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Transition metal peroxo complexes relevant to metalloproteins

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ABSTRACT A series of dioxygen complexes of copper, iron, and manganese have been synthesized using bulky tripodal nitrogen ligands, hydrotris(3,5-dialkyl-1-pyrazolyl)borate, as models for the active sites in the metalloproteins. A binuclear copper peroxo complex having μ-η^2:η^2 coordination mode serves as an accurate model of the active site of oxy-hemocyanin. The reactivities of the complex and related peroxo copper complexes suggested a new radical type reaction mechanism for tyrosinase catalysis. Dioxygen complexes of iron are designed to provide structural and mechanical information for a non-heme oxygen carrier and monooxygenase, hemerythrin and tyrosine hydroxylase. A mononuclear dioxygen complex of manganese represents the first artificial example of hydrogen bond between the peroxide and proton in the ligand and binuclear manganese complexes do the models for the active intermediates of dioxygen evolution sites for PSII.

INTRODUCTION

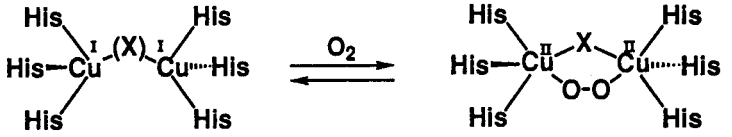
Numerous metalloproteins function to carry or activate dioxygen in biological systems. Iron, copper and manganese are most abundant metal ions found at the active centers of these metalloproteins functioning in the dioxygen metabolism and synthesis of model complexes to mimic their structures and functions has been an urgent target for bioinorganic chemistry.

The chemistry of (porphyrinato) iron-dioxygen complexes has been developed extensively and our current understanding on the structures and functions of heme proteins has been advanced on the basis of these synthetic model works. In contrast, the details of non-heme proteins still remain to be solved. We have focused our endeavor on the synthesis and characterization of dioxygen complexes of copper, iron and manganese with non-porphyrin ligands. Our synthetic strategy lies on the utilization of a novel hindered tris(pyrazolyl)borate. It provides a N_3 pyramidal ligand array which closely mimics the coordination environment often found in these types of metalloproteins. We have successfully isolated and characterized a series of copper, iron and manganese peroxo complexes, which may serve as models for the metalloproteins including *hemocyanin*, *tyrosinase*, *hemerythrin*, *tyrosine* hydroxylase, methane monooxygenase and oxygen evolving site of PSII. The overview of these synthetic model approaches established recently in our laboratory will be presented.

PEROXO COPPER COMPLEXES

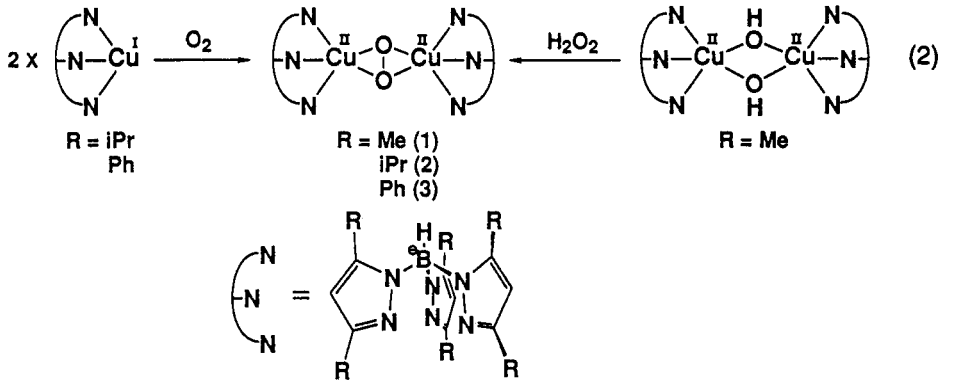
Synthetic Model for Oxy-hemocyanin Hemocyanin(Hc) is a dioxygen carrier for arthropods and mollusks and its active site is compared of a pair of copper ions to which dioxygen is bound symmetrically as a peroxide ion. Oxy-Hc has been characterized by abnormally low ν(O-O) frequency (ca. 750 cm^{-1}),

two absorption bands at ca. 350nm (~20000/2Cu) and ca. 580nm (~1000) and dimagnetism due to the strong magnetic coupling between two copper(II) ions. On the basis of magnetism, it has been long believed that an endogenous bridging ligand (X) other than dioxygen exists between two copper ions and dioxygen is bound in a cis-coordination mode as shown in eq. 1.



