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SYNTHETIC APPROACH TO THE STRUCTURE AND FUNCTION OF COPPER PROTEINS

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I. Introduction

The critical role of copper in biological systems has been recognized for a long time. Copper is an essential component for living systems, although excess intake causes symptoms such as Wilson's disease. Numerous copper-containing proteins are now known and can be categorized based on their functions as follows:

1. Electron-transfer carrier: plastocyanin, azurin, pseudoazurin
2. Dioxygen carrier: hemocyanin
3. Oxygenation: tyrosinase, dopamine β-hydroxylase, phenylalanine hydroxylase, peptidylglycine α-amidating monooxygenase

4. Oxidation: galactose oxidase, amine oxidase, ascorbate oxidase, laccase, cytochrome *c* oxidase
5. Reduction: nitrite reductase, nitrous reductase
6. Disproportionation: superoxide dismutase
7. Unknown: stellacyanin, umecyanin, cucumber basic blue copper protein

Based on spectroscopic properties, mainly electron paramagnetic resonance (EPR), the active sites of copper proteins have been classified into three groups, types I, II, and III. This nomenclature was originally applied to blue oxidases to distinguish the four copper ions contained in these proteins. The original classification has been extended to the copper sites of other proteins. The recent increase in structural information on the copper sites in proteins has, however, revealed greater diversity in the type of copper site. For instance, the type III and type II sites in ascorbate oxidase are in close proximity, forming a trinuclear site, in which all three copper ions are essential for the reactivity. Some proteins, once believed to contain a copper site with normal spectroscopic properties, and thus referred as type II, have been shown to contain copper coordinated by an unusual side chain. Therefore, in this review, new nomenclature is used to classify the copper sites more precisely with respect to their structural features and spectroscopic properties. The definitions are as follows:

Type I. Mononuclear copper having a trigonal basal plane with an N_2S ligand donor set (S denotes thiolate sulfur from cysteine); exhibits unusual spectroscopic properties: (1) a strong absorption band at ~600 nm, (2) a small $A_{\parallel}$ value of <70 G, and (3) a high reduction potential (generally >250 mV).

Type II. Mononuclear copper site exhibiting a normal EPR spectrum. This type, however, can be divided into the following three classes:

Type IIA: The ligand donors come from ordinary protein residues, such as histidine imidazole, cysteine thiolate, and water (or hydroxide).

Type IIB: The ligand donors include unusual protein side chains.

Type IIC: The copper is bridged to another metal ion, forming a hetero-dinuclear metal site.

Type III. EPR-silent binuclear copper site that binds dioxygen as peroxide and exhibits unusual physicochemical characteristics: (1) diamagnetism, (2) two characteristic absorption bands at 350 and 580 nm, and (3) a low O—O vibration stretching frequency (~750 cm^{-1}).

Type IV. Trinuclear copper site having an isosceles triangle shape; two copper ions are strongly magnetically coupled.

The classification introduced in this review (type I–type IV) should cover all structural types of copper sites known to date. For instance, based on this nomenclature, ascorbate oxidase contains type I and type IV, and nitrite reductase contains type I and type II (more precisely, type IIA). Galactose oxidase has a type IIB site.

Except for type II copper sites, which exhibit only the usual spectroscopic features, the structures and properties of other types of copper sites are noteworthy not only from the biological but also the inorganic point of view. Extensive synthetic work has been undertaken in order to clarify the active site structures and the origins of the unusual spectroscopic characteristics of these copper proteins. These studies are based on the premise that the chemistry of copper proteins is mostly dependent on the coordination structure of the copper sites. The benefit of this synthetic model approach in understanding the structures and functions of heme iron and iron–sulfur proteins was demonstrated more than 10 years ago by Holm and Ibers (*1*). Accordingly, the synthesis, characterization, and reactivity of low-molecular-weight copper complexes with artificial ligands designed to mimic structurally the copper sites in proteins have been a major topic in current bioinorganic chemistry. An overview of these synthetic explorations is provided in this review. In particular, the unusual structural and spectroscopic features of type III copper have been the subject of numerous investigations. These synthetic endeavors have been previously reviewed (*2–4*); therefore, this review will focus on the progress achieved since then, in large part by the author's group.

The structures of type II copper sites have not as yet generated as much interest, but the hetero-dinuclear structures of type IIC copper sites and the unusual protein donor ligands found in type IIB copper sites are noteworthy. The synthetic model approach to gain insight into the structures and functions of these types of copper sites will be also described. The diverse functions of a variety of proteins containing type IIA copper sites inspired many chemists to mimic the functions with synthetic copper complexes, even though the spectroscopic properties of the complexes are not unusual. Results of these studies will be reviewed and different aspects of the reactions relating to enzymatic catalysis will be discussed.

II. Synthetic Models for Type I Copper

The unusual spectroscopic properties of type I copper have been particularly fascinating to many inorganic chemists. The most striking

feature associated with the type I copper site is its intense blue color resulting from an absorption at ~600 nm. The molar absorption coefficients (ε) of the band are located in the range of 3000–7000 liter mol^{-1} cm^{-1}, much higher than the visible band observed for ordinary copper(II) complexes; the *d–d* band appears at 600–800 nm with $\varepsilon < 700$ M^{-1} cm^{-1}. The EPR spectra of copper proteins containing type I copper are also unusual. Some proteins exhibit axial-type signals with anomalously small $A_{\parallel}$ values of less than 70 G, whereas others exhibit rhombic signals. Other characteristics of type I copper include the high reduction potential and complicated features of the Raman spectrum in the region of 300–500 cm^{-1}. There have been numerous reviews on blue copper protein chemistry (*5–9*).

By the mid-1970s, extensive spectroscopic investigations led to a consensus that type I copper has a distorted tetrahedral coordination structure containing a copper–cysteine thiolate bond. The most solid evidence for the existence of the Cu—S bond was provided by X-ray photoelectron spectroscopy (XPS) (*10*). The spectrum of native plastocyanin in the sulfur 2*p* binding energy region indicated two resolved peaks. The higher energy band was attributed to the thiolate sulfur bound to the copper ion. The Raman lines observed in the region of 300–500 cm^{-1} were suggested to be associated with the Cu—S bond, although this complicated feature was not fully understood (*11–16*). Furthermore, detailed spectroscopic explorations on Co^{2+}-substituted stellacyanin and plastocyanin (*17–19*) suggested that the cobalt ion is coordinated to a thiolate sulfur in a tetrahedral ligand environment, thus supporting the proposed structure for the type I copper site.

To substantiate the model proposed, a considerable effort was made to synthesize and characterize structurally a copper complex that closely mimics the spectroscopic characteristics of type I copper. Some of the initial work focused on the synthesis of a copper complex having a tetrahedral ligand environment. Because copper(II) strongly prefers planar tetragonal geometry rather than tetrahedral, such complexes were scarcely known except for the classical example of $[CuX_4]^{2-}$ (*20, 21*). The strategy often taken was to use a bidentate ligand in which a hindered substituted group could easily be introduced. For instance, the bis-chelated copper(II) complex with *N*-salicylidenemethylaminate is planar (*22*), whereas the complex with *N*-salicylideneisopropylaminate is distorted tetrahedral (*23*). The alkyl group causes a distortion around the copper ion, resulting in a coordination geometry close to tetrahedral. The EPR spectra of a series of such CuN_4 and CuS_4 complexes were examined (*24–26*), and it was seen that the $A_{\parallel}$ value decreased with an increase in the distortion from planar to tetrahedral,

suggesting that the unusually small $A_{\parallel}$ value of type I copper can be associated with the tetrahedral distortion.

To mimic the physicochemical properties of type I copper more completely, several copper(II) complexes containing an S donor ligand were synthesized. These models are represented in Table I and Fig. 1. A series of cyclic thioether ligands were tested to see whether they could mimic the characteristics of type I copper (*27, 28*). Some of these complexes were found to give rise to a strong band at ~600 nm (ε = 1000–3000 M^{-1} cm^{-1}), with a high reduction potential comparable to that of type I copper. However, X-ray structure determinations indicated that the coordination geometry is planar (*29*). Thus these complexes are not satisfactory models structurally. Sugiura and co-workers have succeeded in preparing a series of model complexes with peptide ligands containing an S donor (*30–34*). Among these complexes, a complex with *N*-mercaptoacetyl-L-histidine (MAH) is of particular interest because the UV–vis and EPR spectra were reasonably similar to those of type I copper (*32*). Unfortunately, the crystal structure of the complex was not determined. Thompson *et al.* prepared a copper thiolate complex by using a hindered tripodal ligand hydrotris(3,5-dimethyl-1-pyrazolyl)borate [$HB(3,5\text{-}Me_2pz)_3$] (*35–37*). Because the tripodal facial ligand provides a rigid pyramidal N_3 array around the copper, it was predicted that the resulting copper(II) thiolate complex could possess a tetrahedral coordination environment with an N_3S ligand donor set. In fact, the structure of the corresponding complex with Co^{2+} was determined by X-ray crystallography, which ascertained the structure as expected (*38*). The thiolate copper(II) complex was unstable thermally, but the low-temperature spectrum exhibited an intense band at 588 nm with ε = 3900 M^{-1} cm^{-1}, mimicking the

TABLE I

SYNTHETIC MODELS FOR BLUE COPPER PROTEINS[a]

Compound	UV–vis (nm)	$A_{\parallel}$ in EPR (G)	$E_{1/2}$ vs. SCE (V)	Ref.
$[Cu([14]aneS_4)]^{2+}$	570(1900), 390(8200)	164	0.69	*28, 29*
$Cu(H_2O)(MAH)$	598(830), 438(970)	100	—	*32*
Cu(MAHH)	513(330)	172	—	*33*
$[Cu(SIm)_2]_2$	620(630)	60	—	*50*
$Cu(ITPDTP)_2$	920(400), 575(3610), 407(4100)	121	—	*51*
$Cu(SC_6H_4\text{-}p\text{-}NO_2)(HB(3,5\text{-}Me_2pz)_3)$	588(3900)	163	—	*36*
$Cu(SC_6H_4\text{-}p\text{-}NO_2)(HB(3,5\text{-}Me_2pz)_2(SC_7H_7))$	595(4100)	187	—	*37*
$[Cu(PMMI)_2]_2(CLO_4)_2$	616(—), 414(—), 382(—), 330(—)	150	0.33	*52*

[a] Cu(II) oxidation state.

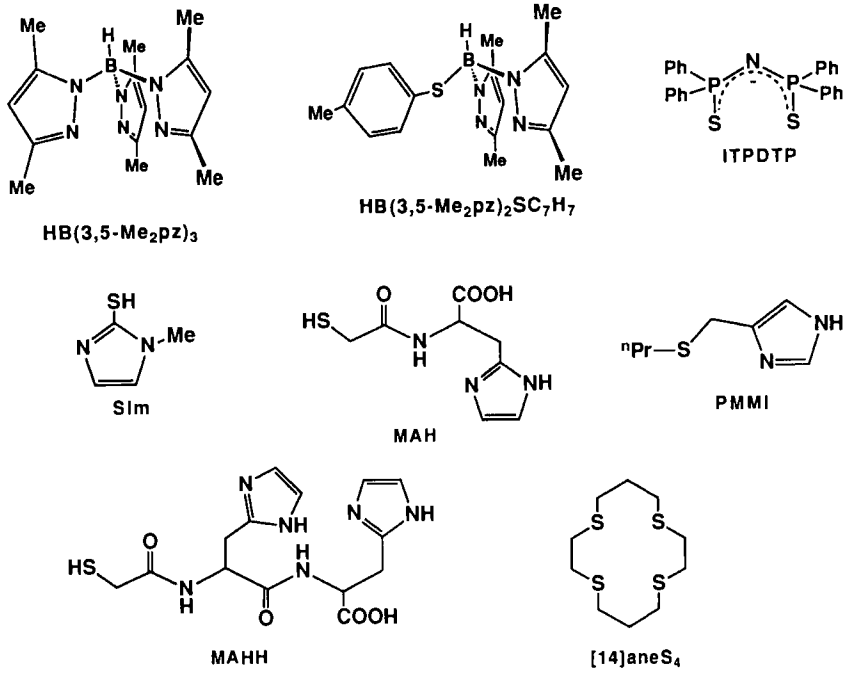


FIG. 1. Structures of ligands for the complexes summarized in Table I.

absorption spectrum of type I copper. However, the EPR spectrum was typical of a tetragonal but not of a tetrahedral complex: $g_{\|} = 2.29$, $g_{\perp} = 2.07$, $A_{\|} = 160$ G. The inconsistencies in the EPR spectrum were puzzling.

In 1978, the first crystal structure of a type I copper protein, plastocyanin from poplar (*Populus nigra*), was reported by Colman *et al.* (*39*). As predicted earlier on the basis of the spectroscopic studies (*40*), the coordination structure of the copper site was best described as distorted tetrahedral. The ligand donors consist of two histidyl nitrogens, a cysteine sulfur, and a methionine sulfur. The Cu—S* (S* denotes thioether sulfur from methionine) bond distance is considerably elongated. Since this work was completed, several other copper proteins containing type I copper have been structurally characterized by single-crystal X-ray analyses. These include algal (*Enteromorpha prolifera*) plastocyanin (*41*), *Alcaligenes denitrificans* azurin (*42*), *Alcaligenes faecalis* pseudoazurin (*43*), cucumber basic blue copper protein (*44*), ascorbate oxidase (*45*), and nitrite reductase (*46*). All coordination structures of the type I copper sites are essentially very similar—distorted tetrahedral coordi-

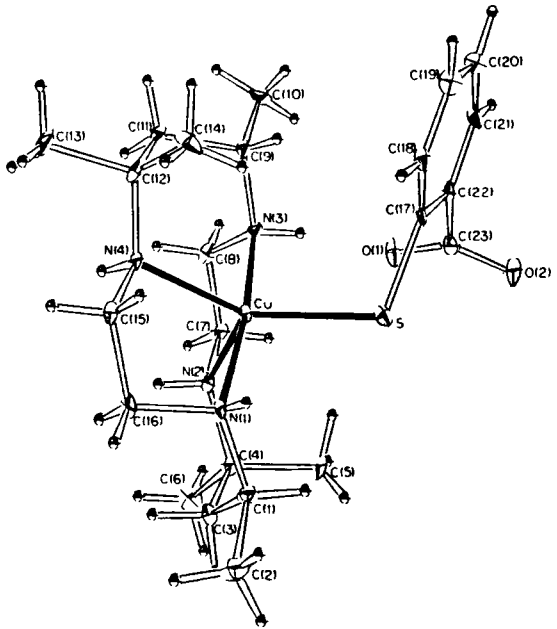
nation with an N_2SS^* ligand donor set. In azurin, there is also a weakly coordinated peptide carbonyl group, which may serve as a fifth ligand. Penfield *et al.* carried out detailed spectroscopic studies using a single crystal of plastocyanin to examine the unusual spectroscopic feature (*47*). The results, in conjunction with SCF-Xα calculations (*48*), indicated that the ground state of the *d* orbital can be ascribed to $d_{x^2-y^2}$ with C_{3v} or a symmetrically lower ligand environment, the long axis being directed along the Cu—S* bond. The most intense band at ~600 nm was assigned to the S $p\pi \rightarrow$ Cu $d_{x^2-y^2}$ LMCL band.

A considerable number of crystal structures of type I copper sites in proteins are now available, so there may be no particular advantage in the synthetic model approach to prove the coordination structure of type I. Yet, inorganic chemists still have an opportunity to utilize the spectroscopic and structural bases established by model studies to understand the precise electronic structure of type I copper. One should keep in mind that the generally accepted interpretation derived from spectroscopic and theoretical studies on the proteins (*47–49*) has not been definitely proved experimentally. A systematic comparison of a series of copper(II) thiolate complexes having an unusual distorted coordination structure is required for a conclusive description of the electronic structure of the type I copper. The synthetic approach is ultimately the most adequate way to clarify how the ligand donors and geometry affect the electronic property and function of type I copper as an electron transfer center.

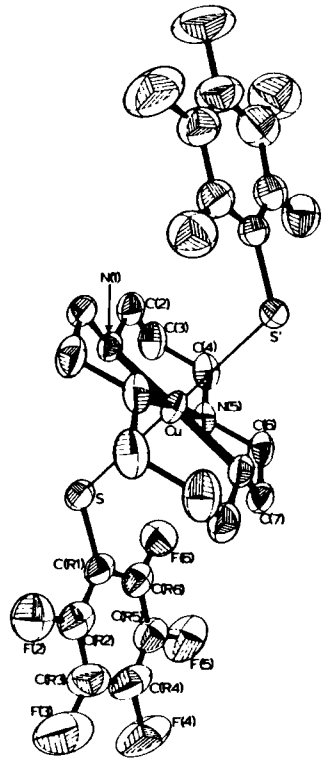
The main difficulty in synthesizing an accurate model for type I copper comes from the instability of the copper(II)–thiolate bond. This bond easily undergoes homolysis, causing reduction of the copper(II) ion with formation of disulfide as follows:

$$2Cu^{II}—SR \rightarrow 2Cu^{I} + RSSR$$

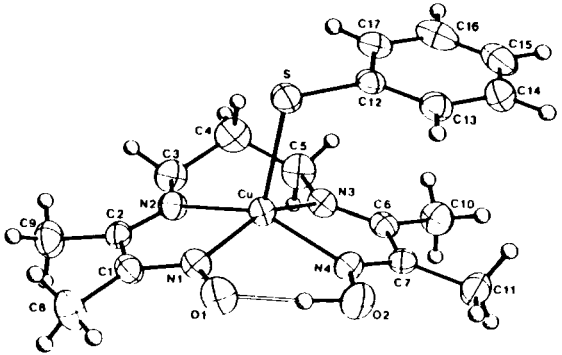
Incomplete structural characterization of the complexes listed in Table I (*28, 29, 32, 33, 36, 37*) is mainly due to this decomposition. However, if a suitable ligand system is employed, it is possible to stabilize and inhibit the facile decomposition of the Cu—S bond. Recently, there have been reports on mononuclear copper(II) thiolate complexes whose structures were determined by X-ray crystallography. Table II summarizes the structural and spectroscopic data on these complexes (see Fig. 2) (*53–59*). Three crystal structures of monomeric complexes having an N_4S ligand donor set are available. The coordination geometries vary from square–pyramidal to trigonal–bipyramidal. In each case, the thiolate sulfur occupies the apical position, and the Cu—S distances are located in the range of 2.31–2.42 Å (*53, 54, 57*). A strong S $\rightarrow$ Cu(II)



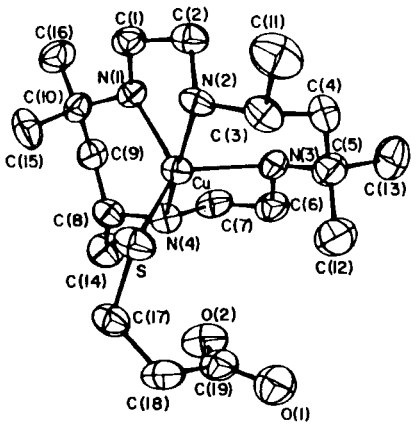
[Cu(tetb)(SC_6H_4-o-CO_2)]·H_2O



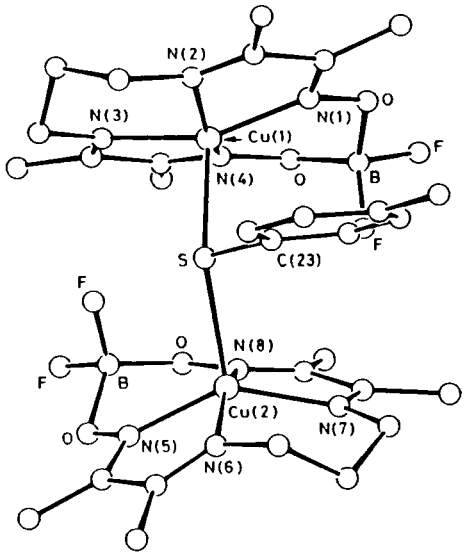
Cu(cyclam)(SC_6F_5)$_2$



$CuL_1(SC_6H_5)$

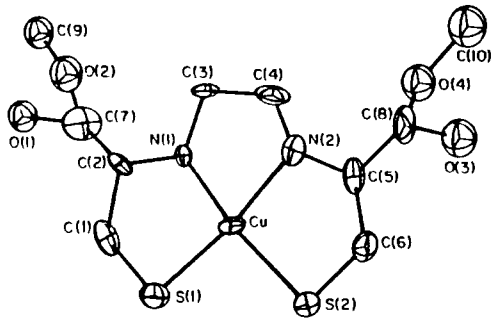


$[Cu(tetb)(SCH_2CH_2CO_2)]\cdot 2MeOH$

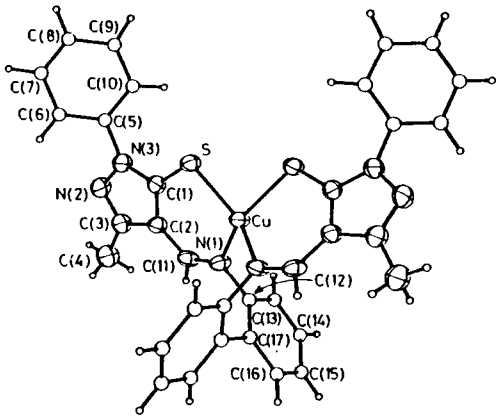


$[Cu(cyclops)_2(SC_6H_4\text{-}p\text{-}Me)]\cdot(ClO_4)$

FIG. 2. Representative crystal structures of thiolate cupric complexes. Reprinted with permission from Refs. *53–59*.



