Site Model for Lipoxygenases

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([1]Osaka Univ.; [2]Nagoya Inst. Tech.; [3]Univ. Tokyo; [4]Nagoya Univ.)

[*Inorg. Chem.* in press]

In our preliminary communication (*Angew. Chem. Int. Ed. Engl.* **37**, 2102 (1998)), we have reported the first example of X-ray analysis of a mononuclear six-coordinate (hydroxo)iron(III) non-heme complex, $[Fe^{III}(tnpa)(OH)(RCO_2)]ClO_4$ {tnpa = tris(6-neopentylamino-2-pyridylmethyl)amine, **1**: $R = C_6H_5$}, which has a characteristic *cis* (hydroxo)–Fe^{III}–(carboxylato) configuration that models the *cis* (hydroxo)–Fe^{III}–(carboxylato) moiety of the proposed (hydroxo)iron(III) species of lipoxygenases. In this full account, we report structural and spectroscopic characterization of the *cis* (hydroxo)–Fe^{III}–(carboxylato) configuration by extending the model complexes from **1** to $[Fe^{III}(tnpa)(OH)(RCO_2)]ClO_4$ (**2**: $R = CH_3$ and **3**: R = H) whose *cis* (hydroxo)–Fe^{III}–(carboxylato) moieties are isotopically labeled by $^{18}OH^-$, $^{16}OD^-$, $^{18}OD^-$, $^{12}CH_3{}^{12}C^{18}O_2^-$, $^{12}CH_3{}^{13}C^{16}O_2^-$, $^{13}CH_3{}^{12}C^{16}O_2^-$, $^{13}CH_3{}^{13}C^{16}O_2^-$, and $H^{13}C^{16}O_2^-$. Complexes **1**, **2**, and **3** are characterized by X-ray analysis, IR, EPR, and UV/Vis spectroscopy, and electrospray ionization mass spectrometry (ESI-MS).

IX-C Aqueous Organometallic Chemistry

The chemistry in aqueous media is presently undergoing very rapid growth because of many potential advantages such as alleviation of environmental problems associated with the use of organic solvents, industrial applications (*e.g.*, introduction of new biphasic processes), and reaction-specific pH selectivity. We have investigated pH-dependent reactions in aqueous media.

IX-C-1 pH-Dependent H_2-Activation Cycle Coupled to Reduction of Nitrate Ion by Cp*Ir Complexes

OGO, Seiji; NAKAI, Hidetaka; WATANABE, Yoshihito

[*J. Am. Chem. Soc.* **124**, 597 (2002)]

This paper reports a pH-dependent H_2-activation promoted by Cp*Ir complexes {Cp* = η^5-$C_5(CH_3)_5$}. In a pH range of about 1 to 4, an aqueous HNO_3 solution of $[Cp^*Ir^{III}(H_2O)_3]^{2+}$ (**1**) reacts with three equivalents of H_2 to yield a solution of $[(Cp^*Ir^{III})_2(\mu\text{-}H)_3]^+$ (**2**) as a result of heterolytic H_2-activation. The hydrido ligands of **2** display protonic behavior and undergo H/D exchange with D^+. Complex **2** is insoluble in a pH range of about –0.2 (1.6 M HNO_3/H_2O) to –0.8 (6.3 M HNO_3/H_2O). At pH –1 (10 M HNO_3/H_2O), a powder of **2** drastically reacts with HNO_3 to give a solution of $[Cp^*Ir^{III}(NO_3)_2]$ (**3**) with evolution of H_2, NO, and NO_2 gases. D-labeling experiments show that the evolved H_2 is derived from the hydrido ligands of **2**. These results suggest that oxidation of the hydrido ligands of **2** couples to reduction of NO_3^-. To complete the reaction cycle, complex **3** is transformed into **1** by increasing the pH of the solution from –1 to 1. Therefore, we are able to repeat the reaction cycle using **1**, H_2, and pH gradient between 1 and –1. A conceivable mechanism for the H_2-activation cycle with reduction of NO_3^- is proposed.

IX-C-2 pH-Dependent Cross-Coupling Reactions of Water-Soluble Organic Halides with Organoboron Compounds Catalyzed by an Organometallic Aqua Complex $[(SCS)Pd^{II}(H_2O)]^+$ {SCS = C_6H_3-2,6-$(CH\text{-}SBu^t)_2$}

NAKAI, Hidetaka; OGO, Seiji; WATANABE, Yoshihito

[*Organometallics* **21**, 1674 (2002)]

This paper reports on the first example of pH-dependent cross-coupling reactions of water-soluble organic halides {3-*X*(C_6H_4)CO_2H, where *X* = Cl, Br, and I} with organoboron compounds {$PhB(OH)_2$ and Ph_4BNa} to form 3-Ph(C_6H_4)CO_2H, catalyzed by a mononuclear organometallic aqua complex [(SCS)$Pd^{II}(H_2O)]_2(SO_4)$ {[**1**]$_2$·(SO_4), SCS = C_6H_3-2,6-($CH_2SBu^t)_2$} in basic media (8 < pH < 13, $NaHCO_3$/NaOH buffers). The structure of **1**·(PF_6) was unequivocally determined by X-ray analysis. The reactions show unique pH-selectivity depending upon the organoboron compounds, *i.e.*, the rate of the reactions with $PhB(OH)_2$ shows a sharp-maximum around pH 10, though the rate of the reactions with Ph_4BNa shows a flat-maximum in a pH range of about 8 to 11. The pH-dependence is discussed on the basis of the pK_a values of [**1**]$_2$·(SO_4) and $PhB(OH)_2$.

IX-C-3 pH-Dependent Transfer Hydrogenation of Ketones with HCOONa as a Hydrogen Donor Promoted by (η^6-C_6Me_6)Ru Complexes

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([1]Osaka Univ.; [2]Nagoya Univ.)

[*Organometallics* **21**, 2964 (2002)]

The paper reports on the development of a new class of water-soluble organometallic catalysts for pH-dependent transfer hydrogenation. An organometallic aqua complex [(η^6-C_6Me_6)Ru^{II}(bpy)(H_2O)]$^{2+}$ (**1**, bpy = 2,2'-bipyridine) acts as a catalyst precursor for pH-dependent transfer hydrogenation of water-soluble and -insoluble ketones with HCOONa as a hydrogen donor in water and in biphasic media. Irrespective of the solubility of the ketones toward water, the rate of the transfer hydrogenation shows a sharp maximum around pH 4.0 (in the case of biphasic media, the pH value of the aqueous phase is adopted). In the absence of the reducible ketones, as a function of pH, complex **1** reacts with HCOONa to provide a formato complex [(η^6-C_6Me_6)Ru^{II}(bpy)(HCOO)]$^+$ (**2**) as an intermediate of β-hydrogen elimination and a hydrido complex [(η^6-C_6Me_6)Ru^{II}(bpy)H]$^+$ (**3**) as the catalyst for the transfer hydrogenation. The structures of **1**(PF_6)$_2$, **2**(HCOO)·HCOOH, and of [(η^6-C_6Me_6)$Ru^{II}(H_2O)_3]SO_4$·$3H_2O$ {**4**(SO_4)·$3H_2O$}, the starting material for the synthesis of **1**, were unequivocally determined by X-ray analysis.