(1)

The striking and unusual magnetic and spectral characteristics of oxy-Hc have fascinated many inorganic chemists, and the synthesis of μ-peroxo dicopper (II) complex modeling the active site of oxy-Hc has been a long-standing challenge in bioinorganic chemistry. Despite extensive efforts, however, no synthetic complex that satisfies the physicochemical characteristics of oxy-Hc had been reported before we presented a novel μ-η^2:η^2-peroxo complex, $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$ (1). The complexes **1~3** can be synthesized either by dioxygen addition to monomeric copper (I) complexes or by H_2O_2 treatment of μ-oxo or di-μ-hydroxo dicopper(II) complexes (2). They contain a peroxide ion as the sole bridging ligand, and, remarkably, exhibit all physicochemical characteristics of oxy-Hc as summarized in Table 1.



(2)

TABLE 1 Physicochemical data for μ-peroxo copper (II) complexes (**1~3**), Oxy-Hemocyanin, and Oxy-Tyrosinase.

Complex	Absorption bands (nm)	ν (O-O) (cm^{-1})	Cu---Cu (Å)	Magnetism
$[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$ (1)	338, 530	731	---	diamag.
$[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$ (2)	349, 551	741	3.56	diamag.
$[Cu(HB(3,5\text{-}Ph_2pz)_3)]_2(O_2)$ (3)	355, 542	759	---	diamag.
Oxy-Hemocyanin	340, 580	744 ~ 752	3.5 ~ 3.7	diamag.
Oxy-Tyrosinase	345, 600	755	ca. 3.6	diamag.

The detailed structure of **2** was determined by X-ray crystallography which established the doubly side-on coordination of the peroxide ion, referred to as μ-η^2:η^2. The Cu-Cu distance of **2** is 3.56 Å (3), which is almost identical to the 3.6 Å estimated for the Cu-Cu separation in oxy-Hc by EXAFS.

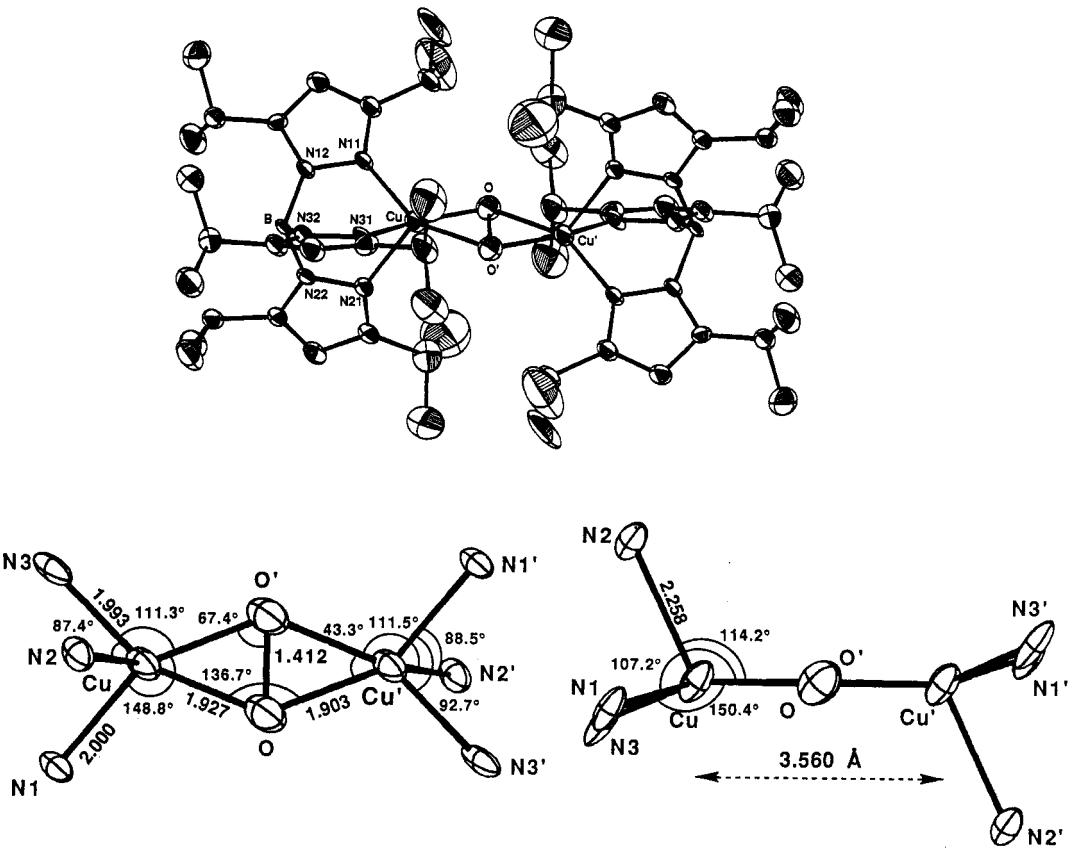
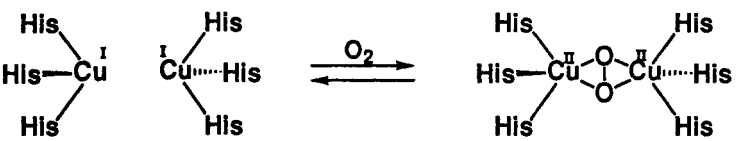