$Cu(SCH_2CH(CO_2Me)NHCH_2\text{-})_2$



CuL_2

FIG. 2. (*Continued*)

TABLE II

STRUCTURAL PARAMETERS AND PROPERTIES OF Cu(II) THIOLATE COMPLEXES

Compound	Coordination	Cu—S length (Å)	UV–vis (nm)	EPR parameter	Ref.
[Cu(tet b)(SC_6H_4-*o*-CO_2)]·H_2O	N_4S trigonal–pyramidal	2.359(4)	360(2300), 418(1000), 430(sh) 590(sh), 730(900), 920(sh)	g_1 = 2.074 g_2 = 2.086 g_3 = 2.117	*53*
$CuL_1(SC_6H_5)$	N_4S square–planar	2.424(1)	355(5200), 428(5490)	—	*54*
Cu(cyclam)(SC_6F_5)	N_4S_2 distorted octahedal	2.940(1)	520(77)	$A_\parallel$ = 205 G $g_\parallel$ = 2.200 $g_\perp$ = 2.050	*55*
[Cu_2(cyclops)$_2$(SC_6H_4-*p*-Me)](ClO_4)	N_4S square–pyramidal	2.506(7)	360(sh), 415(6480)	—	*56*
[Cu(tet b)($SCH_2CH_2CO_2$)]·2MeOH	N_4S trigonal–bipyramidal	2.314(1)	370(—), 420(sh), 724(—)	g_1 = 2.047 g_2 = 2.07 g_3 = 2.163	*57*
CuL_2	N_2S_2 distorted tetragonal	2.245(1)	535(3000), 656(1500)	$A_\parallel$ = 137 G $g_\parallel$ = 2.155 $g_\perp$ = 2.036	*58*
Cu($SCH_2CH(CO_2Me)NHCH_2$–)$_2$	N_2S_2 distorted tetrahedral	2.230(5)	345(5800), 400(sh), 545(1000)	$A_\parallel$ = 182 G $g_\parallel$ = 2.126 $g_\perp$ = 2.039	*59*

LMCT band is observed at ~420 nm, with a few other bands in the visible region whose assignments were not unambiguously determined. Unfortunately, the anisotropic EPR parameters of the solution samples were not determined, whereas the powder spectrum of the complex with tet b (see Section VI for explanation of abbreviations) was reported to exhibit rhombic signals. A thiolate-bridged dinuclear copper(II) complex also possesses an N_4SCu unit with a square–pyramidal geometry. The thiolate again sits at the apical position, whereas the Cu—S distance is slightly more elongated (*56*). A dithiolate complex having a distorted octahedral structure is also known (*55*). In this complex, two thiolate groups sit in the apical positions in a trans configuration. The unusually long Cu—S distance (2.94 Å) is ascribed to the trans effect. As is consistent with the weak interaction between the copper and thiolate, the complex does not give rise to an intense S → Cu(II) charge transfer band. Neither the coordination geometries nor the spectroscopic properties of these thiolate complexes resemble those of type I copper. However, two structurally more appropriate models have been reported—two dithiolate complexes having a distorted tetrahedral geometry (*58, 59*). Both complexes have an N_2S_2 ligand donor. These structural-type complexes are of special interest because the type I copper in cytochrome *c* oxidase, the so-called Cu_A, is suggested to possess an N_2S_2 ligand donor set rather than N_2SS^*. The Cu_A exhibits distinctively smaller $A_\parallel$ and $g_\parallel$ values than those known for other type I coppers. From the X-ray absorption edge (*60*), extended X-ray absorption fine structure (EXAFS) (*61*), EPR, and electron–nuclear double resonance (ENDOR) studies, Stevens *et al.* (*62*) proposed that the Cu_A site consists of a CuN_2S_2 unit with considerable Cu(I)–thiyl radical (*63*) or covalent Cu(II)–thiolate bond (*64*) contribution. The complex reported by Anderson *et al.* (*58*) has a structure close to tetrahedral, with a dihedral angle (CuN_2/CuS_2) of 52°, whereas the complex synthesized by Bharadwaj *et al.* (*59*) has a dihedral angle of only 21°. As predicted, the latter complex exhibited an EPR spectrum typical for conventionally tetragonal copper(II) complexes. The former complex gave the usual axial signals as well, despite its more tetrahedral distortion. Thus, neither complex can mimic the characteristic EPR spectrum of type I copper. Nevertheless, both complexes give rise to a strong band at ~550 nm, similar to that of type I copper to some extent. Another notable point is that the Cu—S distances of these complexes, 2.245 and 2.230 (2.262) Å, are considerably shorter than those found in the five- or six-coordinate thiolate copper complexes.

In order to mimic the spectroscopic characteristics of type I copper, both the stable Cu—S bond and the tetrahedral distortion should be

modeled satisfactorily. However, as already described, it is not easy to control such a tetrahedral environment around the copper(II) ion. In the preceding examples, the ligand hindrance was applied to distort the planar tetragonal geometry so that it became tetrahedral, though most ligands were not effective enough. Accordingly, the resulting complexes did not possess a complete tetrahedral coordination structure; the dihedral angle was limited up to 75° (*65*). In order to satisfy both requirements for mimicking the structure of type I copper, the ideal ligand is, ironically, a protein chain. A number of metalloproteins are known to possess a tetrahedral ligand environment consisting of mainly histidyl nitrogens. Pantolianon *et al.* prepared an Ag—Cu-substituted SOD in which the copper occupied the tetrahedral zinc site in the native enzyme; the copper ion exhibited an unusual EPR spectrum with $A_{\parallel} = 97$ G, which is reasonably similar to the spectra observed for type I copper (*66*). The ligand pocket of alcohol dehydrogenase, consisting of two histidines and two cysteines, has also been used as a ligand to mimic the properties of type I copper (*67–69*). More recently, a tripodal ligand site available in insulin was applied to stabilize Cu—SR species, which exhibited a close resemblance in both absorption and the EPR spectrum: UV–vis, 377 ($\varepsilon = 1400\ M^{-1}\ cm^{-1}$), 408 (1400), 626 (2000), and 910 nm (500); EPR, $g_{\parallel} = 2.281$, $g_{\perp} = 2.079$, $A = 76$ G (*70*). These results confirm the consensus that the distorted tetrahedral coordination environment with a copper–thiolate bond is the essential structural factor responsible for the unusual spectroscopic properties of type I copper.

We have recently found that a series of tetrahedral complexes Cu(X)(HB(3,5-iPr$_2$pz)$_3$) (X = Cl$^-$, Br$^-$, OAr$^-$, OOR$^-$) can be readily isolated by using a very hindered tris(pyrazolyl)borate ligand HB(3,5-iPr$_2$pz)$_3$ (*71–73*). For instance, Cu(Cl)(HB(3,5-iPr$_2$pz)$_3$) was prepared by the simple reaction of $CuCl_2$ and KHB(3,5-iPr$_2$pz)$_3$ in CH_2Cl_2 (*71*). Figure 3 represents the molecular structure of the complex. The mean bond distances of Cu—N and Cu—Cl are comparable, 1.98–2.13 Å, and the dihedral angles around the copper are approximately 90°. The complex is stable in the solid state and in noncoordinating solvents such as CH_2Cl_2, toluene, and pentane. However, because the copper(II) ion favors tetragonal geometry rather than tetrahedral, in the presence of a trace amount of a coordinating substrate, e.g., acetone, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), and *N,N*-dimethylformamide (DMF), Cu(Cl)(HB(3,5-iPr$_2$pz)$_3$) binds the substrate, forming a five-coordinate adduct. The DMF adduct Cu(Cl)(DMF)(HB(3,5-iPr$_2$pz)$_3$) was structurally characterized by X-ray crystallography. As shown in Fig. 4, the coordination structure is best described as square–pyramidal

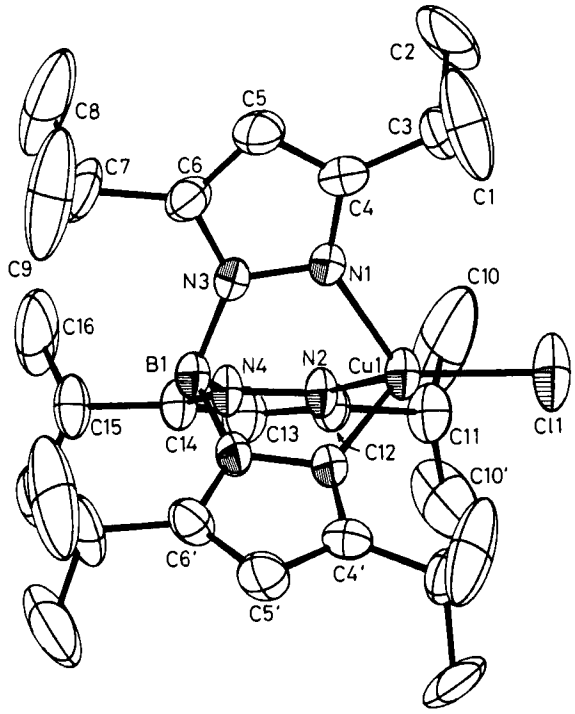


FIG. 3. ORTEP view of Cu(Cl)(HB(3,5-iPr$_2$pz)$_3$). Reprinted with permission from Ref. *71*.

with a basal plane consisting of two nitrogens from the tris(pyrazolyl) borate, a chlorine, and an oxygen from DMF. Hence, the other nitrogen atom from the pyrazolylborate ligand is regarded as an apical ligand with a Cu—N distance that is considerably elongated. Reflecting the structural change, the *d–d* band of 996 nm is shifted to 758 nm for the square–pyramidal DMF adduct. Also, the characteristic reverse EPR signal of Cu(Cl)(HB(3,5-iPr$_2$pz)$_3$) (g_1 = 2.34, g_2 = 2.20, g_3 = 2.01, A_3 = 103 G) associated with the dz^2 ground state changes drastically to an axial-type signal ($g_\parallel$ = 2.30, $g_\perp$ = 2.07, $A_\parallel$ = 142 G) typical for tetragonal complexes.

These experimental results prompted reconsideration of the inscrutable result reported for Cu(SC$_5$H$_4$-*p*-NO$_2$)(HB(3,5-Me$_2$pz)$_3$) (*36*). The absorption spectrum of the complex was reasonably similar to that of type I copper, whereas the EPR spectrum was typical for a tetragonal complex. Because the EPR was recorded in THF at 77 K, our observa-

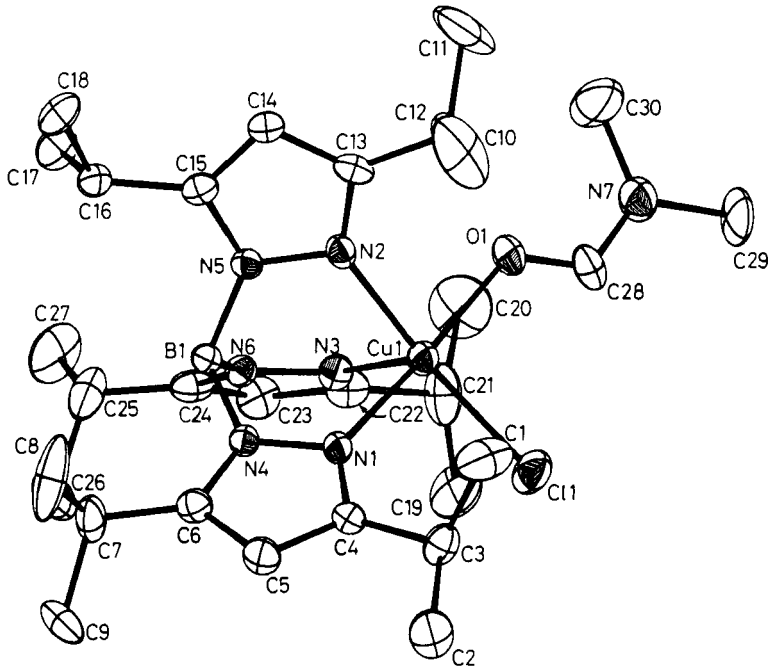


FIG. 4. ORTEP view of Cu(Cl)(DMF)(HB(3,5-$iPr_2pz)_3$). Reprinted with permission from Ref. *71*.

tion that tetrahedral Cu(Cl)(HB(3,5-$iPr_2pz)_3$) easily forms a five-coordinate adduct with a solvent such as THF implied that the tetragonal-type EPR signal may result from the formation of such a adduct. Therefore, by using a more hindered ligand, HB(3,5-$iPr_2pz)_3$, which was expected to stabilize a tetrahedral thiolate complex more effectively, we attempted to synthesize the complex in a noncoordinating solvent. The synthesis was accomplished in CH_2Cl_2 by acid/base reaction between [Cu(HB(3,5-$iPr_2pz)_3$)]$_2$$(OH)_2$ and thiol (*71, 74*); the reaction of Cu(Cl)(HB(3,5-$iPr_2pz)_3$) with NaSR resulted in rapid reduction of the copper(II) ion. Even with HB(3,5-$iPr_2pz)_3$, the thiolate complexes were not stable enough to be characterized, but with hindered (tBuSH, Ph_3CSH) or acidic (C_6F_5SH) thiols, moderately stable thiolate complexes of intensely blue color were successfully obtained. The absorption and EPR spectra of the most stable complex, Cu($SCPh_3$) (HB(3,5-$iPr_2pz)_3$), are shown in Figs. 5 and 6, respectively (*74*). The complex exhibits a very strong band at 625 nm, with additional bands at 440 and 918 nm; the band at 357 nm is presumably due to a decomposed

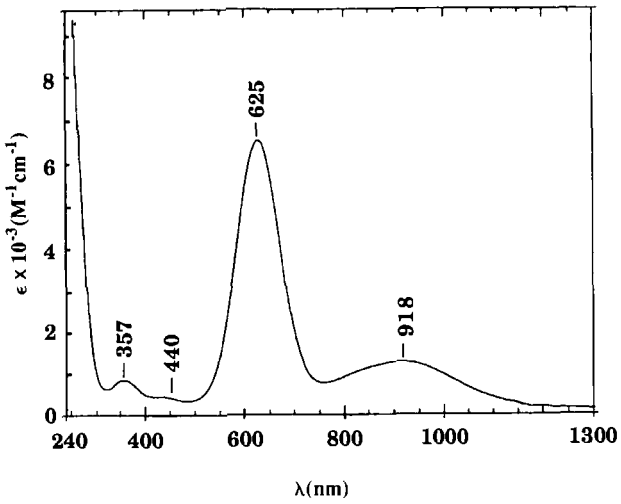


FIG. 5. Electronic spectrum of $Cu(SCPh_3)(HB(3,5\text{-}iPr_2pz)_3)$ in CH_2Cl_2 at room temperature.

product. All these features are very similar to those of type I copper. The EPR gives an axial signal whose parameters are also comparable to those known for plastocyanin and azurin. The properties of the complexes, including the reduction potentials, are summarized in Table III and are compared with those of type I copper proteins. Striking similarities between these complexes and type I copper proteins in their

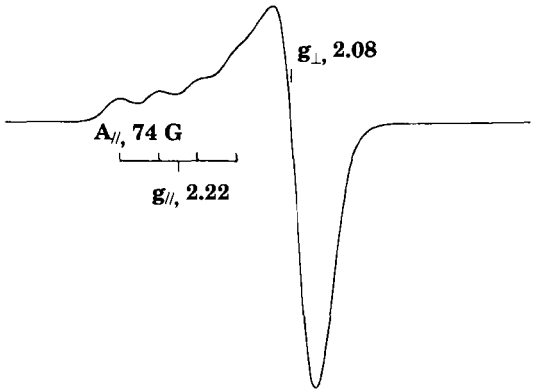


FIG. 6. EPR spectrum of $Cu(SCPh_3)(HB(3,5\text{-}iPr_2pz)_3)$ in CH_2Cl_2 at 77 K.

TABLE III

PROPERTIES OF Cu(SR)(HB(3,5-iPr$_2$pz)$_3$), AZURIN, AND PLASTOCYANIN

Complex	Absorption bands (nm)[a]	$g_\parallel$	$g_\perp$	$A_\parallel$(G)	Redox potential (V)
LCuSCPh$_3$	440(240) 625(6600) 918(1200)	2.23	2.07	71	−0.12
LCuSC$_6$F$_5$	420(200) 665(3600) 960(600)	2.30	2.10	52	0.26
LCuS*t*Bu	608(3000) 900(450)	2.21	2.08	70	−0.11
Azurin	467(270) 625(3500) 820(390)	2.26	2.05	58	0.30
Plastocyanin	460(590) 597(4900) 770(1700)	2.24	2.05	63	0.37

[a] Values in parentheses are for ε (M^{-1} cm^{-1}).

spectroscopic properties are evident, although the reduction potentials of these model complexes are considerably lower than those of type I copper. This is presumably due to the strong electron-donating properties of HB(3,5-iPr$_2$pz)$_3$ and the thiolate ligands employed. In fact, the reduction potential of Cu(SC$_6$F$_5$)(HB(3,5-iPr$_2$pz)$_3$) is higher than is seen with the other complexes. The solvent (CH_2Cl_2) utilized for the CV measurements may be also responsible for the low reduction potentials, because the reduction potential recorded in an aprotic solvent is generally lower than that determined in a protic medium.

The preliminary crystal structure of Cu(SCPh$_3$)(HB(3,5-iPr$_2$pz)$_3$) is presented in Fig. 7 (*74*). The monomeric structure with an N_3S ligand donor set was confirmed. Although the Cu—S and Cu—N distances are all comparable, the coordination geometry is not regular tetrahedral and is best described as trigonal–pyramidal with one nitrogen atom from the tris(pyrazolyl)borate ligand as an apical ligand. Accordingly, the trigonal basal plane consists of two nitrogens and the thiolate sulfur. The copper almost sits on the basal plane; the distance between the copper and the N_2S plane is 0.20 Å, whereas the separation predicted for a regular tetrahedral is ~0.7 Å. The relevant structural parameters of the complex and type I copper sites in proteins are compared in Table IV. The Cu—S distance of the complex is much shorter than those

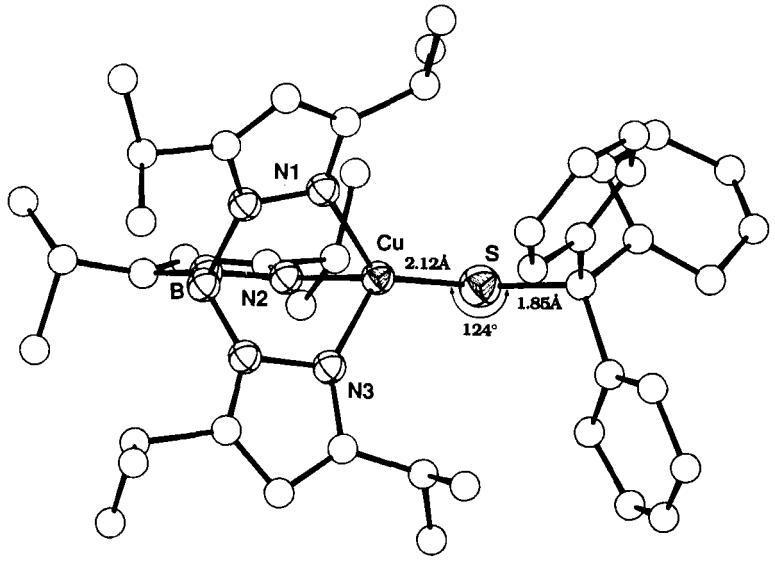


FIG. 7. Preliminary X-ray structure of $Cu(SCPh_3)(HB(3,5\text{-}iPr_2pz)_3)$.

reported for other thiolate complexes (Table II) and is almost the same as those found in proteins. The most notable feature is that the CuN_2S moiety of the complex is structurally very close to that of azurin. Both the coordination structures of the complex and azurin can be referred as trigonal; more precisely, the structure of the complex is described as trigonal–pyramidal, and that of azurin may be referred to as elongated trigonal–bipyramidal. On the other hand, the structural features of the CuN_2S moieties in pseudoazurin and cucumber basic blue copper protein are distinctive. In these proteins, the copper ion position is further away from the N_2S basal plane. Thus, the separations between the copper and the basal plane in the pseudoazurin and cucumber basic blue copper protein are 0.43 and 0.39 Å, respectively, whereas in $Cu(SPh_3)(HB(3,5\text{-}iPr_2pz)_3)$ and azurin, the separations are shorter than 0.20 Å. Hence, the coordination structures of pseudoazurin and cucumber basic blue copper are referred to as distorted tetrahedral rather than trigonal–pyramidal. It is of interest that $Cu(SCPh_3)(HB(3,5\text{-}iPr_2pz)_3)$ exhibits an axial-type EPR spectrum, as does azurin, whereas pseudoazurin and cucumber basic blue copper protein give rhombic EPR signals. These experimental data suggest that the distinct differences in EPR signals are predominantly associated with the structural differences in the CuN_2S chromophore. Alternatively, the existence of apical

TABLE IV

STRUCTURAL COMPARISON OF Cu(II)N2S CHROMOPHORES IN $Cu(SCPh_3)(HB(3,5\text{-}iPr_2pz)_3)$ AND BLUE COPPER PROTEINS

Bond	$Cu(SCPh_3)L$	Azurin	*Populus* plastocyanin	*Enteromorpha prolifera* plastocyanin	Pseudoazurin	Cucumber basic blue copper protein[a]
Cu–N1 (Å)	1.97	2.08	1.91	1.89	2.16	1.90
Cu–N2 (Å)	2.03	2.00	2.07	2.17	2.13	2.13
Cu–S (Å)	2.12	2.15	2.06	2.12	2.16	1.99
S–Cu–N1 (deg)	135	135	132	125	136	139
S–Cu–N2 (deg)	124	119	123	120	112	110
N1–Cu–N2 (deg)	98	105	97	104	100	99
Cu/N1N2S plane (Å)	0.20	0.12	0.36[a]	0.37[a]	0.43	0.39[a]
Cu–S* (Å)	2.05[b]	3.11	2.82	2.92	2.76	2.62
Ref.	*74*	*42*	*303*	*41*	*43*	*44*

[a] Data provided by H. C. Freeman.

[b] The distance between the copper and apical nitrogen atom.

ligands seems not to affect the spectroscopic properties of type I copper significantly when the copper(II) ion positions are close enough to the N_2S plane (<0.2 Å). In the model complex, the apical nitrogen atom is bound to the copper apparently more rigidly than the methionine sulfur or peptide carbonyl in azurin, yet the spectroscopic characteristics of the complex are remarkably similar to those of azurin. Conversely, rhombic EPR signals are observed for pseudoazurin and cucumber basic blue copper protein, although the copper sites of both proteins consist of N_2SS^* ligand donors, as does azurin. On the other hand, the structural features of the CuN_2S units in plastocyanins are not typical for trigonal structures, and they give axial EPR spectra. The separations between the copper and the N_2S plane are 0.36 and 0.37 Å, which may be slightly shorter than those found in pseudoazurin and cucumber basic blue copper protein, but clearly longer than that reported for azurin. In plastocyanins, however, the Cu—S* bond distances are considerably longer than those found in pseudoazurin and cucumber basic blue copper protein. Therefore, the coordination structure of the CuN_2S chromophores in plastocyanins is still regarded as trigonal rather than distorted tetrahedral, as pointed out previously on the basis of spectroscopic and theoretical studies (*47, 48*).

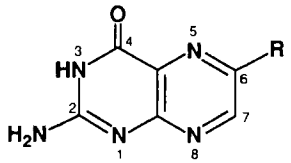
Even though there is a possibility that the apical methionine ligand may control the reduction potential, the definite role still remains ambiguous (*75*). Synthetic studies with model compounds, in conjunction with protein work by site-directed mutagenesis, should be undertaken to further address this point.

III. Synthetic Models for Type II Copper

A. Type IIA

1. Copper Complexes Containing Pterin and PQQ

Type IIA copper is defined as a monomeric copper site with a tetragonal ligand environment, exhibiting the spectroscopic features of conventional synthetic Cu(II) complexes. The coordination structure of the type IIA site is not remarkable from an inorganic point of view. What is noteworthy is the interaction between the copper ion and a prosthetic group or substrate. The electron uptake or activation of a substrate that occurs through this interaction plays an essential role in the catalytic cycle, yet the structural and mechanistic details are not certain. Therefore, the synthetic model approach may provide useful information for understanding the catalytic processes.



1 R = -CH(OH)CH(OH)CH_3

2 R = -CH_2NH-  -CONHCHCH$_2$CH$_2$COOH (COOH)

3 R = -COOH

Phenylalanine hydroxylase (PAH) from *Chromobacterium violaceum* is known to contain a type II copper (*76, 77*). The enzyme also contains the reduced form of biopterin **1** as a cofactor (*78*). EPR studies suggest that the pterin is bound to the copper through N5 at an equatorial position (*79*). As a model for the active site of PAH, Kohzuma *et al.* reported the first structurally characterized example of a pterin-containing copper(II) complex (*80*). The complex was obtained from a mixture of $Cu(NO_3)_2$, bpy, and folic acid **2** at pH >10 under aerobic conditions. The side chain of folic acid was cleaved, yielding a ternary complex Cu(bpy)(PC)(H_2O) (PC denotes pterin-6-carboxylate **3**), whose crystal structure is given in Fig. 8. As estimated for PAH, N5 is coordinated to the copper(II) ion, forming an equatorial plane together with two nitrogens from bpy and water. The carbonyl O4 and a carboxylate oxygen at the side chain in PC serve as apical ligand donors. Recently two other X-ray structures of pterin copper(II) complexes, $Cu(ethp)_2$(phen) and Cu(pterin)(HB(3-Phpz)$_3$), were reported (*81*). In both cases, the pterin serves as a bidentate mono-anion ligand that is bound to the copper through the N5 and O4 atoms. The structural parameters of these complexes are compared in Table V. A smooth trend is clear where a decrease in the Cu—O distance parallels a longer C—O distance. Pterin deprotonation redistributes electron density in the pyrim-

TABLE V

BOND DISTANCES (Å) OF Cu(II) COMPLEXES CONTAINING PTERIN

Bond	Cu(bpy)(PC)(H_2O)	$Cu(ethp)_2$(phen)	Cu(pterin)(HB(3,5-$Ph_2pz)_3$)
Cu–O4	2.499	2.303	1.968
C4–O4	1.238	1.250	1.282
C4–N3	1.339	1.349	1.318
C2–N3	1.359	1.335	1.378
C2–N1	1.361	1.325	1.333

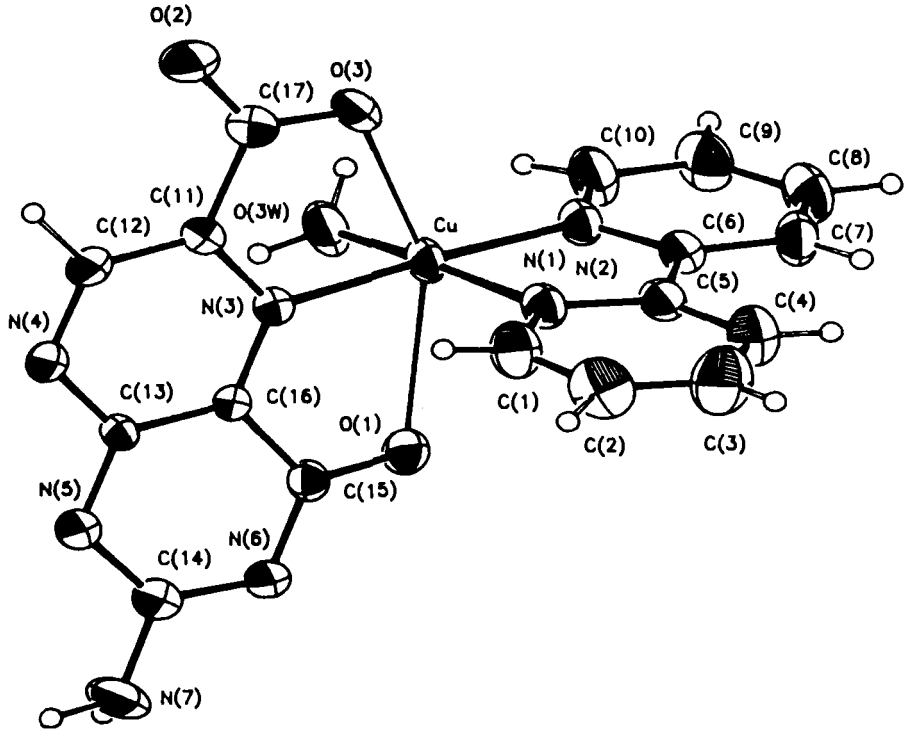
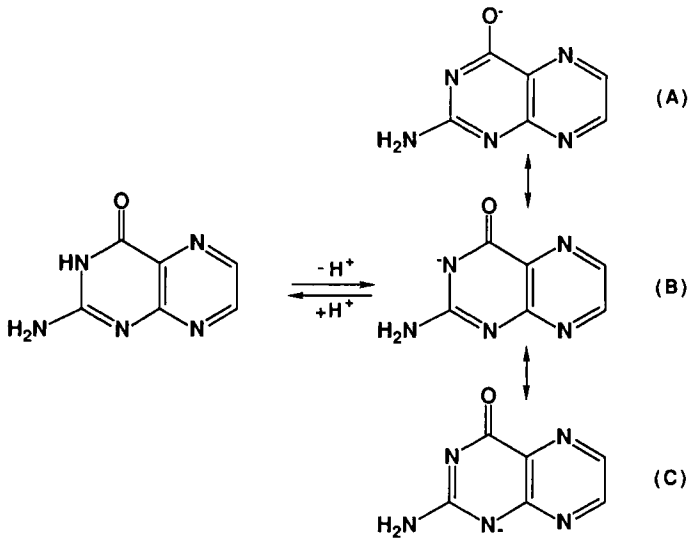


FIG. 8. ORTEP view of Cu(PC)(bpy)(H_2O). Reprinted with permission from Ref. *80*.

idine ring as schematically indicated in Scheme 1. The structural feature of Cu(bpy)(PC)(H_2O) is consistent with the resonance structure of C in Scheme 1, whereas Cu(pterin)(HB(3-$Ph_3pz)_3$) prefers structure A. The structure of Cu(ethp)$_2$(phen) is ascribed to resonance forms B and C, combined to delocalize the negative charge over the region N1–C2–N3. The structural difference between Cu(bpy)(PC)(H_2O) and Cu(pterin)(HB(3-$Phpz)_3$) was ascribed to the orientation of the pterin ring. In Cu(PC)(bpy)(H_2O), the pterin ring is directed perpendicular to the equatorial plane around the copper so that O4 sits at the apical position. On the other hand, both N5 and O4 occupy the equatorial positions in Cu(pterin)(HB(3-$Phpz)_3$). These synthetic studies clearly indicate that the most accessible ligand donors in pterin are N5 and/or O4, though coordination of a pterin N1 was recently found in [Cu(bpy)(LM)](NO_3)H_2O (LM = lumazine) (*82*).

Copper-dependent amine oxidases contain a type II copper (*83–85*). The yellow–pink color of amine oxidase implies the existence of another organic cofactor whose identification has not yet been unambiguously



SCHEME 1.

determined. A plausible candidate is the PQQ cofactor previously suggested, or its derivative (*86, 87*). Although the proposal seems unlikely based on the data presented recently (*vide infra*), ternary copper(II) complexes containing PQQ have been prepared and characterized as a model for the active site of amine oxidase (*88*). One of the Cu–PQQ complexes was reported to be effective for benzylamine oxidation to benzaldehyde, although PQQ by itself can catalyze nonenzymatic oxidations of amines.

2. Mononuclear Copper Superoxo and Peroxo Complexes

It is reasonable to suggest involvement of a monomeric superoxo copper(II) complex in oxygen incorporation reactions catalyzed by monooxygenases containing type IIA copper. As described above, copper-dependent PAH contains a type II copper. Dopamine β-hydroxylase (DBH) (*89, 90*) and the recently isolated peptidylglycine α-amidating monooxygenase (PAM) (*91, 92*) represent other examples of this family. The first monomeric superoxo copper(II) complex was reported by Nappa *et al.* (*93*). Reaction of KO_2 with an N_4 macrocyclic complex Cu(tet b)$^{2+}$ in DMSO gave an EPR-silent complex that was identified as a monomeric superoxo copper(II) complex. The *d–d* band observed at 672 nm and CV data were also consistent with the structure. Formation of a superoxo complex from a copper(I) precursor was demonstrated by Thompson (*94*). The reaction of a monomeric copper(I)–

ethylene complex $Cu(C_2H_4)(HB(3,5\text{-}Me_2pz)_3)$ with dioxygen gave $Cu(O_2)(HB(3,5\text{-}Me_2pz)_3)$ as a purple solid. The identification of the product was accomplished on the basis of its diamagnetic property and IR results using $^{18}O_2$. The complex exhibited an intense absorption band at 524 nm ($\varepsilon = 600\ M^{-1}\ cm^{-1}/Cu^{2+}$), which was assigned to an $O_2^- \rightarrow Cu(II)$ LMCT. However, except for the IR results, the properties of the complex are identical to those of μ-η^2:η^2 peroxo dicopper(II) complex $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$ (*95*) (*vide infra*). Hence, there is a debate as to whether the reported complex might be the μ-peroxo dicopper rather than a monomeric superoxo complex. The low-temperature reaction of a monomeric copper(I) complex $[(TPA)Cu(RCN)]^+$ with dioxygen has been carefully examined (*96*). When the reaction was carried out between -90 and -75°C, formation of a species that exhibits characteristic bands at 410 nm ($\varepsilon = 4000\ M^{-1}\ cm^{-1}$) and 747 nm ($\varepsilon = 1000\ M^{-1}$) was noted. The complex is formed transiently and is transformed into a stable *trans*-μ-1,2-peroxo dicopper(II) complex that possesses distinct absorption bands (the details are given in Section IV,A). In conjunction with kinetic experiments, the transient species was suggested to be a monomeric superoxo complex. Despite their apparent biological relevance, so far only these three complexes have been characterized as superoxo complexes. The difficulty in isolating and characterizing the complexes is due to the facile formation of the μ-peroxo dicopper(II) complex, which results from the coupling of the superoxo species with the copper(I) precursor. The reaction of KO_2 with a copper(II) complex may be a better option to avoid formation of the μ-peroxo complex, yet the basicity of KO_2 often causes reduction of the copper(II) ion. Therefore, design of a suitable ligand system is an important requirement for further studies on this type of complex.

In light of the accepted mechanism for cytochrome *P*-450 (*97–100*), a superoxo–Cu(II) intermediate is further reduced, leading to dioxygen activation. Accordingly, a monomeric peroxo or hydroperoxo copper(II) complex serves as a synthetic model for these intermediates of copper-containing monooxygenases. However, no well-characterized complexes of these types are available to date. Formation of a monomeric hydroperoxo or acylperoxo complex was reported to occur when a *trans*-μ-1,2-peroxo complex, $[(Cu(TPA))_2(O_2)]^{2+}$, was treated with H^+ or $RC(O)^+$, but no details of the structures and properties of the complexes were provided (*101*). A related complex, a monomeric acylperoxo copper(II) complex, was synthesized (*102*). Low-temperature reaction of a dimeric copper(II) hydroxide complex, $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(OH)_2$, with 2 equivalents of *m*-CPBA (3-chloroperoxybenzoic acid) yielded a monomeric acylperoxo complex whose structure was characterized by

IR and EPR. Although the complex reacts with PPh_3 instantaneously to give $OPPh_3$ with a monomeric benzoate complex, it does not oxidize any other substrates, including cyclohexene. The corresponding alkylperoxo complex $Cu(OOCMe_2Ph)(HB(3,5\text{-}iPr_2pz)_3)$ was also obtained in a similar manner and its structure was established by X-ray crystallography (*103*). Again, the complex does not exhibit high oxo-transfer reactivity.

One may expect high oxo-transfer reactivities for these complexes, because the corresponding acylperoxo iron(III) complex with a porphyrin ligand is known to be very effective for oxo-transfer reactions (*104*). Nevertheless, both of the copper analogs were inactive except for PPh_3 oxidation. It is surprising that the acylperoxo complex does not react with cyclohexene even at room temperature, whereas free *m*-CPBA readily epoxidizes cyclohexene even at $-20°C$. As a related complex, a dinuclear acylperoxo copper(II) complex was reported (*105*). The complex is also ineffective for oxo-transfer reactions except PPh_3. It is known that alkylperoxide and acylperoxide coordinated to an early transition metal ion are very effective for electrophilic oxo-transfer reactions. Thus a variety of catalytic systems have been developed for olefin epoxidations, for which the main component is an early transition metal such as Ti. The high reactivity of alkylperoxide and acylperoxide coordinated to early transition metals is explained simply in terms of the strong Lewis acidity of the metal ions, which enhances the electrophilicity of the coordinated peroxides. Conversely, late transition metal ions are much less electrophilic, so that most of the complexes cannot directly incorporate an oxygen into the double bond of an olefin. Examples are the alkylperoxo complexes of Pd (*106, 107*), Co (*108, 109*), and Pt (*110*). In reactions with olefins, these complexes give the ketone instead of the epoxide because the oxidation proceeds via initial coordination of olefin followed by alkyperoxide insertion into metal–carbon bonds (*111*). If there is no open coordination site available, they are inactive or decomposed via homolysis of the M—O or O—O bond, although one exception, an acylperoxo platinum(II) complex, was reported to be effective for olefin epoxidation (*112*). Iron(III) and manganese(III) ions presumably possess the medium character. Accordingly, the porphyrin complexes do not incorporate the oxygen atom directly into olefins, rather they require heterolysis of the O—O bond to become effective for the reaction, though under certain reaction conditions direct oxo transfer can occur (*113*). It is generally accepted that the resultant oxo species are strongly electrophilic because of the unusually high oxidation state of the central metal ion, formally iron(V) or manganese(V), and they are extremely effective in the epoxidation of olefins.

Turning to the complexes of copper(II), copper(IV) is not stable and heterolysis of the O—O bond of the peroxide to form the copper(IV) oxo complex does not occur. In addition, the Lewis acidity of the copper(II) ion is not high enough to enhance the electrophilicity of the coordinated alkyl- or acylperoxide to promote direct oxo-incorporating reactions. With these points in mind, the inert activity of alkyl- and acylperoxo copper(II) complexes, experimentally observed, is understandable, and it is quite unlikely that the mechanism of copper monooxygenase parallels that of cytochrome *P*-450.

Now the question is how the hydroperoxo intermediate, most likely formed as an intermediate in copper monooxygenase catalysis, can oxidize the substrate. Before reaching a conclusion, we may need more data on the general reactivity of monomeric hydroperoxo copper(II) complexes. However, all of the data obtained so far suggest a mechanism involving the homolysis of the O—O bond. Such a homolytic cleavage is well established for a number of alkylperoxo complexes, including alkylperoxo iron(III) complexes with porphyrin ligands (*114*). Because copper(II) ion is known to be a good catalyst in the Haber–Weiss decomposition of alkylhydroperoxide and hydrogen peroxide, homolysis of the O—O bond is quite likely to occur for the hydroperoxo copper(II) intermediate. In fact, our experimental results indicated that both monomeric acylperoxo and alkylperoxo copper(II) complexes undergo spontaneous decomposition via the radical mechanism. Because the tendency of a peroxide to undergo homolysis is in the order acylperoxide $<$ hydroperoxide $\leq$ alkylperoxide, the results support O—O bond homolysis. The OH· radical that is formed is then responsible for oxidation catalyzed by the copper monooxygenases. This does not necessarily mean that the enzymatic reaction proceeds via a free-radical mechanism such as in the Fenton reaction; rather, a concerted mechanism within the copper coordination sphere is more likely. But it must be emphasized that the character of the reaction is essentially of a radical type. Based on many physico-organic investigations (*115–117*), it has been proposed that the mechanism for DBH is as indicated in Fig. 9. The mechanism is entirely consistent with that described above, and provides further insight into the mechanism of tyrosinase (to be discussed later).

3. Models for Nitrate Reductase

The crystal structure of nitrite reductase (NiR) from *Achromobacter cycloclastes* was recently reported by Godden *et al.* (*46*). The protein contains both a type I and a type II copper site. The type I center has a distorted tetrahedral structure typical of type I copper, whereas the

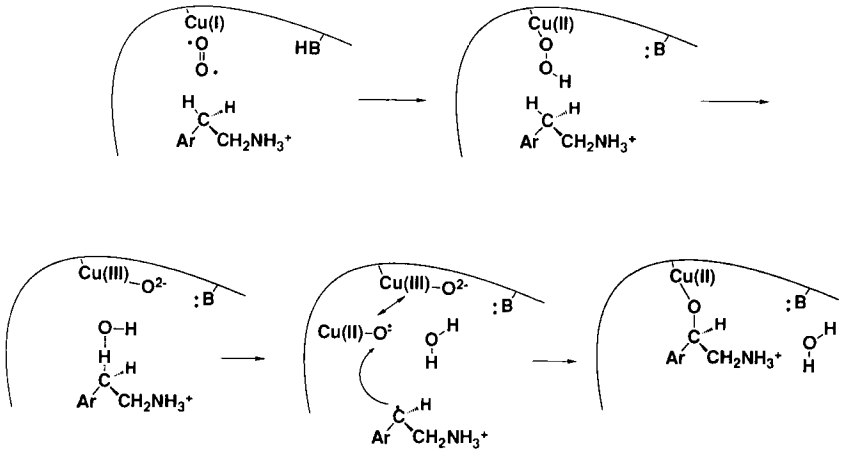


FIG. 9. Proposed reaction mechanism of DBH (*115–117*).

type II site has an unusual distorted tetrahedral ligand array consisting of three histidines and water or hydroxide. Nitrite reductases catalyze NO_2^- reduction to NO, which is subsequently converted to N_2 by nitrous reductase; in some cases N_2O is produced (*118*). As a model for the substrate-binding intermediate for NiR, a monomeric nitrite copper(II) complex $Cu(NO_2)(HB(3\text{-}tBupz)_3)$ was prepared with a hindered N_3 ligand (*119*). The nitrite group is coordinated to the copper in a bidentate manner via the two oxygen atoms. In NiR, reduction of NO_2^- is suggested to involve initial dehydration of bound nitrite to yield a copper nitrosyl, E–Cu–NO^+ (*120*). To mimic the enzymatic reaction, $Cu(NO_2)(HB(3\text{-}tBupz)_3)$ was treated with trimethylsilyl triflate, but no N—O bond scission was observed; instead, simple replacement of the nitrite with triflate was noted. This negative result may imply that the coordination of nitrite in NiR is different from that in the model complex, possibly involving N-coordination (*119*). Although nitrosyl complexes of other metal ions are well known, few copper complexes are available. A monomeric nitrosyl complex $Cu(NO)(HB(3\text{-}tBupz)_3)$ was successfully prepared and structurally characterized by Carrier *et al.* (*121*). The complex was obtained by NO addition to a copper(I) precursor. On the other hand, Paul *et al.* reported formation of a dicopper(II) nitrosyl complex, whose structure was determined by X-ray crystallography, by treating the dinuclear copper(I) complex $[Cu_2(XYL—O—)]^+$ with NO^+ (*122*). The dinuclear structure apparently differs from the type II site in NiR, yet the reaction chemistry of the complex retains the biological relevance, because NiR undergoes a two-electron redox reaction associ-

ated with the combined Cu(II)/Cu(I) couples of the type II and type I copper centers. In fact, some interesting reactions are seen with the dicopper complexes. The reaction of a dicopper(I) complex with NO_2^- in the presence of 2 equivalents of H^+ resulted in formation of the nitrosyl dicopper(II) complex with H_2O (*123*). This reaction mimics the stoichiometry of the reaction catalyzed by NiR. The reaction of the dicopper(I) complex with NO was further explored, resulting in the transient formation of a purple complex, presumably Cu(II)–$(NO^-)_2$–Cu(II), followed by formation of a μ-oxo dicopper(II) complex with N_2O. On the basis of these observations a new mechanism was proposed wherein the initially formed NO_2^- adduct is hydrated, giving a nitrosyl copper(II) intermediate, and the two coordinated nitrosyl groups at the type II copper site are then coupled to give N_2O and O^{2-}.