Fig. 1 Molecular structure of μ-η^2:η^2-peroxo complex **2**, $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2O_2$.

Thus, we proposed a new model for the dioxygen binding in Hc: dioxygen is bound between two copper ions simply in a μ-η^2:η^2 configuration without any endogenous ligand as eq. 3 (4).



(3)

Very recently, this proposal was conclusively supported by the crystal structure of oxygenated *Limulus* II hemocyanin determined by X-ray diffraction studies by Magnus et al. (5).

Mechanism of Tyrosinase Tyrosinase (Tyr) is a copper-containing monooxygenase which catalyzes the oxidation of monophenol to o-quinone.

The active site of Tyr is also composed of two copper ions. When dioxygen is bound, Tyr gives similar characteristic spectra to those of oxy-Hc. Hence, the coordination mode of the activated dioxygen in oxy-Tyr is reasonably suggested to be a planar μ-η^2:η^2. In order to provide mechanistic insight into the reaction mechanism of Tyr, the reaction of the complex **2** with hindered phenols and the reactivity of the related peroxo copper complex, Cu(tBuOO)(HB(3,5-iPr$_2$pz)$_3$) (**4**) were investigated (6). The anaerobic reaction of **2** with hindered phenols gave diphenoquinones in the similar manner as does Tyr and the aerobic reaction gave benzoquinones as well as diphenoquinones. The complex **2** is not very stable in solution at room temperature and undergoes spontaneous homolytic O-O bond cleavage of peroxide ion to give radical species Cu(II)-O• which abstracts allylic hydrogen of cyclohexene and phenoxy hydrogen. This initiates radical reactions in the presence of dioxygen. However, the homolytic cleavage of O-O bond may be depressed in Tyr if the two copper ions are hold by the protein chains so as to reverse the cleavage. Another observed reaction is acid/base replacement between the acidic phenol and the basic peroxide to give a phenoxo intermediate which undergoes reductive Cu-O bond cleavage, resulting in the formation of phenoxo radical. We think that the latter reaction plays a key role in the enzymatic reaction. Since only one phenol is accessible at the substrate binding pocket in Tyr, the initially formed intermediate is a phenoxo-hydroperoxo compound. On the basis of the reactivity of Cu(tBuOO)(HB(3,5-iPr$_2$pz)$_3$), it seems highly likely that the hydroperoxide intermediate undergoes Cu-O bond homolysis. It is reasonable to assume that the phenoxo radical and HOO• couple each other instantaneously in the coordination sphere of the dinuclear copper site to give a hydroperoxobenzoquinone which is converted to benzoquinone completing the catalytic cycle as illustrated in Fig. 2.