B. Type IIB

As described already, amine oxidase was suggested to contain PQQ as a prosthetic group. However, very recently Klinmann and co-workers indicated that the prosthetic group involved in the amine oxidase from bovine serum (BSAO) is not PQQ but topa (6-hydroxydopa) (*124–126*). The finding is of interest for a number of reasons. First, it represents a new type of cofactor that is bound covalently to a protein chain. Second, it has been recognized that the 6-hydroxy derivatives of dopa, dopamine, and related compounds are neurotoxic. Third, how does topa form? The origin of topa is possibly correctly ascribed to dopa or tyrosine. Here, what is noteworthy is the fact that the biosynthesis of catecholamines including dopa requires two copper-containing monooxygenases, PAH and DBH. In addition, tyrosinase, a monooxygenase containing a type III copper site, is also effective for tyrosine oxidation. Thus topa may be formed via self-oxidation of a coordinated tyrosine promoted by the copper ion.

As the first example of an oxidase containing only a type II copper, the crystal structure of galactose oxidase (GOase) at 1.7 Å resolution was recently reported (*127*). The protein side chains from two histidines and two tyrosines serve as ligand donors. At pH 4.5, consistent with spectroscopic features, the copper has a square–pyramidal ligand environment with a basal plane consisting of two histidine nitrogens, a tyrosine phenoxide oxygen, and an oxygen atom from an exogenous acetate (Fig. 10). The other tyrosine is positioned at the apical site. At pH 7.0 GOase is active and the copper is best described as distorted

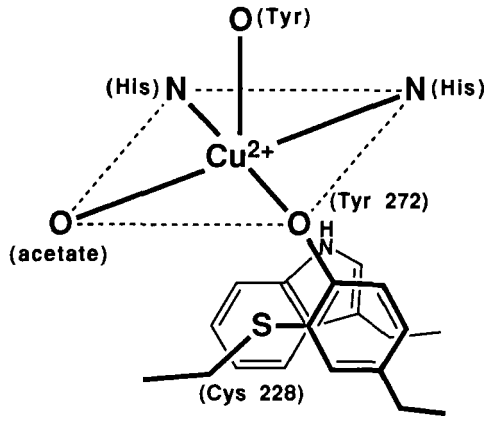


FIG. 10. The active site structure of GOase (*127*).

tetrahedral with the four protein residues attached. It is of interest that a number of copper(II) complexes with Salen type ligands were tested for the ability to function in the aerobic oxidation of primary alcohols, thus mimicking the reaction of GOase; a complex having a distorted tetrahedral environment with a N_2O_2 ligand donor set was found to be the most effective (*128*). A unique structural feature seen in the X-ray structure of GOase is a novel thioether bond, linking Cys-228 and Tyr-272 in a stacking interaction with Trp-290.

Both BSAO and GOase catalyze two-electron oxidations of substrates along with dioxygen reduction to hydrogen peroxide. There has for a long time been a question as to how these enzymes can catalyze a two-electron transfer from organic substrate to dioxygen, whereas the type II copper is capable of undergoing only a one-electron Cu(II)/Cu(I) change. In GOase, the participation of the Cu(III)/Cu(I) couple was previously proposed as an explanation for the two-electron reaction (*129*), but recent spectroscopic investigations have excluded this possibility, instead indicating the existence of a tyrosine radical as a redox component (*130, 131*). In BSAO, detailed EPR studies revealed the presence of a Cu(I)–semiquinone state that may correspond to a fully reduced state of the enzyme (*132*). Incorporation of this new information leads to the proposal that topa in BSAO, or Tyr-272 linked to Cys-228 in GOase, functions as an organic redox center alongside the type II copper. The overall redox processes are therefore interpreted as shown in Fig. 11. Substantiating these mechanisms using synthetic models will be a new challenge for inorganic chemistry in the future.

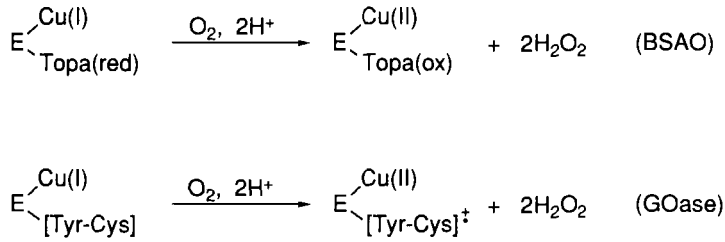


FIG. 11. Hypothetical redox mechanisms of BASO and GOase.

C. TYPE IIC

1. *Structural Models for SOD: Dinuclear Cu–Cu and Cu–Zn Complexes Bridged with Imidazolate*

Superoxide dismutases (SODs) are a ubiquitous group of enzymes whose physiological role is to protect cells from the toxic effects of the superoxide ion by catalyzing the following disproportionation reaction (*133, 134*).

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

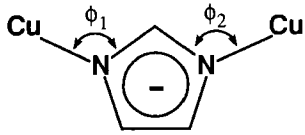
Three types of SODs, Cu,ZnSOD, MnSOD, and FeSOD, are now known. Cu,ZnSOD is found in almost all eukaryotic cells, whereas MnSOD and FeSOD occur in mitochondria and prokaryotic cells, respectively. The active sites of both MnSOD (*135*) and FeSOD (*136*) are monomeric, whereas the active site of Cu,ZnSOD (*137*) contains a quite distinctive hetero-dinuclear Cu–Zn core bridged by imidazolate. The copper is coordinated to four nitrogen atoms from histidines and water and has a distorted square–pyramidal geometry; the zinc is surrounded by three histidyl nitrogens and a carboxylate oxygen atom from aspartic acid and is tetrahedral. Because of their accessibility, it is possible to introduce a variety of metal ions, and Cu,Cu- or Cu,Ag-substituted SODs have been the subject of many spectroscopic investigations (*138–140*). The unique core structure of Cu,ZnSOD has also stimulated inorganic chemists to synthesize structural models. Because of the difficulty in obtaining hetero-dinuclear complexes, most efforts have been focused on the synthesis of a model for Cu,CuSOD, which is obtained by substitution of the zinc by copper (*141–143*). Cu,CuSOD exhibits the same catalytic activity as the native Cu,ZnSOD. Selected examples are given in Fig. 12 and in Table VI with corresponding structural parameters. Cu,CuSOD exhibits antiferromagnetic properties ($J = -26$ cm^{-1}) (*141*), thus the magnetic properties of the dicopper(II) complexes are of particular interest. As shown in Table VI, all μ-imidazolate dicopper

TABLE VI

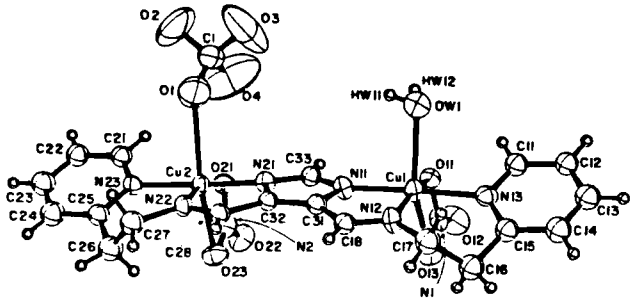
REPRESENTATIVE EXAMPLES OF SOD MODELS

Compound	J (cm^{-1})	ϕ_1, ϕ_2 (deg)	α_1, α_2 (deg)	Cu—Cu (Å)	Ref.
$[Cu_2(bpim)]^{3+}$	−81.8	143, 142	5, 11	6.14	*151*
$[(TMDT)_2Cu_2(Im)(ClO_4)_2](ClO_4)$	−25.8	129, 129	92, 90	5.94	*145*
$[Cu_2(L_{30})(Im)]^{3+}$	−21.0	134, 129	69, 79	5.99	*146, 152*
$[Cu_2L_{24}(Im)]^{3+}$	−21.2	128, 133	89, 90	5.97	*146, 153*
$[Cu(pmdt)]_2(2\text{-MeIm})(ClO_4)_3$	−38.1	121, 121	90, 90	5.66	*154, 155*
$[Cu_2(Im)(ImH)_2 \subset A]^{3+}$	—	125, 125	—	5.94	*156*
$[L_3Cu_2(Im)]^{3+}$	−19.3	129, 127	93, 91	5.92	*157*
$\{[Cu(AE)](Im)[Ni(AE)]\}^{+}$	+21.5	129, 129	—	5.93	*150*
$\{[Cu(tren)](Im)[Zn(tren)]\}^{3+}$	—	—	—	5.92	*148*

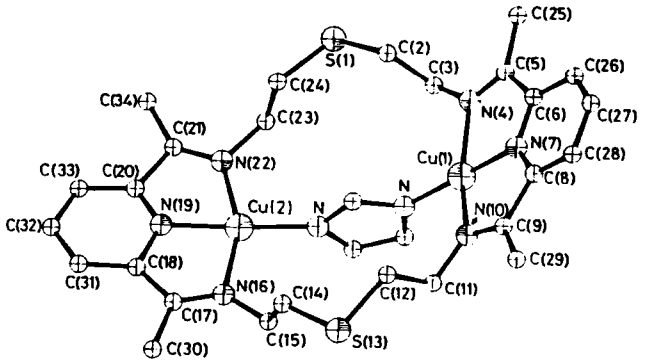
complexes are antiferromagnetic, with J values varying from ~ -20 to -80 cm^{-1}. The coordination geometries of the complexes are best described as tetragonal except for $[Cu_2(Im)(ImH)_2 \subset A]^{3+}$, which is trigonal–bipyramidal. The notable structural feature of these model complexes is that the dihedral angles (α_1, α_2) between the imidazolate ring and the tetragonal basal planes are close to either 0° or 90°. Thus, the antiferromagnetic interactions are suggested to occur predominantly through a σ-exchange pathway and not through π interaction. The J values correlate with the angles ϕ_1 and ϕ_2, which are defined as illustrated (*144*):



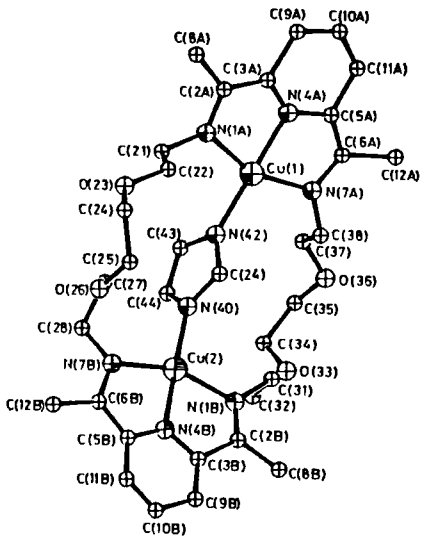
An increase in the size of these angles is expected to cause the stronger coupling. In fact, $[Cu_2(bpim)]^{3+}$, whose angles are distinctively larger than the others, gives the highest $-J$ value. However, the trend is not strict, presumably because other structural changes also affect the magnetism. For instance, the angle between the two tetragonal planes around the copper ions has been shown to be a structural factor correlating with the J value (*145, 146*). Alternatively, the magnetism reflects the delicate structural change that may affect the J values not only through σ interaction but also π interaction. Other details on dicopper(II) models for SOD prior to 1981 have been reviewed by Strothkamp and Lippard (*147*). Two hetero-dinuclear Cu–Zn complexes were recently reported (*148, 149*), and a related Cu,Ni complex (*150*) has also



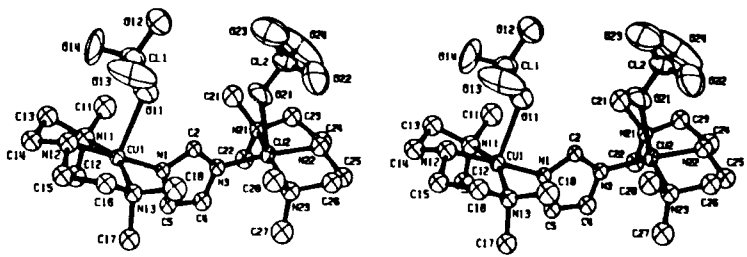
$[Cu_2(bpim)]^{3+}$



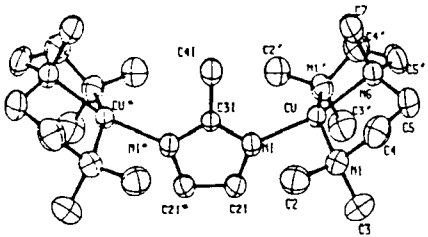
$[Cu_2(L_{24})(Im)]^{3+}$



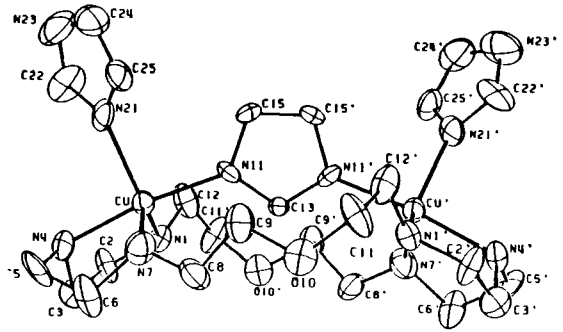
$[Cu_2(L_{30})(Im)]^{3+}$



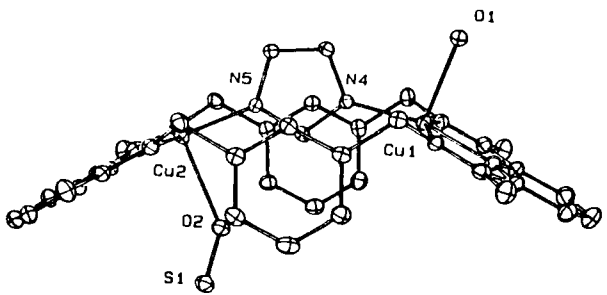
$[(TMDT)_2Cu_2(Im)(ClO_4)_2](ClO_4)$



$[Cu(pmdt)]_2(2\text{-MeIm})(ClO_4)_3$

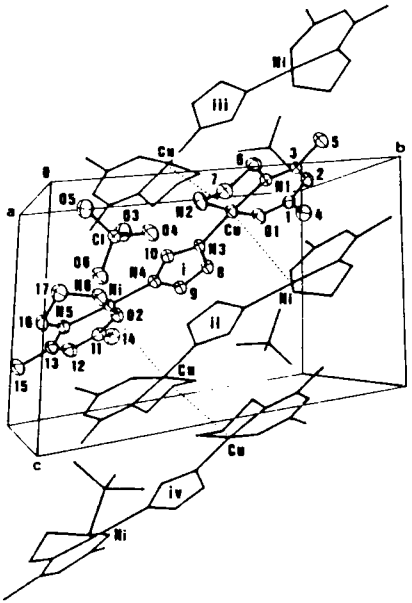


$[Cu_2(Im)(ImH)_2 \subset A]^{3+}$

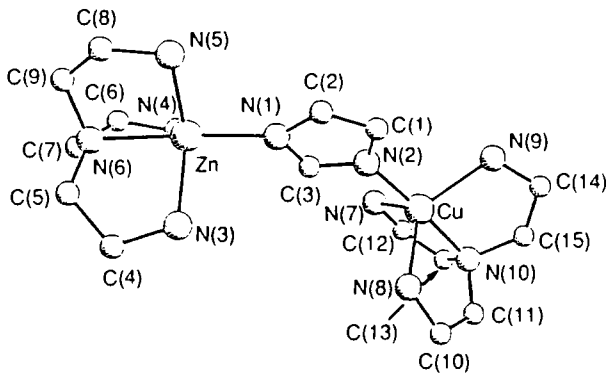


$[L_3Cu_2(Im)]^{3+}$

FIG. 12. Representative crystal structures of SOD model compounds. Reprinted with permission from Refs. *145*, *148*, *150–153*, and *155–157*.



{[Cu(AE)](Im)[Ni(AE)]}$^{+}$



{[Cu(tren)](Im)[Zn(tren)]}$^{3+}$

FIG. 12. *(Continued)*

been structurally characterized. However, both the copper and zinc (or nickel) ions in these complexes (*148–150*) are coordinated to identical ligands. Accordingly, the ligand environment of the zinc is dissimilar to that of the zinc in native Cu,ZnSOD. In summary, synthesis of an accurate structural model of native Cu,ZnSOD remains to be achieved. Moreover, there have been no attempts directed at functionally mimicking Cu,ZnSOD catalysis. Thus, the usefulness of synthetic models in understanding the catalytic mechanism of Cu,ZnSOD and the functional roles of copper and zinc is limited.

2. Functional Mimics for SOD

Superoxide is believed to play a role in several diseases and in inflammation, carcinogenesis, and aging (*158*). The application of SODs as pharmaceutical reagents has thus attracted much attention in recent years. Although beneficial effects of SODs in clinical settings have been reported, their limitations have also become apparent (*159*). SODs are easily filtered by the kidney and do not easily enter cells due to their high molecular weight. Also a problem are the toxic effects when the protein is recognized as antigen. To overcome these disadvantages, development of a low-molecular-weight metal complex capable of mimicking SODs *in vivo* has been proposed. The activities of a variety of copper complexes have been tested extensively in this regard because the copper site of Cu,ZnSOD is believed to be responsible for the binding and dismutation of superoxide ion. Examples are $Cu(salicylate)_2$ (*160–162*), Cu(Gly-His-Leu) (*163*), $Cu_{14}[(penicillamine)_{12}Cl]$ (*164*), Cu(polyamine) (*165*), and Cu(cimetidine) (*166*). Some of these complexes were reported to be more effective than native Cu,ZnSOD. However, the instability of the complexes *in vivo* is a serious problem. For instance, the Cu(II) ion is known to be catalytically very effective for superoxide dismutation *in vitro,* yet in living cells it is easily deactivated due to its conversion to a hydroxide form or its chelation by some reagent, for example, albumin. Clearly, for pharmaceutical use, the SOD mimic must satisfy the conditions of being stable, nontoxic, and active *in vivo.* One persistent problem comes from the fact that there is no reliable method of assaying the SOD activity *in vivo.* Even *in vitro,* definite determination of SOD activity is not easy to accomplish because of the instability of superoxide. The most reliable method is probably pulse radiolysis or the stopped-flow method in conjunction with some suitable source of superoxide ion, such as KO_2. However, such methods are not convenient for most inorganic and pharmaceutical chemists, and the assay of SOD activity usually depends on indirect methods, such

as that using cytochrome *c*. The reliability of these indirect methods is somewhat questionable. Thus, establishing a simple experimental method to determine SOD activity, especially *in vivo,* is very desirable to enable development of pharmaceutically relevant copper complexes.

3. *Cytochrome c Oxidase Models: Dinuclear Cu–Fe Complexes*

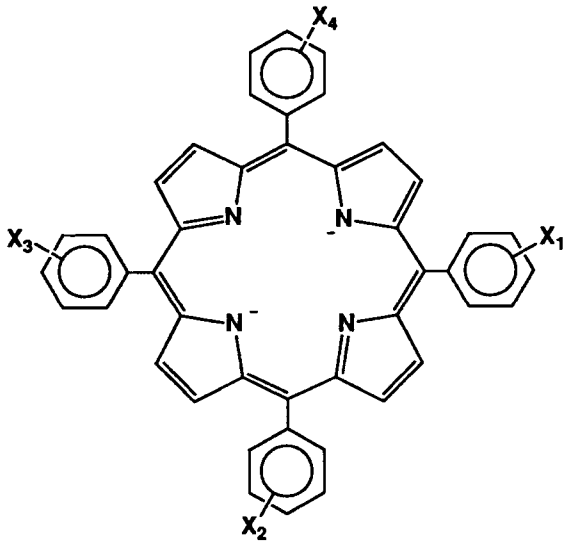
Cytochrome *c* oxidase (CcO) plays an indispensable role in respiration, being responsible for the reduction of O_2 to H_2O, with four electrons supplied from cytochrome *c*, which is positioned at the end of the electron transfer chain for respiration (*188*). The resting state of CcO contains at least two heme iron(III) and two copper(II) ions (*189, 190*) that exhibit EPR signals associated with one heme iron and a type I copper. The unusual feature is that the other heme iron, so-called a_3, and the type II copper are EPR silent. In a widely accepted scenario, this is ascribed to a strongly antiferromagnetic interaction between heme a_3 and the type II copper ($-J > 200\ cm^{-1}$), although other interpretations, such as ligand-mediated spin relaxation, are possible (*191–193*). On the basis of the EXAFS results, which indicated an Fe—Cu separation of only ~3 Å, the existence of an unknown bridging ligand, most likely sulfur, has been suggested (*194–197*).

This fascinating proposal prompted many chemists to attempt the synthesis and magnetic characterization of hetero-dinuclear Fe—Cu complexes. Typical examples are given in Fig. 13 and Table VII with the magnetic data. A variety of dinuclear complexes containing a bridge, e.g., imidazolate, thiolate, phenoxide, oxide, and halide, have been synthesized in order to clarify the general properties of the bridging ligand as a magnetic mediator. The most extensively tested are imidazolate bridging complexes. As seen with dicopper(II) complexes, however, the magnetic interaction through imidazolate is not very strong, the J value ranging from 0 to $-20\ cm^{-1}$. Although Chunplang and Wilson (*179*) reported a μ-imidazolate Mn(II)–Cu(II) complex that is strongly antiferromagnetic ($-J > 200\ cm^{-1}$, EPR silent), reevaluation of the magnetic properties is necessary, because careful investigation of a similar complex by Koch *et al.* has initiated a suggestion that Mn(III)–Cu(II) and not Mn(II)–Cu(II) may be present due to air oxidation (*185*). The Fe—Cu separation in μ-imidazolate complexes is in the range of 6–7 Å, which is significantly longer than that determined for CcO. Together with the observed weak magnetic coupling, such a structure does not seem to fit the Fe—Cu core in CcO. Regarding the antiferromagnetic interaction, among these models, the complexes having a diphenoxo bridge exhibit the highest $-J$ values, although the antiferromagnetic coupling is still not strong enough to become EPR

TABLE VII

HETERO-DINUCLEAR Fe—Cu COMPLEXES AS STRUCTURAL MODELS FOR CYTOCHROME *c* OXIDASE

Compound	Bridging ligand	Fe—Cu (Å)	Spin state	μ_B (temperature)	J (cm^{-1})	Ref.
$Fe(C_{18}H_{16}N_6)(bipym)Cu(acac)_2$	Bipyrimidyl	—	Fe(II) $S = 2$, Cu(II) $S = \frac{1}{2}$	5.46 (300 K)	0	*167*
$[Fe(P)–Cl–Cu(N_4)](ClO_4)_2·2H_2O$	Chloride	4.96	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	5.61 (330 K)	0	*168*
$[Fe(P)–OH–Cu(N_4)]^{2+}$	Hydroxide	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	5.2 (300 K)	0	*169*
$(Bu_4N)[Mn(Im)Co(FF)]·3THF$	Imidazolate	—	Mn(II) $S = \frac{5}{2}$, Co(II) $S = \frac{1}{2}$	6.42 (r.t.)	−5	*170*
$(Por)FeCu(O)(OAc)(N_x)$	Oxo	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	2.7 (300 K)	−101	*171*
$[(TPP)Fe(O)Cu(IMDH)]^+$	Oxo	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	4.23 (298 K)	—	*172*
$[FeCu(protopor)]·H_2O$	Hydroxide	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	4.1 (r.t.)	—	*173*
$\{[ClFe(TPP)Cu(IMD)](BF_4)_2(PSH)\}^+$	Imidazolate	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	2 (300 K)	−15	*174*
$\{CuFe[(fsa)_2en]Cl(H_2O)(MeOH)\}·MeOH$	Diphenoxide	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	—	−80	*175*
$Fe(salpen)CuCl_2$	Diphenoxide	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	5.2 (r.t.)	−70	*176*
$(UroTPP)FeCu(acac)_2$	Imidazolate	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	5.67 (298 K)	0	*177*
$[Fe(P)(CN)Cu(N_4)](ClO_4)_2·3H_2O$	Cyanide	—	Fe(III) $S = \frac{1}{2}$, Cu(II) $S = \frac{1}{2}$	2.95	+0.25	*178*
$\{Mn(TPP)[Cu(IMD)]\}BF_4$	Imidazolate	—	Mn(II) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	5.11	<-200	*179*
$(Py)(TPP)Cr(O)Fe(TMP)$	Oxo	3.6	Fe(III) $S = \frac{5}{2}$, Cr(III) $S = \frac{3}{2}$	5.11	150	*180*
$(TPPS)FeCu(GGH)$	Imidazolate	—	—	—	—	*181*
$Cu[(prp)_2en]Fe(hfa)_2$	Diphenoxide	—	Fe(II) $S = 2$, Cu(II) $S = \frac{1}{2}$	5.0 (180 K)	−6	*182*
$Fe(TPP)Cu(aib_3)$	Carboxylate	—	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	5.5 (300 K)	Weak	*183*
$\{[Fe(TPP)(THF)][Cu(MNT)_2]\}^-\{Fe(TPP)(THF)\}^+$	Thiolate	4.04	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	7.26 (220 K)	0	*184*
$Mn(TPP)Cu(Imid)$	Imidazolate	—	Mn(II) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	5.8 (r.t.)	−6	*185*
$[Fe(TPP)Cu(Imid)]B_{11}CH_{12}·3DMF·3H_2O$	Imidazolate	6.0	Fe(III) $S = \frac{1}{2}$, Cu(II) $S = \frac{1}{2}$	3.5	+22	*186*
$(TBA)\{[Fe(p\text{-}Cl_4TPP)]_2[Cu(MNT)_2]_2\}·2C_6H_6$	Thiolate	3.76	Fe(III) $S = \frac{5}{2}$, Cu(II) $S = \frac{1}{2}$	6.4	—	*187*

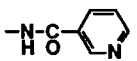


TPP : $X_1=X_2=X_3=X_4=$ H

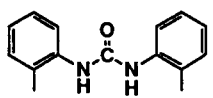
TMP: $X_1=X_2=X_3=X_4=$ *p*-OMe

p-Cl_4TPP : $X_1=X_2=X_3=X_4=$ *p*-Cl

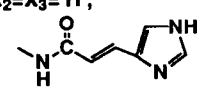
TPPS : $X_1=X_2=X_3=X_4=$ *p*-SO_3

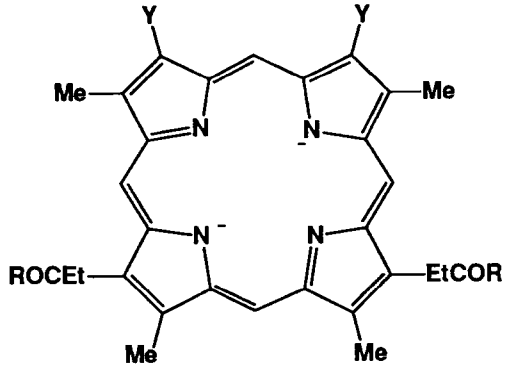
(P) : $X_1=X_2=X_3=X_4=$ 

FF : $X_1=X_2=X_3=$ H , $TPPX_4–Z–X_4TPP$

Z= 

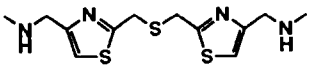
UroTPP : $X_1=X_2=X_3=$ H ,

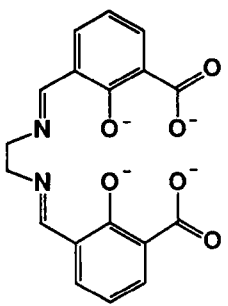
$X_4=$ 



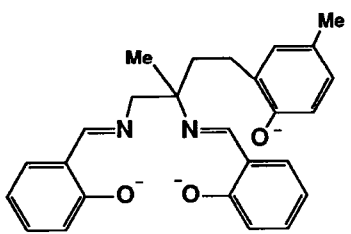
Protoporphyrin : Y=Me, R=OH

Strapped porphyrin : Y=Et, R–(S)–R

(S) = 



$(fsa)_2$en



salpen

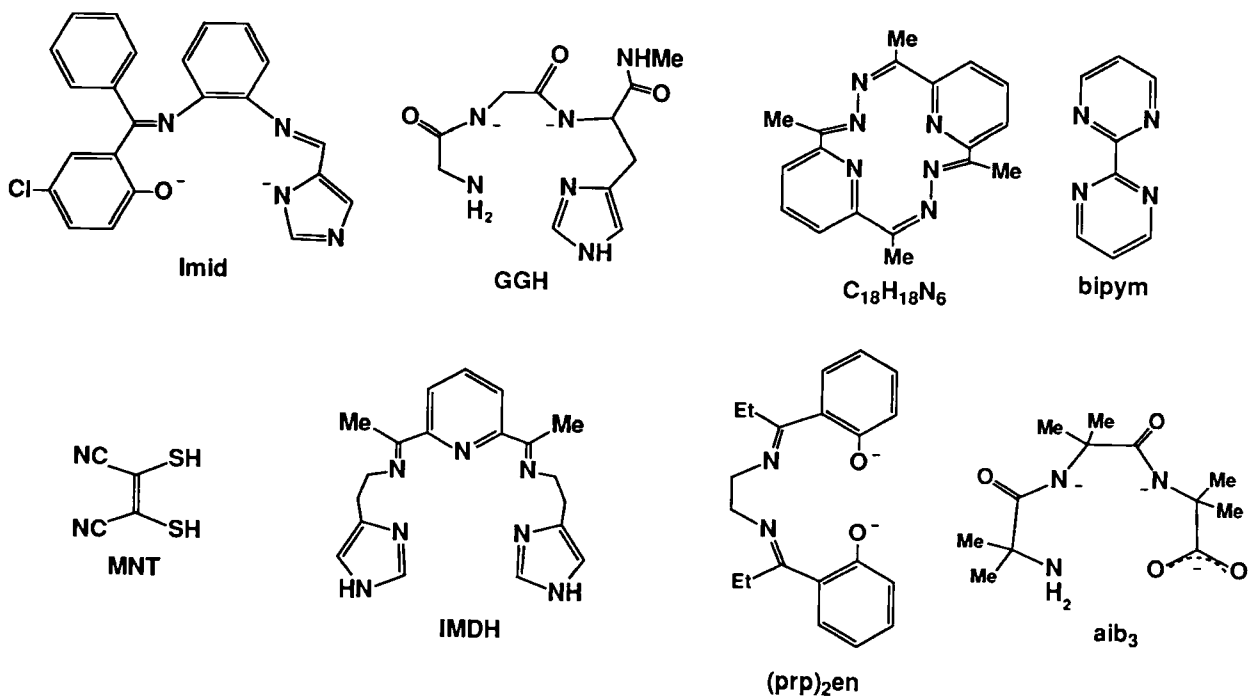


FIG. 13. Structures of ligands for the complexes given in Table VII.

silent as in CcO. None of these diphenoxo complexes, however, contain a porphyrin ligand. Considering the highly tetragonal nature of porphyrin ligands, a diphenoxide linkage between the copper and the heme iron a_3 in CcO does not seem feasible. On the basis of EXAFS analyses (*194–197*), the most likely bridging ligand is sulfur. Two μ-thiolate complexes have been synthesized and structurally characterized recently (*184, 187*). Although sulfur is expected to mediate a strong magnetic coupling, the model complexes exhibit no magnetic coupling or ferromagnetic property, thus providing rather negative evidence. Another good candidate as the bridging ligand may be hydroxide or oxide. Though hydroxide bridging gives a small $-J$ value (*173*), relatively large magnetic couplings are observed for μ-oxo complexes (*171, 172, 180*).

Despite much effort, surveys of the magnetic properties of a series of hetero-dinuclear Fe–Cu complexes are still incomplete; thus factors affecting the role of bridging ligands as mediators for antiferromagnetic interaction are not yet very clear. In order to better understand the trends, which may lead to an evaluation of the bridging ligand in CcO, examples of other model compounds containing bridging ligands, especially oxide and sulfide, could be a welcome addition to those given in Table VII. In particular, model complexes whose structures are determined by X-ray crystallography are preferable, because the magnetism is affected not only by the kind of ligand donor but also by other structural features, including the coordination geometry of the copper and iron and the orientation of bridging ligands to the highest occupied molecular orbitals (HOMOs) of the metal ions. From this point, complexes that do not contain a porphyrin-iron moiety may not provide sufficient insight into the dinuclear site structure in CcO. Develoment of functionalized porphyrin ligands containing a side arm for copper coordination would provide more appropriate models. An excellent ligand was reported recently (*198*).

The short Fe–Cu distance (~3 Å) and the EPR-silent property of the resting state of CcO are beyond any present understanding acquired through model studies. None of the model compounds give a sufficiently short metal-to-metal distance nor are EPR silent. There is much controversy about the origin of the bridging ligand. If the bridging ligand causing the strong magnetic interaction of the resting state of CcO is endogenous, the question naturally arises as to the existence of the ligand in both the oxidized and reduced states of CcO. Unfortunately, no experimental data on the nature of the bridging ligand in the oxidized and reduced states of CcO have yet been obtained. As a fascinating hypothesis, the possibility that dioxygen may bridge as peroxide be-

tween the type II copper and heme a_3 in oxidized CcO has been proposed several times (*194, 199, 200*). On this assumption, an oxo bridge originating from the peroxide may serve as the most reasonable candidate for the resting state of CcO, whereas EXAFS data are indicative of a sulfur. However, one should keep in mind that recent resonance Raman studies on the transiently formed dioxygen adduct of CcO suggested the terminal coordination of a peroxide (hydroperoxide) at the heme a_3 (201).

IV. Synthetic Models for Type III Copper

A. μ-Peroxo Dinuclear Copper Complexes as Models for Oxyhemocyanin and Oxytyrosinase

Hemocyanin (Hc) is a widely occurring oxygen transport protein in invertebrates, arthropods, and mollusks. Hc contains a type III copper that binds dioxygen reversibly as peroxide (*202–204*). One of the striking characteristics of oxy-Hc is its diamagnetic property, which is ascribed to the strong antiferromagnetic coupling between the two copper(II) ions ($-2J > 600$ cm^{-1}) (*205–206*). Another notable feature of oxy-Hc is its unusually intense absorption bands at 350 nm (ε = 20,000 M^{-1} cm^{-1} per dimer) and 580 nm (ε = 1000 M^{-1} cm^{-1}), instead of the weak d–d band at 600–700 nm normally observed for copper(II) complexes. Tyrosinase (Tyr) was the first monooxygenase to be discovered; it is effective for monophenol oxidation and plays an essential role in melanin biosynthesis (*207*). The active site of Tyr also consists of a pair of copper ions that form a relatively stable dioxygen adduct with properties similar to those of oxy-Hc.

In order to provide a structural basis for understanding the unique dinuclear structure and the striking spectroscopic properties of oxy-Hc and oxy-Tyr, the synthesis of model μ-peroxo dinuclear copper(II) complexes has been the focus of much recent research. A key question, the focus of much attention throughout these studies, is the origin of the diamagnetism of oxy-Hc. A huge number of investigations dealing with the synthesis and magnetic properties of dinuclear copper complexes have been reported. The chemistry of dinuclear copper complexes relating to oxy-Hc has been reviewed (*2, 3, 4*). The interested reader is directed to these reviews, which cover the literature prior to 1988; the present review focuses on the progress achieved since then. Another review dealing with very recent results on copper/dioxygen chemistry is also available (*208*).

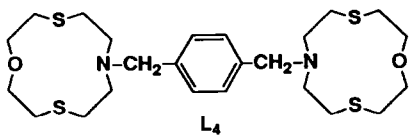
Despite many efforts, the synthesis, isolation, and characterization of μ-peroxo copper complexes has proved difficult to achieve. The Cu(II)–O_2^{2-}–Cu(II) moiety is, in general, difficult to construct, because of the facile reduction of the copper(II), as Cu(II)-SR, to copper(I), with release of dioxygen. The first conclusive evidence for the formation of a μ-peroxo copper(II) complex was provided by Karlin *et al.* in 1984 (*215*). Before that time a few μ-peroxo copper(II) complexes had been reported, but structural assignments were unfortunately debatable because of incomplete characterization (*232*). Karlin *et al.* synthesized a dinuclear copper(I) complex (Cu—Cu separation of 3.6 Å, comparable to that of deoxy-Hc) with a dinucleating ligand containing a bridging phenoxo group. They indicated that it reacts with dioxygen quasireversibly, forming a μ-peroxo dicopper complex whose structure was established on the basis of the O–O stretching vibration frequency (803 cm^{-1}) ascertained by resonance Raman spectroscopy (*215, 216*). In a detailed Raman study using mixed isotopically labeled dioxygen (*233*), it was concluded that the coordination mode of the peroxide is asymmetrical and terminal; and thus different from the mode suggested for oxy-Hc. The first X-ray structure of a μ-peroxo dicopper(II) complex was reported by Jacobson *et al.* (*226*). The complex was prepared by the self-assembly of a monomeric copper(I) complex with TPA and dioxygen. As shown in Fig. 15, the two copper(II) ions are bridged solely by a peroxide, the coordination mode being described as *trans*-μ-1,2. The structural feature of this complex, including the longer Cu—Cu separation of 4.36 Å, is apparently different from that of oxy-Hc, although its diamagnetic property (*224*) shed light on a new facet—that peroxide alone can mediate a strong magnetic interaction between two copper(II) ions. The physicochemical properties of these and other μ-peroxo complexes are compared in Table VIII (*209–231*). In most cases, the μ-peroxo complexes are prepared by dioxygen addition to dinuclear copper(I) precursors, but in some cases they are derived from monomeric copper(I) complexes. A variety of dinucleating ligands containing a phenoxo and alkoxo bridge have been employed in the syntheses (see Fig. 14). The predominant ligand donors are nitrogen atoms from amine, pyridine, pyrazole, or even imidazole groups. Also utilized were other ligands, such as thioether sulfur. In most cases, complete magnetic and spectroscopic data are not available so that a straight comparison of the properties with those of oxy-Hc and oxy-Tyr is not possible. One of the most interesting complexes is $[Cu_2(N_4)(O_2)]^{2+}$, reported by Karlin *et al.* (*218, 219*). The complex was prepared from a dicopper(I) complex in which each copper is coordinated to three nitrogen atoms as in Hc. The complex is diamagnetic and the absorption spectrum is,

TABLE VIII

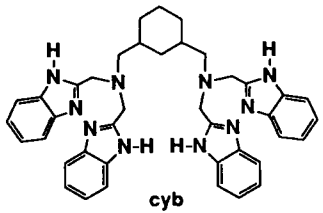
PROPERTIES OF μ-PEROXO BINUCLEAR Cu(II) COMPLEXES

Compound	UV–vis (nm)	ν(O—O)(cm^{-1})	Cu—Cu (Å)	Magnetism	Ref.
$[Cu_2L_4(O_2)]^{2+}$	390(—), 600–680(—)	—	—	EPR active	*209*
$\{[Cu(IMDH)]_2(O_2)\}^{2+}$	410(sh)	—	—	EPR inactive	*210, 211*
$[Cu_2L'_{30}(O_2)]^{2+}$	654(250)	—	—	—	*212*
$\{[Cu(cyb)]_2(O_2)\}^{2+}$	550–600(540)	—	—	—	*213*
$\{[Cu(TEEN)]_2(O_2)(H_2O)\}^{2+}$	630(—)	825	—	EPR inactive	*214*
$\{Cu_2(XYL–O–)(O_2)]^{2+}$	385(2900), 505(6000), 610(sh)	803	3.3[a]	—	*215–217*
$[Cu_2(N_3)(O_2)]^{2+}$	365(15,000), 490(5250), 600(1200), >850(—)	—	3.2[a]	EPR inactive	*218, 220, 221*
$[Cu_2(N_4)(O_2)]^{2+}$	360(15,000), 458(5000), 550(1200), 775(200)	—	3.4[a]	Diamagnetic	*218–221, 224*
$[Cu_2(N_5)(O_2)]^{2+}$	360(21,400), 423(3600), 520(1200), >600(—)	—	—	EPR inactive	*218, 221, 222*
$[Cu_2(N_3OR)(O_2)]^{2+}$	350(20,000), 485(1400), 600(880), 875(sh)	—	3.3[a]	Diamagnetic	*220–221, 223–224*
$[Cu_2(XYL–F)(O_2)]^{2+}$	360(18,700), 435(4400), 515(1300), 787(700), 925(600)	—	—	—	*221, 222*
$[Cu_2(UN)(O_2)]^{2+}$	360(11,000), 520(1000), 600(sh)	—	—	—	*225*
$\{[Cu(TPA)]_2(O_2)\}^{2+}$	440(2000), 525(11,500), 590(7600), 1035(160)	834	4.35	Diamagnetic	*224, 226*
$\{[Cu(L–im)]_2(O_2)\}^{2+}$	346(2200), 450(1450), 500(1900), 650(600)	—	2.8 or 4.3[a]	—	*227*
$[Cu_2(tpmc)(O_2)]^{2+}$	472(2900), 780(900)	820 and 771	—	EPR active	*228*
$[Cu_2(Py)_4N_6O(O_2)]^{+}$	390(4800), 500(6500), 630(750)	—	—	—	*229*
$[Cu_2(Py)_2(Pz)_2N_6O(O_2)]^{+}$	400(2500), 505(4900), 630(3400)	—	—	—	*229*
$[Cu_2(Py)_2(DMP)_2N_6O(O_2)]^{+}$	390(3400), 510(6700), 630(1600)	—	—	—	*229*
$Cu_2[Bz(NMI)_2N]_2(O_2)$	360(—), 450(—), 550(—)	—	—	—	*230*
$Cu_2(FD_2DIEN_2)(O_2)$	360(—), 504(1060)	—	—	—	*231*

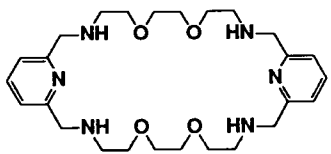
[a] Estimated by EXAFS.