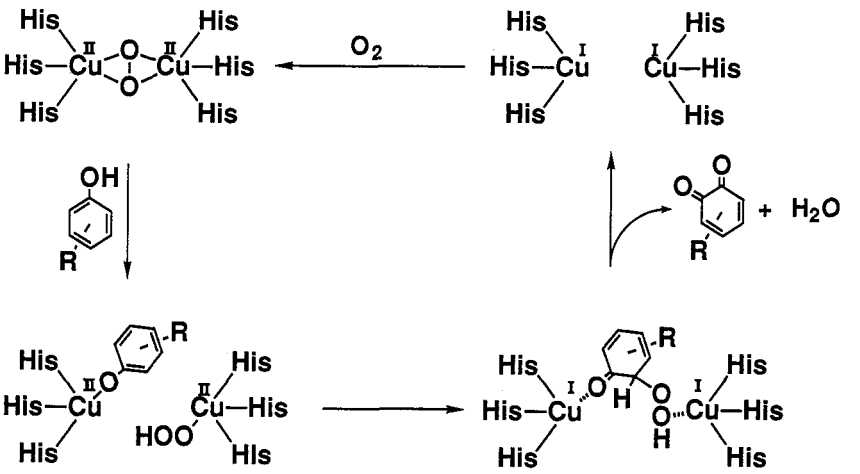
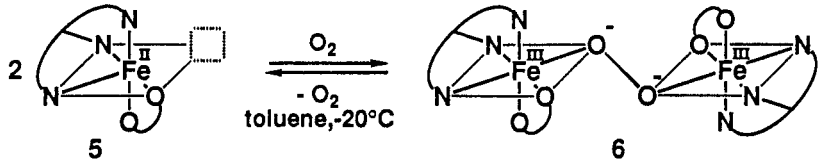


Fig. 2 Proposed mechanism of tyrosinase catalysis.

This scheme proposed by us does not agree with mechanism such as direct electrophilic attack of peroxide ion to aromatic C-H bond (7,8). Both μ-η^2:η^2 peroxo dicopper complexes and copper alkylperoxo (9) or acylperoxo complexes (10) do not exhibit any electrophilic oxo-transfer reactivity to unsaturated carbon atom. Although the definite evidence has not been obtained, we believe that the radical coupling mechanism is more conceivable for the reaction mechanism of Tyr (11).

PEROXO IRON COMPLEXES

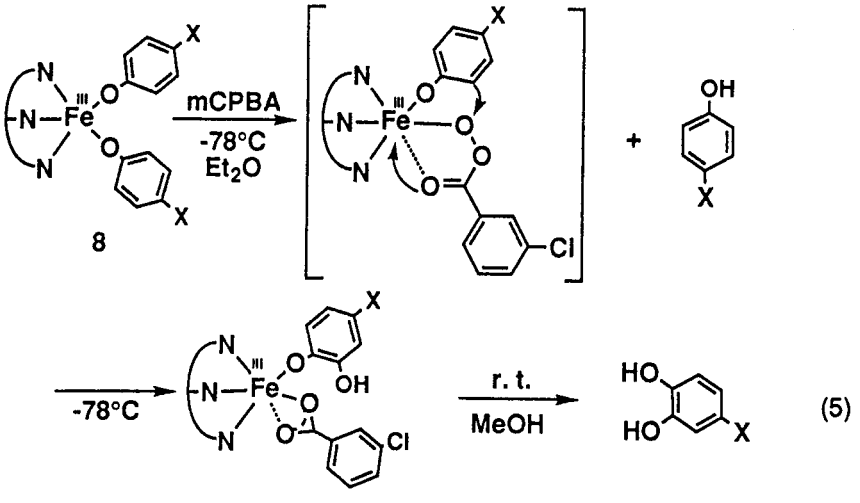
Reversible Dioxygen Binding by Non-heme Iron Complex As the structural analogue of non-heme iron oxygen carrier, hemerythrin (Hr), a five-coordinate carboxylato iron(II) complex Fe(PhCOO)(HB(3,5-$iPr_2pz)_3$) (**5**) was prepared using hydrotris(3,5-diisopropyl-1-pyrazolyl) borate. The complex reacts with a variety of σ-donor such as pyridine to form a stable adduct, whereas it does not interact with CO as just like Hr. It was found that complex **5** binds dioxygen reversibly at -20 °C in toluene (12). The dioxygen adduct was characterized by dioxygen consumption stoichiometry and by spectroscopic methods, UV-vis, resonance Raman and EXAFS, by which the adduct was identified as a μ-peroxo diiron (III) complex.