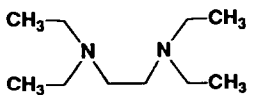
L_4



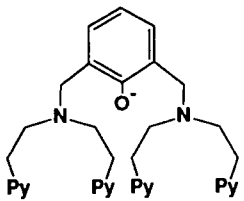
cyb



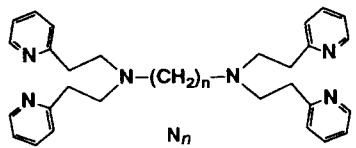
L'_{30}



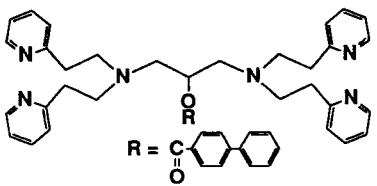
TEEN



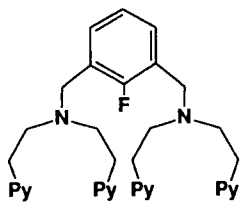
XYL-O



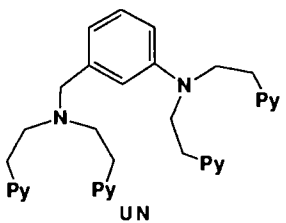
N_n



N_3OR



XYL-F



UN

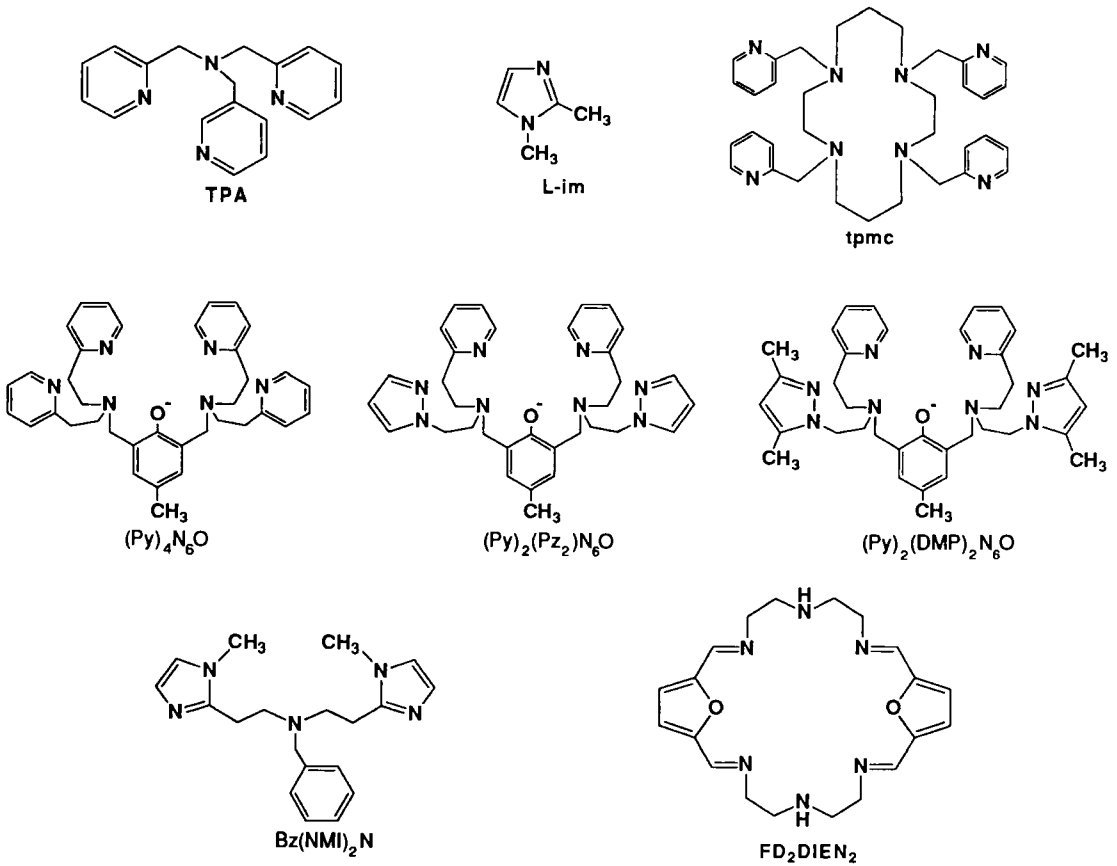


FIG. 14. Structures of ligands for the complexes given in Table VIII.

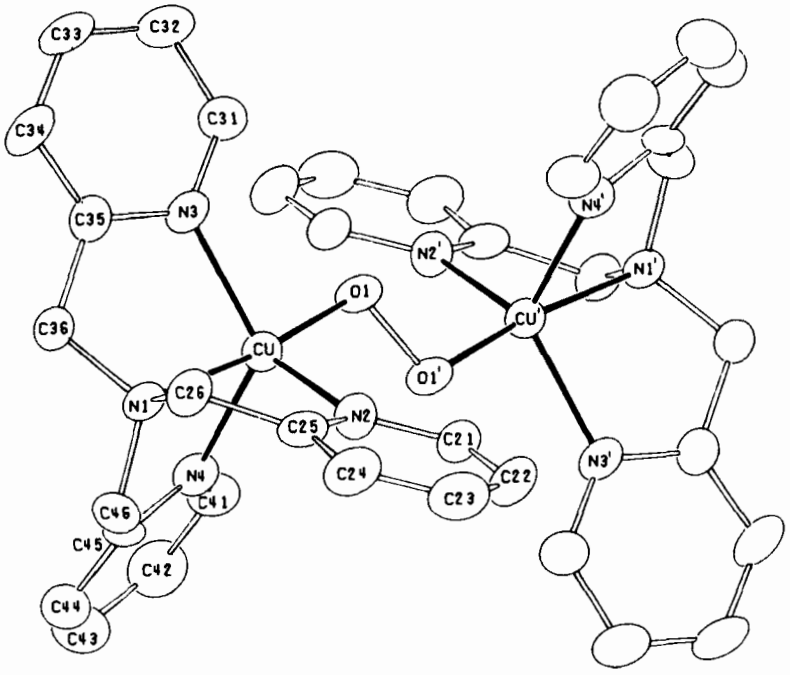


FIG. 15. ORTEP view of $\{[Cu(TPA)](O_2)\}^{2+}$. Reprinted with permission from Ref. *226*.

to some extent, similar to that of oxy-Hc. Unfortunately, the crystal structure is not available. The coordination structure of the peroxide was suggested as bent μ-η^2:η^2 based on the EXAFS result (the Cu—Cu separation was 3.4 Å, shorter than the 3.6 Å of oxy-Hc), but the possibility of *cis*-μ-1,2 was not excluded. It is notable that other μ-peroxo complexes, which contain an N_3–N_3 ligand frame, also give rise to absorption bands whose features are partly similar to those of oxy-Hc. Recently, as an accurate structural model for deoxy-Hc, a copper(I) complex with a tripodal imidazole ligand was synthesized and its dioxygen adduct was characterized (*227*). Again, the properties do not resemble those of oxy-Hc. Overall, the success in mimicking the characteristics of oxy-Hc and oxy-Tyr with these synthetic models is limited. These negative results imply a structural uniqueness of the Cu(II)(O_2^{2-})Cu(II) chromophore in proteins.

In 1988, the author's group reported the formation of a μ-peroxo dicopper complex by low-temperature treatment of the μ-oxo di-

copper(II) complex $[Cu(HB(3,5\text{-}Me_2pz)_2)]_2O$ with H_2O_2 (*95*). The complex, identified as $[Cu(HB(3,5\text{-}Me_2\text{-}pz)_3)]_2(O_2)$ based on FD-MS, mimics for the first time all the physicochemical characteristics of oxy-Hc; these properties include diamagnetism, a characteristic absorption spectrum, and an unusually low O–O stretching frequency. Subsequently, an analogous complex, $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$, was prepared by the reaction of H_2O_2 with the di-μ-hydroxo complex or by direct O_2 addition to the corresponding monomeric copper(I) complex (*235*). The structure of $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$ was determined by X-ray crystallography, which definitely established the novel coordination mode of the peroxide ion as $\mu\text{-}\eta^2:\eta^2$ (Fig. 16). The properties of these $\mu\text{-}\eta^2:\eta^2$ complexes, including $[Cu(HB(3,5\text{-}Ph_2pz)_3)]_2(O_2)$, are summarized in Table IX. The properties observed (*237*), including the Cu—Cu separation, are strikingly similar to those of oxy-Hc and oxy-Tyr, raising the possibility that type III copper also binds dioxygen in such an unusual coordination mode. The possibility of $\mu\text{-}\eta^2:\eta^2$ coordination of the peroxide has been proposed before as a plausible model for oxy-Hc. However, the structural model did not receive much attention, because there were no X-ray crystal structures of such complexes, except for the oxophilic *f*-block elements, lanthanum (*238*) and uranium (*239*).

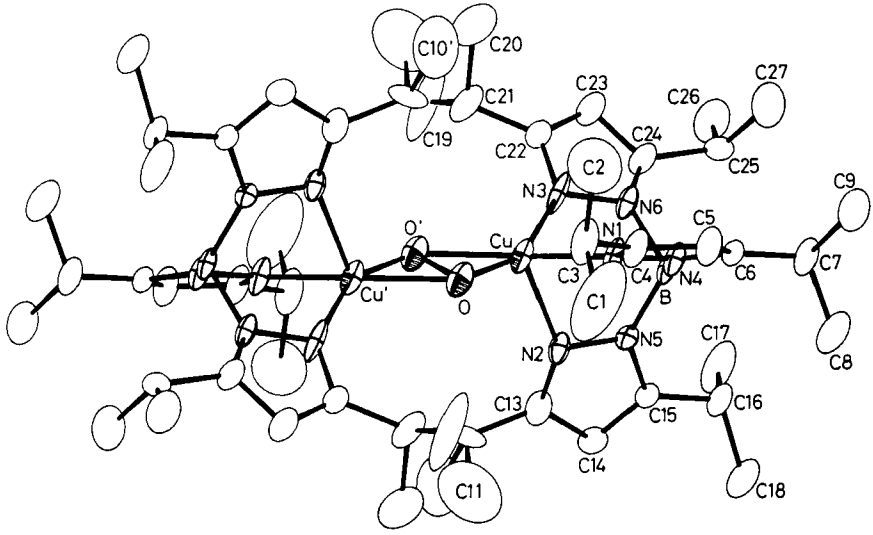


FIG. 16. ORTEP view of $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$. Reprinted with permission from Ref. *235*.

TABLE IX

PHYSICOCHEMICAL PROPERTIES OF μ-η^2:η^2 PEROXO COMPLEXES AND OXY-Hc AND OXY-Tyr

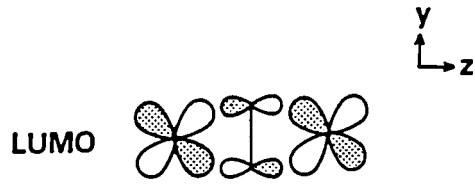
Complex	Magnetic property	Absorption bands (nm)[a]	ν(O—O) (cm^{-1})	Cu---Cu (Å)
$[Cu(HB(3,5\text{-}Me_2pz)_3)]_2$ (O_2)	Diamagnetic	530(840), 338(20,800)	731	—
$[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2$ (O_2)	Diamagnetic	551(790), 349(21,000)	741	3.56
$[Cu(HB(3,5\text{-}Ph_2pz)_3)]_2$ (O_2)	Diamagnetic	542(1040), 355(18,000)	759	—
Oxy-Hc	Diamagnetic	580(1000), 340(20,000)	744–752	3.5–3.7
Oxy-Tyr	Diamagnetic	600(1200), 345(18,000)	755	~3.6

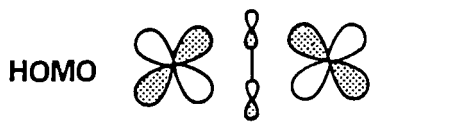
[a] Values in parentheses are ε (M^{-1} cm^{-1}).

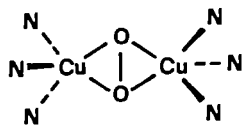
The structure of the Cu_2O_2 chromophore of oxy-Hc or oxy-Tyr has been the subject of extensive spectroscopic studies for quite some time. On the basis of EXAFS results, Cu—Cu separations were estimated to be 3.5–3.7 Å for oxy-Hc (*240, 241*), 3.5 Å for deoxy-Hc (*241, 242*), and 3.6 Å for oxy-Tyr (*243*). Recently, a single-crystal X-ray analysis of deoxy-Hc from lobster was successfully completed, and the Cu—Cu separation was determined to be 3.54 Å (*244, 245*). The ligand donors around each copper ion are identified as three histidyl nitrogen atoms. No other ligand donors (such as water) were found. The resonance Raman study using mixed isotopically labeled dioxygen clearly indicated that dioxygen is bound at the dinuclear copper site of Hc as peroxide in a symmetric fashion (*246*). These experimental results, together with the diamagnetic property, led to the generally accepted consensus that dioxygen is bound to the dicopper site in a *cis*-μ-1,2 structure with an unidentified endogenous bridging ligand (*202–204*). The endogenous ligand, which is the predominant mediator responsible for the strong magnetic interactions, was initially believed to be a tyrosine phenoxide. However, the X-ray crystal structure of deoxy-Hc excluded this possibility, and hydroxide is currently the most likely candidate (*247, 248*). Although there is no synthetic example of copper having such a structure, μ-OH-*cis*-μ-1,2-peroxo cobalt complexes are known (*249, 250*), supporting the proposal as reasonable. Furthermore, detailed spectroscopic studies on met-azide-Hc (*251, 253*), which is antiferromagnetic, were performed, leading to the conclusion that met-azide-Hc has a μ-RO-*cis*-μ-1,3-azido core structure. Because the azide ion favors a coordination structure similar to that of peroxide, the results strongly support the consensus. However, no direct experimen-

tal evidence for the existence of the endogenous ligand has been obtained.

The existence of an endogenous bridging ligand was proposed to account for the diamagnetism of oxy-Hc, because it was believed that magnetic coupling through peroxide alone was not sufficiently strong. However, it is now apparent that peroxide ion in a μ-$\eta^2:\eta^2$ mode can mediate a strong magnetic interaction, causing diamagnetism (*237*). The occurrence of the diamagnetism through a μ-$\eta^2:\eta^2$ peroxide was interpreted on the basis of extended Hückel calculations (*237*). The calculations were performed for a simplified model $\{[(NH_3)_3Cu]_2(O_2)\}^{2+}$ whose structural parameters were taken from the crystal structure of $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$. Figure 17 indicates the molecular orbitals for $[(NH_3)_3Cu]_2(O_2)^{2+}$. Each $(NH_3)_3Cu^{2+}$ fragment carries five frontier orbitals primarily made up of Cu 3*d* orbitals, which split in a three-below-two pattern. For the d^9 Cu^{2+} configuration, the singly occupied molecular orbital (SOMO) level is the Cu *yz* orbital with a slight admixture of *xz*. In the presence of the bridging O_2^{2-}, the out-of-phase combination of the $(NH_3)_3Cu^{2+}$ SOMO is destabilized through interaction with O_2 π_σ^*, whereas its in-phase counterpart remains nearly untouched. The strong antibonding nature between Cu *yz* and O_2 π_σ^* is evident in the lowest unoccupied molecular orbital (LUMO):







A

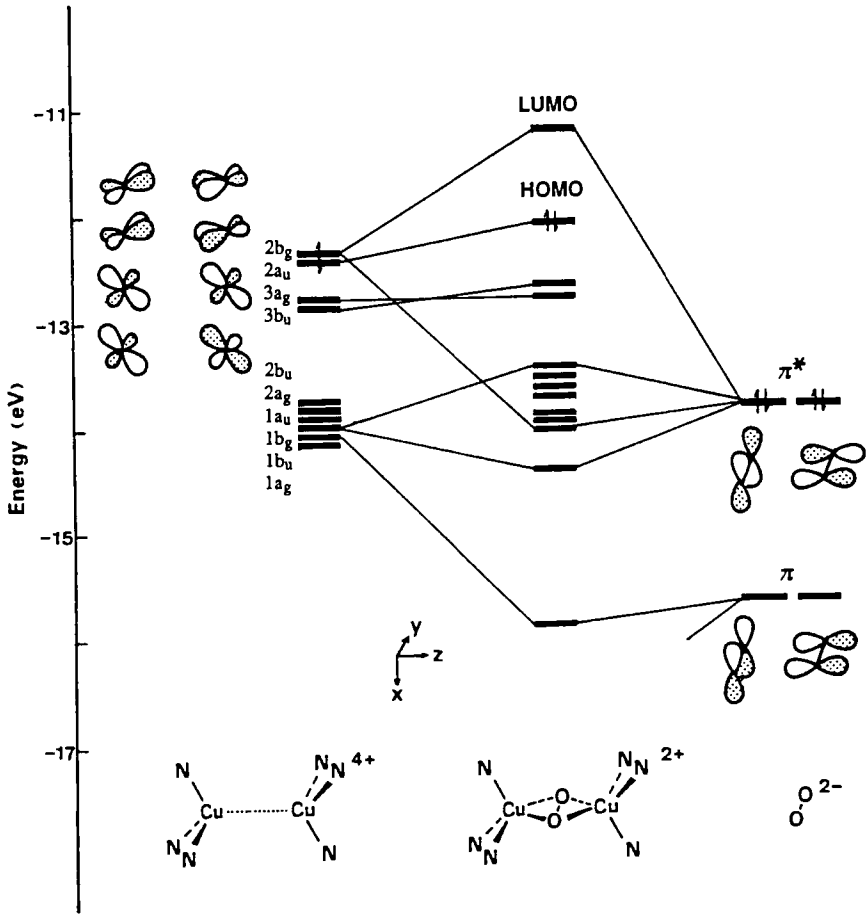


FIG. 17. Interaction diagram for the frontier orbitals of $[(NH_3)_3Cu]_2(O_2)^{2+}$. Two $(NH_3)_3Cu^{2+}$ fragments are combined and have a staggered (D_{3h}) geometry, and the $[(NH_3)_3Cu]_2^{4+}$ composite is mixed with O_2^{2-}.

The resultant large HOMO–LUMO gap of 0.883 eV accounts for the observed diamagnetism of the μ-$\eta^2:\eta^2$ peroxo complexes. More sophisticated MO calculations by SCF-Xα SW were carried out on $\{[(NH_3)_2Cu]_2O_2\}^{2+}$ (*254*). Although the model for the Cu fragment is different, the estimated HOMO–LUMO gap is also enormous, with $-2J = 5660\ cm^{-1}$, providing the theoretical support that a very strong magnetic interaction can be mediated by a μ-$\eta^2:\eta^2$ peroxide ion. Accordingly, given that the coordination of the peroxide in oxy-Hc is μ-$\eta^2:\eta^2$,

the diamagnetic property of oxy-Hc is understandable and the existence of the supposed endogenous bridging ligand is no longer necessary.

Structural comparisons of the $N_3Cu(O_2)CuN_3$ moiety in $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$ and in deoxy-Hc support this possibility favorably. The schematic view of the $N_3Cu\text{---}CuN_3$ moiety of deoxy-Hc is given in Fig. 18. The coordination structures of the two copper(I) ions are close to each other, consisting of two short Cu—N bonds and another distinctly elongated Cu—N bond. The four tightly bound nitrogens (Cu—N distances of 1.95–2.10 Å) together with the two coppers comprise a planar $N_2Cu\text{---}CuN_2$ frame. The apical nitrogens (Cu—N distances of 2.66 and 2.77 Å) coordinate from opposite sides of this plane. These structural features are very similar to the $N_3Cu\text{---}CuN_3$ moiety in $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$ as shown in Fig. 18, except for the shorter Cu—N_{apical} distances. Thus a peroxide ion would fit in the planar $N_2Cu\text{---}CuN_2$

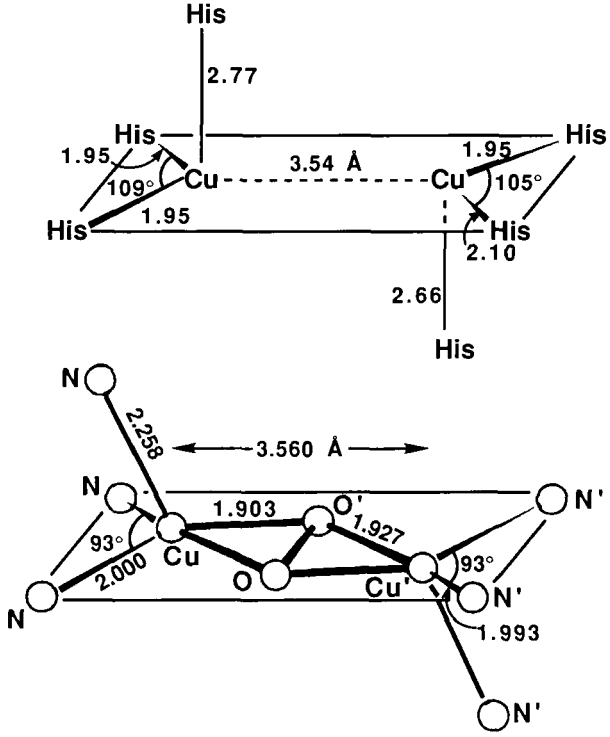


FIG. 18. Structural comparison of the $N_3Cu\text{–}CuN_3$ moiety of deoxy-Hc and the $\mu\text{-}\eta^2:\eta^2$ peroxo complex $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$.

frame in deoxy-Hc to form the $N_3Cu(\mu\text{-}\eta^2:\eta^2O_2)CuN_3$ chromophore in oxy-Hc without serious structural deformation.

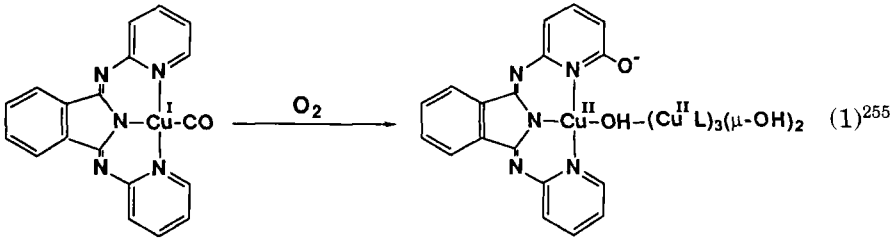
Both μ-1,2 and $\mu\text{-}\eta^2:\eta^2$ peroxo structures can account for the occurrence of two intense bands observed at 350 and 580 nm for oxy-Hc, because two distinct LMCT bands due to O_2^{2-} π_σ^* and O_2^{2-} π_v^* are predicted to split significantly. Detailed theoretical analyses of the $O_2^{2-} \rightarrow$ Cu(II) LMCT bands have been reported by Ross and Solomon (*254*). According to the analyses, the $\pi_\sigma^*-\pi_v^*$ split in $\mu\text{-}\eta^2:\eta^2$ is larger than that of *cis*-μ-1,2, suggesting that the $\mu\text{-}\eta^2:\eta^2$ structure is more likely as a model for oxy-Hc. The low O–O vibrational stretch of oxy-Hc was also interpreted in terms of the $\mu\text{-}\eta^2:\eta^2$ structure based on MO calculations (*254*), which indicated that O_2^{2-} σ^* can act as a π acid. Hence, the back-donation of the copper to weaken the O–O bond of the peroxide ion may result in an unusually low O–O frequency in the $\mu\text{-}\eta^2:\eta^2$ peroxo complex. In contrast, no clear account was addressed for the low O–O frequency of oxy-Hc, assuming the *cis*-μ-1,2 structure.

As mentioned above, both *cis*-μ-1,2 and $\mu\text{-}\eta^2:\eta^2$ remain vital candidates as the coordination mode of the peroxide in oxy-Hc and oxy-Tyr. The $\mu\text{-}\eta^2:\eta^2$ structure does seem, however, more likely because its particular structure gives a simpler and more straightforward account of all the unusual features of oxy-Hc. In order to distinguish these two possibilities conclusively, the synthesis and characterization of a *cis*-μ-1,2 peroxo complex with a hydroxide bridge presents a serious challenge for inorganic chemists. On the other hand, a single-crystal X-ray analysis for oxy-Hc is currently underway and may provide a definitive answer (*234*).

B. Reaction Aspects of μ-Peroxo Dinuclear Copper Complexes Relevant to Tyrosinase Catalysis

Although Tyr was reported as the first monooxygenase more than three decades ago (*207*), few of its mechanistic details have been revealed to date. Because oxy-Tyr apparently contains a Cu_2O_2 chromophore, the oxidation chemistry of synthetic μ-peroxo complexes should shed light on the catalytic mechanism of Tyr. A number of ligand oxidations are now known, wherein aerobic treatment of a copper(I) complex, mostly dinuclear, yields an isolatable dicopper(II) complex having an oxidized ligand. Since the first report by Gagne *et al.* (*255*) in 1979, oxidations including aromatic hydroxylations (*230, 256–260, 264, 275*), oxidative dehydrogenation of amines (*212*), and aliphatic hydroxylations (*214*) have been reported. Some typical reactions are

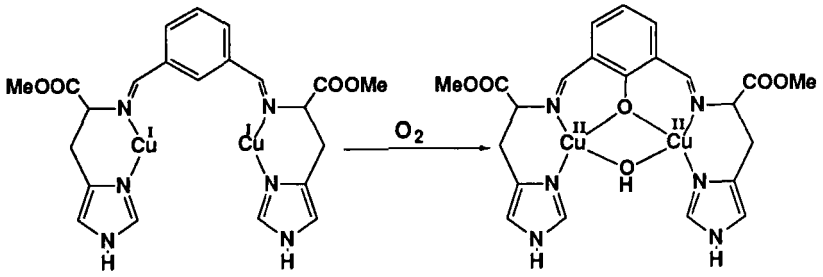
represented in Eqs. (1)–(5). In these reactions, μ-peroxo complexes are suggested as the most plausible intermediate, yet the involvement of such a species has not been proved experimentally, except in one case. In the reaction shown in Eq. (2), no transient intermediate was characterized. However, when a similar asymmetric ligand was employed (*225*), at low temperature under dioxygen, formation of a transient intermediate was noted; the absorption spectrum of this intermediate is similar to that of the related μ-peroxo dicopper(II) complexes that were characterized earlier (*218, 220*). Following initial work on the hydroxylation process [Eq. (5)], Ngwenya *et al.* prepared a complex with a related hexadentate macrocycle in which the phenyl group was replaced with furan (*231*). The dinuclear copper(I) complex reacts with dioxygen, but in this case, the furan moiety was not hydroxylated; instead, a four-electron product, the bis(hydroxo)dicopper(II) complex,



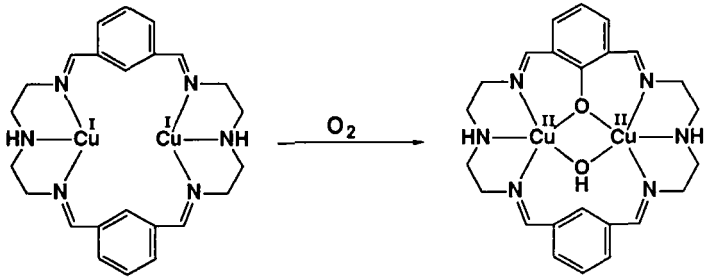
(1)[255]

(2)[256]

(3)[257]



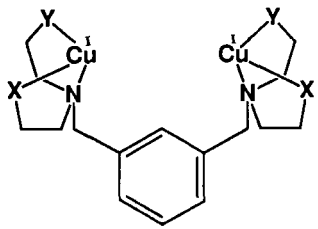
(4)[258,264]



(5)[259]

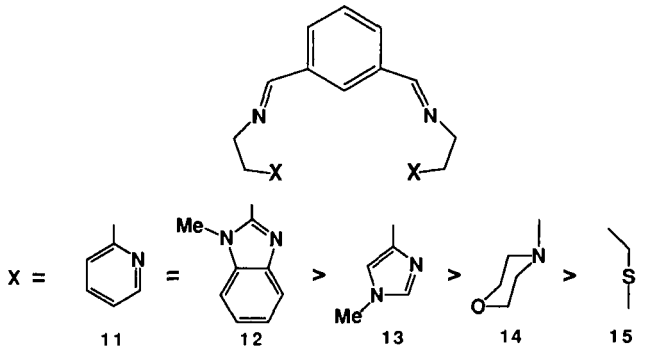
was formed. Observed during this reaction was formation of an intermediate exhibiting two strong bands at 360 and 550 nm. The transient species was identified as a μ-peroxo dicopper(II) complex on the basis of its spectroscopic similarity to that of $\{[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)\}$. In the other ligand oxidation reactions, even indirect evidence for involvement of a μ-peroxo intermediate was not provided, and the mechanistic details remain to be evaluated.

Sorrell *et al.* (*262, 263*) synthesized a series of dicopper(I) complexes in which the dinucleating ligand contains an *m*-xylyl spacer (**4–10**). In complex **4**, which is identical to the one utilized for the reaction given



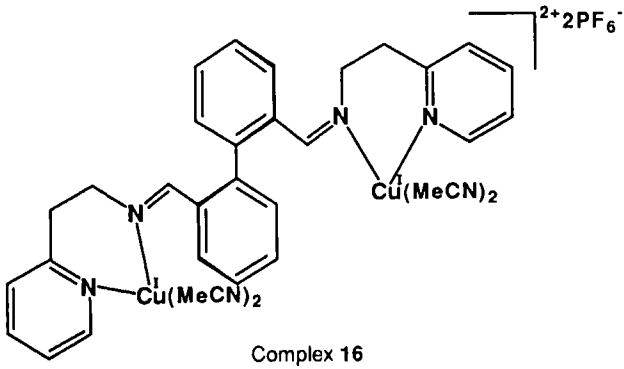
4 X=Y=Py **5** X=Y=Pz **6** X=Y=DMP **7** X=Y=MeIm
8 X=Py,Y=Pz **9** X=Py,Y=DMP **10** X=Py,Y=MeIm

by Eq. (2), hydroxylation of the aromatic ring was observed on dioxygen treatment. However, substitution of pyridyl with pyrazolyl or imidazolyl groups affected the oxidation chemistry drastically. Thus, complexes **5–10** are all inert as far as aromatic hydroxylation is concerned, and instead they undergo a four-electron reduction of dioxygen to give bis(hydroxo)dicopper(II) complexes. Sorrell *et al.* attempted to correlate the tendency for dioxygen binding and activation with ligand basicities and Cu(II)/Cu(I) potentials, but they did not address clearly the substitution effect. Casella *et al.* carried out similar experiments with the bis(imine) dinucleating ligands **11–15** (*265*). All dinuclear copper(I) complexes with these ligands undergo aromatic hydroxylation when exposed to dioxygen, as reported earlier by Gelling *et al.* (*260*). The rate of the reaction was found to be inversely correlated with the basicity of the substituted group.



Given the present level of mechanistic understanding, relevance of these ligand oxidations to Tyr catalysis is not certain. In particular, it is puzzling that none of these systems is effective for the oxidation of externally added substrates. Thus one cannot exclude the possibility that these intramolecular ligand oxidations proceed via some unknown activation pathway of the peroxo intermediate, or even via an intermediate other than the peroxo species. Other oxidation reactions that may be relevant to Tyr/or other copper monooxygenases are the formation of a μ-oxo dinuclear complex following anaerobic reaction of the copper(I) precursor with PhIO. Two such examples are known, and it was suggested that reactions proceed via an oxo copper(III) intermediate (*266, 267*). A related ligand oxidation of a copper(I) complex with PhIO was also noted (*268*). Valentine and co-workers investigated olefin epoxidations with PhIO catalyzed by copper complexes and found that dinuclear complexes are, in general, more effective than mononuclear complexes for

the reaction (*269, 270*). Finally, very recently Reglier *et al.* reported the catalytic oxidation of a phenol as a reaction that mimics Tyr (*271*). In the presence of **16** and triethylamine, 2,4-di-*t*-butylphenol is oxidized to the corresponding benzoquinone with turnovers ranging from 11 to 16 h^{-1}. It is of interest that the reaction undergoes another reaction pathway, in the absence of the amine, to give a radical coupled product of the phenol. The authors suggested that triethylamine deprotonates the phenol to generate a phenoxo μ-peroxo dicopper(II) intermediate. Unfortunately the spectroscopic characterization was not completed.



Complex **16**

In the heme-iron-containing monooxygenase, cytochrome *P*-450, protonation of a mononuclear peroxo iron complex is believed to be a key reaction step. Either the resultant hydroperoxo intermediate or the oxo ferryl porphyrin π-cation radical resulting from heterolysis of the O—O bond of the hydroperoxo intermediate is responsible for the oxo-transfer reactions, as previously described. Because oxy-Tyr contains a peroxide, the parallel mechanism accepted for cytochrome *P*-450 leads to a speculation that a similar protonation step may be involved in Tyr catalysis. Accordingly, Karlin *et al.* studied protonation and acylation of the phenoxo-bridged μ-peroxo complex $[Cu_2(XYL—O—)(O_2)]^{2+}$. The protonated species was characterized in detail and a dinuclear μ-1,1-hydroperoxo structure was proposed on the basis of EXAFS results (*272, 273*). The acylated product was crystallized and the structure was determined as a μ-1,1-acylperoxo dicopper(II) complex (*105*). Interestingly, both of these complexes oxidize PPh_3 to $OPPh_3$ instantaneously, whereas the starting μ-peroxo complex $[Cu(XYL—O—)(O_2)]^{2+}$ is inactive. However, both the hydroperoxo and acylperoxo complexes are not very reactive in terms of oxo-transfer reactions. The reaction with phenols was not described but they are ineffective for olefin epoxidation. The

negative results are consistent with the ineffectiveness of monomeric alkylperoxo and acylperoxo copper(II) complexes described in Section III,A. Therefore, the mechanism of Tyr, as well as other copper monooxygenases, should be distinctly different from that of cytochrome *P*-450. It is highly unlikely that the hydroperoxo intermediate or subsequent heterolytic cleavage of the O—O bond causes oxygen incorporation into the CH bond of the substrate.

As described in Section IV,A, it was suggested that the complex $[Cu_2(XYL—O—)(O_2)]^+$ adopts an asymmetric terminal coordination mode of the peroxide ion with a phenoxo bridge, thus the structure is different from oxy-Tyr. The reactivity of another structural type, the (*trans*-μ-1,2) μ-peroxo complex $[(Cu(TPA))_2(O_2)]^{2+}$, was also explored. The reaction patterns were essentially identical to those seen for $[Cu(XYL—O—)(O_2)]^{2+}$. On the other hand, another complex—$[Cu_2(N_4)(O_2)]^{2+}$ (the structure is a bent μ-η^2:η^2 or *cis*-μ-1,2)—exhibits some distinctive reaction patterns. For instance, the complex oxidizes PPh_3 to $OPPh_3$ quantitatively, whereas the other two complexes give the corresponding Cu(I)–PPh_3 adducts. Whereas $[Cu_2(N_4)(O_2)]^{2+}$ reacts with SO_2 as well as other μ-peroxo complexes to give a μ-sulfate dicopper(II) complex, it does not react with other electrophiles such as H^+, $RC(O)^+$, and CO_2. On the basis of these observations, it was demonstrated that the peroxide in $[Cu_2(N_4)(O_2)]^{2+}$ is nonbasic or electrophilic, whereas the other two are apparently nucleophilic (*101, 274*). It is of interest that among these three μ-peroxo complexes, $[Cu(N_4)(O_2)]^{2+}$ is most like oxy-Tyr in its physicochemical characteristics (*218, 221*). The electrophilic property was thus ascribed to the unique coordination mode of the peroxide, possibly a bent μ-η^2:η^2. But again, $[Cu(N_4((O_2)]^{2+}$ is ineffective for oxo-transfer reactions toward cyclohexene and phenol (*101*). Detailed mechanistic investigations on ligand hydroxylation of $[Cu_2(XYL)]^{2+}$ [given by Eq. (2)] and related complexes were recently accomplished (*276, 277*). When a methyl group was introduced at the 1-position on the aromatic ring in the ligand, migration of the methyl group was noted during the ligand oxidation. Because the pattern is similar to the NIH shift, Karlin *et al.* proposed a mechanism involving a direct electrophilic attack of the peroxide to the CH bond of the aromatic ring.

SCF-Xα calculations have indicated that the μ-η^2:η^2 peroxide is less negative than other structural peroxides (such as *trans*-μ-1,2 or *cis*-μ-1,2) because the π_σ^* donor interacts with the Cu *d* orbitals more strongly than with the others (*254*). In addition, the peroxide σ^* orbital can function as a π acid. Based on these theoretical predictions, Solomon and co-workers (*254, 278*) proposed the electrophilic reaction of the μ-η^2:η^2 peroxide in Tyr with phenol (Fig. 19). Here, the key feature is that the copper ion

binding of dioxygen orients the coordinated peroxide O—O bond so as to align its empty σ^* orbital with the arene substrate π–π orbital, facilitating electrophilic attack and O—O bond cleavage to give the product (*254*). Together with the experimental observations reported by Karlin and coworkers, this mechanism should be seriously considered. But one should keep in mind that most of the peroxo complexes, especially those of the late transition metal ions, are nucleophilic and not electrophilic, as discussed in Section III,A. MO calculations have indicated that μ-η^2:η^2 peroxide is more electrophilic than the other structural peroxide coordinated to copper, but there is no comparison with the experimentally reactive electrophilic peroxide, such as in *m*-CPBA. Therefore, the relatively electrophilic nature of μ-η^2:η^2 peroxide derived from the MO calculations does not necessarily mean that the peroxide in a synthetic complex or Tyr is electrophilic enough to exhibit the high oxo-transfer reactivity. The mechanism for ligand hydroxylation given by Nasir *et al.* includes electrophilic attack of the bent μ-η^2:η^2 peroxide on the arene ring (*277*). However, a number of questions should be addressed before accepting this mechanism. The mechanism is based on the assumption that the μ-peroxo intermediate formed possesses a structure and reactivity comparable to $[Cu_2(N_4)(O_2)]^{2+}$, which was demonstrated to have a bent μ-η^2:η^2 structure and to be electrophilic (*101, 220, 274*). As discussed already, however,

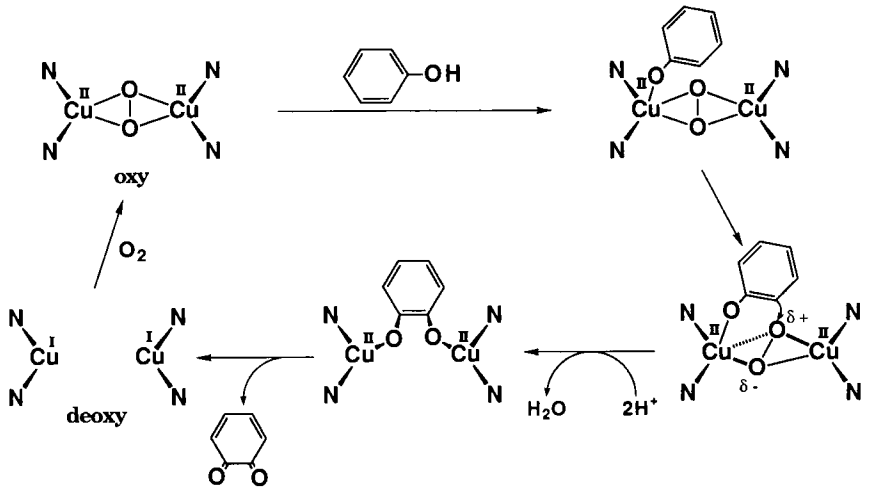


FIG. 19. The reaction mechanism for Tyr proposed by Solomon *et al.* (*254, 278*).

both aspects are not yet firmly established. The bent μ-η^2:η^2 structure has been emphasized strongly since the structural determination of $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$ was accomplished (*235*), but there is still a possibility that the complex contains another coordination mode of the peroxide, such as *cis*-μ-1,2. Moreover, $[Cu_2(N_4)(O_2)]^{2+}$ is ineffective for any other oxo-transfer reactions except PPh_3, thus it is not very electrophilic. Even if the hydroxylation proceeds via a bent μ-η^2:η^2 peroxo dicopper(II) intermediate similar to $[Cu_2(N_4)(O_2)]^{2+}$, a straight extension of the mechanism to the Try catalysis is debatable. Note that the absorption spectrum of $[Cu_2(N_4)(O_2)]^{2+}$ is distinctly different from that of oxy-Tyr or oxy-Hc—it exhibits a strong band at ~460 nm (*219*), which is not observed for oxy-Tyr and oxy-Hc. Moreover, the Cu—Cu separation (3.4 Å) of the complex is considerably shorter than the 3.6-Å separation estimated for oxy-Tyr and oxy-Hc. Therefore, it would appear that the coordination structure of the peroxide in oxy-Tyr or oxy-Hc differs significantly from that in $[Cu_2(N_4)(O_2)]^{2+}$, and there is no guarantee that the chemistry of the ligand hydroxylation parallels the catalytic mechanism of Tyr.

All of the mechanisms postulated for the ligand oxidations discussed above do not involve radical intermediates. However, considering the radical-type mechanism accepted for another copper-containing monooxygenase, DBH, the possibility that Tyr also catalyzes oxidation in a radical manner should be taken into account. The preclusion of the radical mechanism for Tyr seems to reflect the general consensus that Tyr gives only a benzoquinone as the product of monophenol oxidation, whereas diphenoquinone, an apparent radical coupling product, is not produced. *It should be emphasized that this is not true.* Recently, we (*279*) and Pandey *et al.* (*280*) independently found that Tyr gave rise to a radical coupling product when hindered phenols were employed as substrates. For instance, 2,6-dimethylphenol and 2,4-di-*t*-butylphenol are oxidized to the corresponding diphenoquinones mainly by Tyr. Thus, at least under certain reaction conditions, oxidations catalyzed by Tyr proceed via a radical mechanism.