(4)

The structure of the adduct (**6**)was confirmed by variable temperature magnetic susceptibility measurement by SQUID; the dioxygen adduct is antiferromagnetic, with J = -33 cm^{-1} as expected for the dinuclear structure (13). As a more accurate model for Hr, μ-hydro-μ-carboxylato diiron(II) complex, [Fe(HB(3,5-$iPr_2pz)_3$)]$_2$(OH)(PhCOO) (**7**) was synthesized and its interaction with dioxygen was investigated (14).

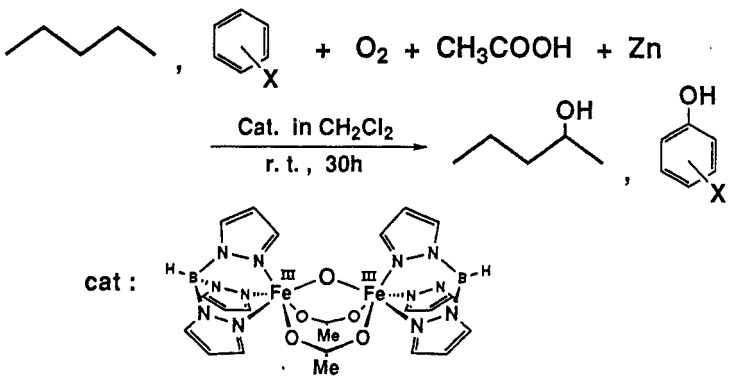
A Reaction Mimic of Tyrosine Hydroxylase Tyrosine hydroxylase (TH) is a tetrahydropterin-dependent non-heme iron monooxygenase which catalyzed the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (dopa). While the reaction mechanism of TH remains unclear, reductive activation of dioxygen involving peroxytetrahydropterin has been suggested (15). A series of bis(phenoxo)ferric complexes, [Fe(HB(3,5-$iPr_2pz)_3$)](OAr)$_2$ (**8**) were prepared by the aerobic oxidation of ferrous complexes and they were treated with 1 equiv. of mCPBA at -78 °C. The reaction gave (catecholato)(carboxylato) complex with liberating 1 equiv. of the phenol. Warming the complexes in MeOH to room temperature resulted in the formation of catechols in quantitative yields. The reaction mimics regioselective hydroxylation of phenols as well as the possible role of pterin hydroperoxide and may offer an ingenious model for TH (16).



(5)

Aerobic Hydroxylation of Alkane and Aromatics Catalyzed by μ-Oxo Diiron Complex

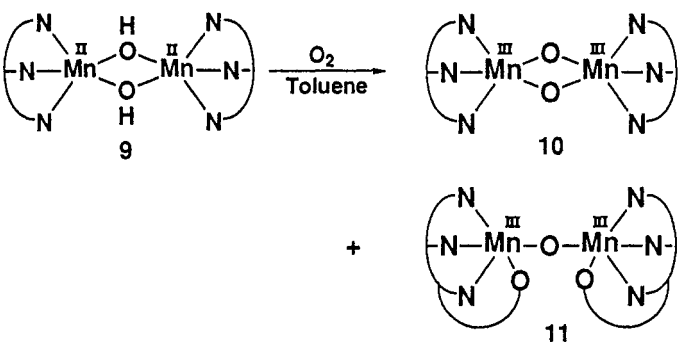
Methane monooxygenase (MMO) has a diiron site like Hr and catalyzes dioxygen hydroxylation of alkanes, especially methane. It was found that μ-oxo diiron complex, catalyzes an aerobic hydroxylation in the presence of proton and electron source (acid and Zn powder). This system can oxidize both alkanes and aromatics and turnover number reaches 12 for the hydroxylation of benzene to phenol at room temperature (17,18). Although the catalytic activity of system is not sufficiently high and the reaction mechanism has not been clarified, the reaction may present the first reaction mimic for MMO by using a μ-oxo diiron complex.