Because the spectroscopic features of μ-η^2:η^2 peroxo complexes $[Cu(HB(3,5\text{-}R_2pz)_3)]_2(O_2)$ are in excellent accord with those of oxy-Tyr, the reaction chemistry of the complexes should shed light on the catalytic mechanism of Tyr directly. Accordingly, the reactivity of $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$ was explored in detail (*281*). Reactions of the complex with CO and PPh_3 cause fast displacements, as has been reported for several other μ-peroxo complexes, resulting in formation of Cu(I) adducts $Cu(CO)(HB(3,5\text{-}Me_2pz)_3)$ and $Cu(PPh_3)(HB(3,5\text{-}Me_2pz)_3)$,

respectively. The complex is not thermally stable and at room temperature, in the absence of a substrate, spontaneously decomposes to a μ-oxo complex $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2O$. The rate of consumption of the μ-peroxo complex was first order with respect to the concentration of the complex. These results are interpreted in terms of the mechanism given in Fig. 20. A notable feature here is the homolysis of the O—O bond of the peroxide, which generates a copper(II) oxygen anion radical. The homolysis of O—O bond is well known for μ-peroxo iron(III) complexes (*282, 283*), but no sound experimental evidence has been provided for copper complexes. In the absence of a substrate, the copper(II) oxygen anion radical couples with a copper(I) species, which is in equilibrium with the μ-$\eta^2:\eta^2$ complex in solution, resulting in the μ-oxo product. On the other hand, the copper(II) oxygen anion radical functions as a weak hydrogen acceptor so as to react with cyclohexene to generate a cyclohexyl radical. Accordingly, in the presence of dioxygen, the μ-$\eta^2:\eta^2$ peroxo complex initiates a classical type of radical chain reaction via the homolysis of the O—O bond, to give rise to oxidized products such 2-cyclohexene-1-one. In the absence of dioxygen, the formed radical ends up by reacting with the solvent, thus 2-cyclohexene-1-chloride is obtained. The products from the aerobic or anaerobic oxidation of hindered phenols with $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$ are summarized in Table X. Under both reaction conditions, the main products are diphenoquinones, and benzoquinones are formed as minor products under

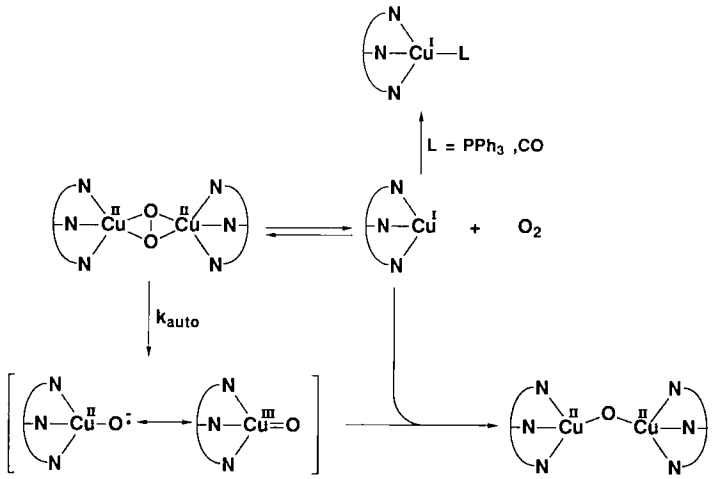
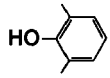
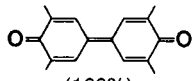
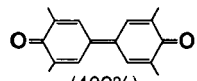
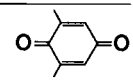
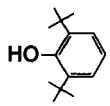
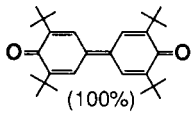
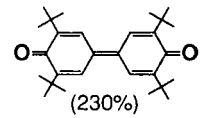
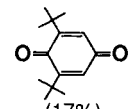
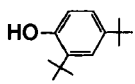
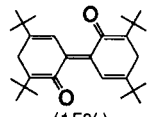
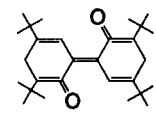
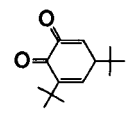


FIG. 20. Homolytic O—O bond cleavage of the peroxide ion in $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$ (*281*).

TABLE X

AEROBIC AND ANAEROBIC REACTIONS OF PHENOLS WITH $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$[a]

Phenol	Argon	Dioxygen (1 atm)	
	 (100%)	 (400%)	 (15%)
	 (100%)	 (230%)	 (17%)
	 (15%)	 (1700%)	 (18%)

[a] Reaction conditions (*281*): 10 mg of $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$, 100 mg of the phenol in 1.0 ml of $CHCl_3$, for 2 h at room temperature.

aerobic conditions. Because diphenoquinones are apparently radical reaction products, the formation of such products may be received as negative evidence for a Tyr mimic. Nevertheless, when the substrates are hindered like those used in the model reactions, Tyr gives primarily radical coupling products (*279, 280*), supporting the close relation of the reaction chemistry of the μ-η^2:η^2 peroxo complex to Tyr catalysis. In order to gain a more detailed mechanistic insight, kinetic investigations of the anaerobic reaction between the peroxo complex and 2,6-dimethylphenol were carried out. The rates of consumption of the peroxo complex and of formation of the diphenoquinone were determined, respectively, as follows.

$$-d[Cu_2O_2]/dt = (k_{auto} + k_1[DMP])[Cu_2O_2]$$

$$\{d[DPQ]/dt\}_{max} = k_2[Cu_2O_2]_0$$

In these equations, Cu_2O_2, DMP, and DPQ denote $[Cu(HB(3,5\text{-}Me_2pz)_3)]_2(O_2)$, 2,6-dimethylphenol, and the diphenoquinone, respectively. The rate expressions clearly indicate the occurrence of two competitive reaction pathways ascribed to (1) the spontaneous O—O bond homolysis of the μ-η^2:η^2 peroxo complex and (2) acid/base replacement of the peroxo group with phenoxide to give a phenoxo copper(II) interme-

diate. Diphenoquinone is formed by the coupling of the phenoxy radical, which is yielded either by H· abstraction from phenol with a copper(II) oxygen anion radical generated by pathway 1 (k_1, Fig. 21) or by homolysis of the Cu—O bond of the phenoxo copper(II) intermediate formed by pathway 2 (k_2). The formation of the phenoxo intermediate was supported by an independent experiment in which the low-temperature reaction of $[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$ with 2 equivalents of *p*-fluorophenol gave an isolatable phenoxo complex $Cu(OC_6H_4\text{-}p\text{-}F)(HB(3,5\text{-}iPr_2pz)_3)$ (*284*), the structure of which was determined by X-ray crystallography. This clearly indicates that the $\mu\text{-}\eta^2:\eta^2$ peroxo complex is nucleophilic, as expected for peroxo complexes of late transition metals. The reaction chemistry of the isolated phenoxo complex supports the reaction mechanism illustrated in Fig. 21 (*284*).

Pathway 1 may not be relevant to Tyr catalysis: homolytic cleavage of the peroxide O—O bond is presumably not the case in Tyr because the two copper ions are held by the protein chains so as to reverse the O—O bond cleavage. But pathway 2 can occur in Tyr as well. A proposal for the mechanism of Tyr is illustrated in Fig. 22. In Tyr, only one phenol can be inserted into the substrate-binding pocket in the protein,

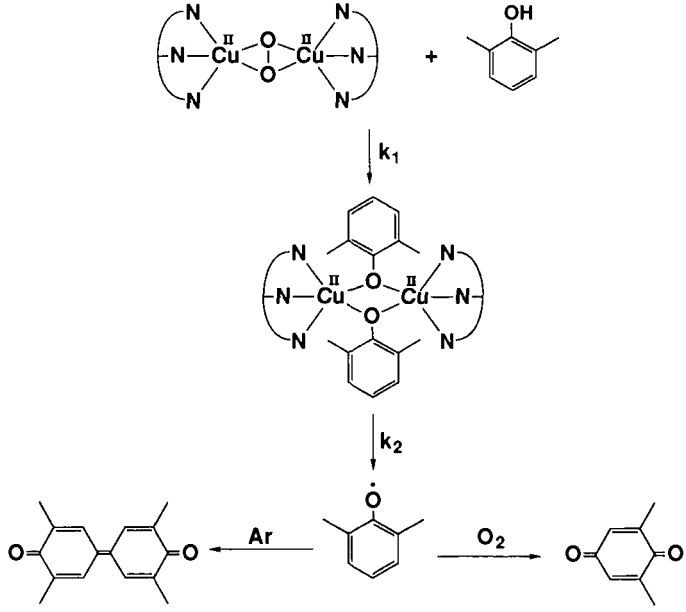


FIG. 21. The mechanism of diphenoquinone formation via a phenoxo intermediate (*281*).

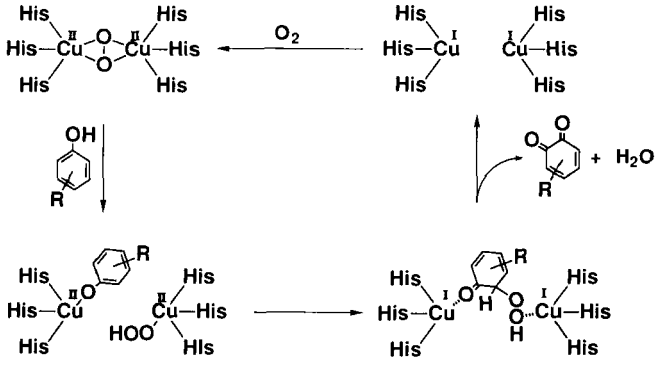
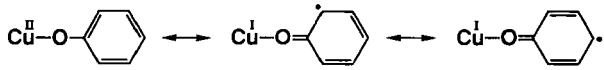


FIG. 22. Proposed mechanism for Tyr catalysis based on the reaction chemistry of μ-η^2:η^2 peroxo complexes.

so the initial intermediate formed is most likely a hydroperoxo–phenoxo intermediate. In the light of reaction patterns observed for the μ-η^2:η^2 complex and the alkylperoxo complexes, which serve as models for the hydroperoxo moiety, the hydroperoxide should undergo a radical reaction. On the other hand, the ortho/para positions of the phenoxo copper(II) moiety are accessible as radical species, because of the contribution of the following radical structures:



Therefore, the radical coupling occurs easily to give a hydroperoxybenzoquinone intermediate, which is converted to benzoquinone, completing the catalytic cycle. When the phenol is hindered, the inner-sphere coupling between the phenoxide and hydroperoxide does not proceed effectively and homolysis of the Cu(II)—OPh bond occurs. The resultant phenoxy radicals couple together to give the diphenoquinone.

V. Synthetic Models for Type IV Copper

The multicopper oxidases (laccase, ascorbate oxidase, and ceruloplasmin) catalyze a four-electron reduction of dioxygen to water (*285–287*). Consistent with the four-electron stoichiometry, the enzymes contain four copper ions. One of the copper ions is type I, causing an intensely blue color of the proteins, thus the enzymes of this family are referred to as blue oxidases. They also contain a monomeric copper site that exhibits normal spectroscopic features, whereas the other two copper

ions are magnetically coupled. Extensive spectroscopic investigations on laccase (*288–294*) led to the conclusion that the normal copper ion plays an important role in exogenous ligand binding. In fact, it is positioned close to the coupled binuclear copper site, forming a trinuclear-like copper site. Recent X-ray crystal work on ascorbate oxidase has demonstrated that this is the case (*45, 295*). Figure 23 shows the schematic view of the trinuclear site, referred as type IV copper in this review. Here, Cu(1) and Cu(2) are the magnetically coupled copper(II) ions. Both Cu(1) and Cu(2) are coordinated by three histidyl nitrogens, whereas Cu(3), the normal copper(II) ion, is coordinated to two histidyl nitrogens. One hydroxide or water coordinated to Cu(3) has also been suggested. The Cu(1)—Cu(2) separation is 3.4 Å, and the Cu(1)—Cu(3) and Cu(2)—Cu(3) separations are comparable, ~4.0 Å. Because no antiferromagnetic interaction between Cu(3) and the coupled dinuclear copper site is detectable, the existence of a bridging ligand between these coppers is highly unlikely, whereas the existence of three ligand donors around Cu(3) seems insufficient to construct a stable ligand field for the copper(II) ion. There may also be additional water or some other ligand. On the other hand, some bridging ligand(s), possibly OH^-, should exist between Cu(1) and Cu(2), which accounts for the strong magnetic coupling between the two copper(II) ions. Despite the remarkably similar structure of the dinuclear copper unit in the type IV site in Hc, the paired copper ions in the type IV site do not appear to bind dioxygen; rather dioxygen seems to be bound between one of the coupled copper ions and Cu(3). Very recently (*296*), a carefully controlled reaction of dioxygen at the type IV site of laccase was explored (in this experiment, the type I copper was replaced by Hg^{2+} for spectroscopic innocence). A peroxide-like intermediate was detected for the first time; it gave characteristic absorption bands at 340 and 470 nm, presumably due to $O_2^{2-} \rightarrow$ Cu(II) LMCT bands. Because the spectrum resembles

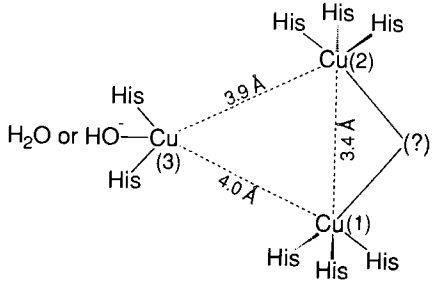


FIG. 23. Structure of the type IV copper site in ascorbate oxidase (*45*).

that of the μ-1,1-hydroperoxo dicopper(II) complex (*229, 273*), the dioxygen adduct was suggested to be a hydroperoxide bridging in the μ-1,1 mode between the type II-like copper and one of the coupled copper ions at the type IV site.

The unique structural motif of the type IV copper site opens a new frontier in synthetic bioinorganic chemistry. A number of trinuclear copper complexes have been synthesized with multidentate N ligands and bridging hydroxides; even complexes having a Cu_3OH unit are known (*297–300*). Studies directed at understanding the magnetic properties and dioxygen activation mechanism of type IV copper have just been initiated (*301–303*).

VI. Abbreviations

[14]aneS_4	1,4,8,11-Tetrathiacyclotetradecane
MAH	*N*-Mercaptoacetyl-L-histidine
MAHH	*N*-Mercaptoacetyl-DL-histidyl-DL-histidine
SIm	1-Methyl-2-mercaptoimidazole
ITPDTP	Imidotetraphenyldithiodiphosphino-*S,S'*
HB(3,5-Me_2pz)$_3$	Hydrotris(3,5-dimethyl-1-pyrazolyl)borate
HB(3,5-Me_2pz)$_2$(SC_7H_7)	Hydrobis(3,5-dimethyl-1-pyrazolyl)(*S-p*-toluenethiolato)borate
PMMI	4-(*n*-Propylmercaptomethyl)imidazole
tet b	rac-5,7,7,12,14,14-Hexamethyl-1,4,8,11-tetraazocyclotetradecane
L_1	3,9-Dimethyl-4,8-diazaundeca-3,8-diene-2,10-dione
cyclam	1,4,8,11-Tetraazacyclotetradecane
cyclops	1,1-Difluoro-4,5,11,12-tetramethyl-3,6,10,13-tetraaza-1-bora-2,14-dioxacyclotetradeca-3,5,10,12-tetraenate
L_2	2,2'-Bis(1-phenyl-3-methyl-5-*t*-butylthiopyrazol-4-ylmethyleneamino)biphenyl
bpy	2,2'-Bipyridine
PC	Pterin-6-carboxylate
ethp	2-Ethylthiopterin
phen	1,10-Phenanthroline
HB(3,5-iPr_2pz)$_3$	Hydrotris(3,5-diisopropyl-1-pyrazolyl)borate
HB(3,5-Ph_2pz)$_3$	Hydrotris(3,5-diphenyl-1-pyrazolyl)borate
bpim	4,5-Bis{[(2-(2-pyridyl)ethyl)imino]methyl}imidazolate
TMDT	1,1,7,7-Tetramethyldiethylenetriamine
ImH	Imidazole
Im	Imidazolate
L_{30}	The 30-membered macrocycle prepared from 2,6-diacetylpyridine and 3,6-dioxaoctane-1,8-diamine
L'_{30}	The 30-membered macrocycle prepared from 2,6-diformylpyridine and 3,6-dioxaoctane-1,8-diamine
L_{24}	The 24-membered macrocycle prepared from 2,6-diacetylpyridine and 3-thiapentane-1,5-diamine

pmdt	*N,N,N′,N″,N″*-Pentamethyldiethylenetriamine
2-MeIm	2-Methylimidazolate
A	1,4,7,13,16,19-Hexaaza-10,22-dioxacyclotetracosane
L_3	The macrocycle prepared from 2,6-diacetylpyridine and *m*-xylylenediamine using $[Cu(\mu\text{-Im})Cu]^{3+}$ as a template
AE	7-Amino-4-methyl-5-aza-3-hepten-2-onato
tren	Tris(2-aminoethyl)amine
$C_{18}H_{18}N_6$	The 14-membered macrocycle prepared from 2,6-diacetylpyridine and hydrazine
bipym	2,2′-Bipyrimidine
acac	Acetylacetone
(P)-(N_4)	α,α,α,α-Tetrakis(*o*-nicotinamidophenyl)porphyrin
FF	*N,N*-Bis(5-(*o*-phenyl)-10,15,20-triphenylporphyrin)urea
TPP	Tetraphenylporphyrin
IMDH	2,6-Bis[1-(2-imidazol-4-ylethlimino)ethyl]pyridine
protopor	3,7,12,17-Tetramethyl-8,13-divinylporphyrin-2,18-dipropionic acid
PS	1,2-Bis(dimethylamino)naphthalene
$(fsa)_2$en	*N,N′*-Bis(2-hydroxy-3-carboxybenzylidene)-1,2-diaminoethane
Salpen	*N,N′*-Disalicylidene-2-methyl-4-(2-hydroxy-5-methylphenyl)-1,2-butanediamine
UroTPP	5-{*o*-[4-Imidazolyl)vinyleneamido]phenyl}-10,15,20-triphenylporphyrin
dap	2,6-Diacetylpyridine
Py	Pyridine
TMP	Tetra-*p*-methoxyphenylporphyrin
TPPS	Tetra-*p*-sulfophenylporphyrin
GGH	Glycylglycyl-L-histidine-*N*-methylamide
$(prp)_2$en	*N,N′*-Ethylenebis(2-hydroxypropiophenoneimine)
hfa	Hexafluoroacetylacetone
aib_3	The tripeptide of α-aminoisobutyric acid
MNT	*cis*-1,2-Dicyanoethylene-1,2-dithiolato
Imid	*N*-(4-Imidazolylmethylidyneimine)-*N′*-(2-hydroxy-5-chlorobenzyliphenoneimine)phenylene
TBA	Tetra-*n*-butylammonium
P-Cl_4TPP	5,10,15,20-Tetra-*p*-chlorophenylporphyrin
L_4	1,4-Bis(1-oxa-4,10-dithia-7-azacyclododecan-7-ylmethyl)benzene
cyb	Bis[*N,N′*-bis(2-benzimidazolylmethyl)amino)methylcyclohexane
TEEN	*N,N,N′,N′*-Tetraethylenediamine
XYL—O—	α,α′-Bis[*N,N*-bis(2-pyridylethyl)amino]-2-olate
N_4	*N,N,N′,N′*-Tetrakis(2-pyridylmethyl)-1,4-diaminobutane
N_3OR	*N,N,N′,N′*-Tetrakis(2-pyridylmethyl)-1,3-diaminopropyl-2-biphenylcarboxylate
XYL-F	α-Bis[*N,N*-bis(2-pyridylethyl)amino]-2-fluoro-*m*-xylene
UN	α-Bis[*N,N*-bis(2-pyridylethyl)amino]-*N,N*-bis(2-pyridylethyl)-*m*-toluidine
TPA	Tris[(2-pyridyl)methyl]amine
L-im	1,2-Dimethylimidazole

tpmc	1,4,8,11-Tetrakis(2′-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane
Py_4N_6O	2,6-Bis{[bis(2-(2-pyridyl)ethyl)amino]methyl}-*p*-cresol
$(Py)_2(Pz)_2N_6O$	2,6-Bis{[(2-pyrazolyl)ethyl][2-(2′-pyridyl)ethyl]aminomethyl}-*p*-cresol
$(Py)_2(DMP)_2N_6O$	2,6-Bis{[[2-(3,5-dimethyl-1-pyrazolyl)ethyl][2-(2-pyridyl)ethyl]amino]methyl}-*p*-cresol
$Bz(NMI)_2N$	*N*,*N*-Bis[2-(1′-methyl-2′-imidazolyl)ethyl]-*N*-benzylamine
FD_2DIEN_2	3,6,9,16,19,22-Hexaazatricyclo[22.2.1.1.12.14]-octacosa-1(26),2,9,11,13,15,22,24-octane

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NOTE ADDED IN PROOF. The X-ray structure of Limulus oxyhemocyanin at 2.4 Å resolution reported by K. A. Magnus (Symposium "Copper Coordination Chemistry: Bioinorganic Perspectives" Baltimore, August, 1992) indicated the μ-η^2:η^2 coordination mode of the peroxide without an endogenous bridging ligand.