(6)

PEROXO MANGANESE COMPLEXES

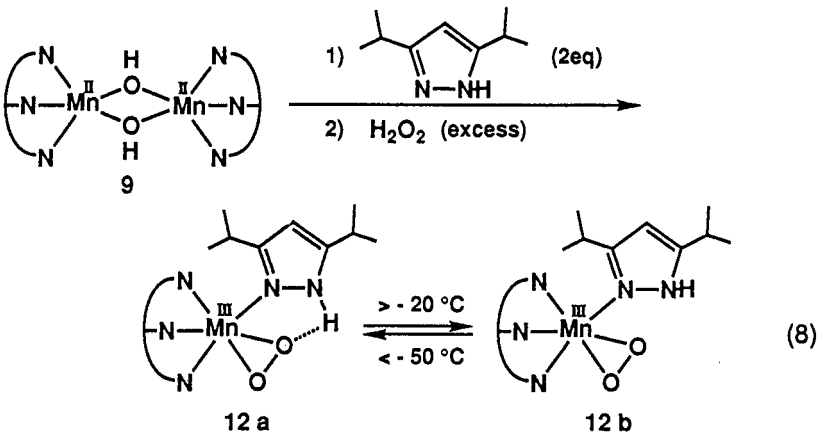
The hindered ligand, $HB(3,5\text{-}iPr_2pz)_3^-$, is again useful in preparing manganese complexes modeling the active sites of manganese proteins. We have synthesized a di-μ-hydroxo manganese complex $[Mn(HB(3,5\text{-}iPr_2pz)_3)](OH)_2$ (**9**) which may serve as a model for the reduced states of ribonucleotide reductase and catalase; both proteins are known to have a dimanganese site. Such a unit may exist in the poly manganese site in the oxygen evolving center (OEC) in photosynthetic system II (PSII). The complex **9**, reacts with dioxygen giving two main products, a five-coordinate di-μ-oxo dimanganese(III) complex, $[Mn(HB(3,5\text{-}iPr_2pz)_3)](O)$ (**10**) and a dimanganese(III) complex (**11**), where one of the isopropyl groups in each tris(pyrazolyl)borate ligand is first hydroxylated and then coordinated as an alkoxo ligand (19,20). The complex **11** is quantitatively obtained by anaerobic oxidation with $KMnO_4$ (19). Transformations of these novel di-μ-hydroxo and di-μ-oxo manganese complexes may correspond to elemental reaction steps in the dioxygen formation from water catalyzed by OEC in PSII.



(7)

The reaction of the complex with excess amount of H_2O_2 in the presence of 3,5-diisopropylpyrazole gives a monomeric side-on peroxo manganese (III) complex, $[Mn(HB(3,5\text{-}iPr_2pz)_3)](3,5\text{-}iPr_2pz)(O_2)$ (**12**).

Complex **12** exhibits thermochromism: at -78 °C in deep blue but in tan brown at -20 °C (21).
The structure of each isomer of **12** was determined by X-ray crystallography and it was found that a hydrogen bond between the peroxide and a pyrazole proton exists only in the blue form.



(8)

To our knowledge, this is the first artificial complex which possesses the hydrogen bond between the coordinated dioxygen and ligand proton. We expect that will serve as a model for the proton transfer to the coordinated dioxygen in the reductive activation of molecular oxygen occurring in many monooxygenase catalysis as well as for oxy-Hr in which such a proton transfer yields a hydroperoxo species.

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