Dalton Perspectives

μ-η^2:η^2-Peroxide in Biological Systems

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Transition metal–dioxygen complexes have attracted the attention of inorganic chemists for quite some time because of their diversity in terms of structure, spectral features and reactivity. Dioxygen complexes play key roles not only in a variety of practical catalytic oxidations but also in the physiologically indispensable metabolism of dioxygen. Some excellent review articles dealing with the structure and reactivity of transition metal–dioxygen complexes are available, to which interested readers are directed.[1]

Among these dioxygen complexes, μ-peroxo dinuclear complexes represent a well known family which can be classified into five types on the basis of their co-ordination modes as shown in Fig. 1. The first two (terminal and μ-1,1) are unsymmetric and few examples have been reported to date. Those most commonly observed, *trans*-μ-1,2 and *cis*-μ-1,2, are isomeric if there is no bridging ligand present other than peroxide. From the point of view of thermodynamics the *trans* structure is generally the more stable, but in the presence of an additional bridging ligand the *cis*-μ-1,2 mode is adopted. A number of structurally characterized examples of these structural types, particularly of cobalt complexes, have been reported.[2] The existence of the last structural type, designated as μ-η^2:η^2, however, has been debated, although crystal structures of μ-η^2:η^2-peroxo complexes of f-block elements (La[3] and U[4]) have been determined, and such structures were proposed for complexes of V,[5] Co[6] and Rh.[7] In 1989, the first crystal structure of a transition-metal μ-η^2:η^2-peroxo complex, $[\{Cu[HB(3,5\text{-}Pr^i_2C_3HN_2)_3]\}_2(O_2)]$ [$HB(3,5\text{-}Pr^i_2C_3HN_2)_3$ = hydrotris(3,5-diisopropylpyrazol-1-yl)borate], was reported.[8] Because of its unusual and fascinating structure, the structure determination opened the door to a new frontier of inorganic chemistry. Subsequently, the existence of a bent μ-η^2:η^2-peroxide was confirmed by X-ray crystallography in a vanadium complex.[9] The physicochemical characterization of the μ-η^2:η^2-peroxo copper complex also made an impact on bioinorganic chemistry,[10] since its very unusual spectral features provided new insight into the structure of dioxygen binding in the naturally occurring copper proteins haemocyanin and tyrosinase, both of which contain a dicopper site which binds/or activates dioxygen as a peroxide.[11,12]

This article presents a perspective on the synthesis, structure and reactivity of the μ-η^2:η^2-peroxo copper complex and its biological relevance to haemocyanin and tyrosinase, together with a brief discussion on the possible involvement of this particular structural type of peroxide in other biological systems.

Background

Haemocyanin is a ubiquitous dioxygen carrier for invertebrates. It contains a dinuclear copper site to which dioxygen can be bound as peroxide. A study by resonance-Raman spectroscopy with mixed labelled dioxygen established that the peroxide co-ordination was symmetric,[13] and thus the co-ordination mode adopted fell into three possible types, *i.e.* *trans*-μ-1,2, *cis*-μ-1,2 or

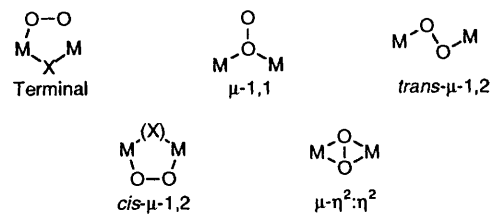
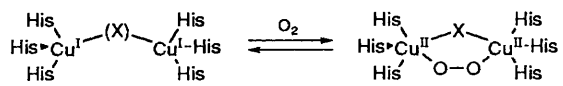


Fig. 1 Co-ordination modes of peroxide adopted in dinuclear complexes



Scheme 1 His = histidine

μ-η^2:η^2, although μ-η^2:η^2 had not been considered seriously before 1989 because of the lack of a structurally characterized complex. Whereas the two copper ions are divalent in the dioxygen binding state (so called oxyhaemocyanin), oxyhaemocyanin is EPR silent. It is in fact, diamagnetic at room temperature, suggesting an enormously strong antiferromagnetic coupling ($-2J > 600$ cm^{-1}) between the two copper(II) ions. Furthermore, instead of d–d bands normally observed at 600–700 nm for copper(II) complexes, oxyhaemocyanin exhibits two intense absorptions at *ca.* 350 (ε *ca.* 20 000) and *ca.* 580 nm (*ca.* 1000 dm^3 mol^{-1} cm^{-1}), both attributable to a $O_2^{2-} \longrightarrow Cu^{II}$ ligand-to-metal charge-transfer (l.m.c.t.) band. The occurrence of the diamagnetism and the unusual absorption spectrum are the focus of much interest, while other intriguing features associated with the Cu^{II}–O_2^{2-}–Cu^{II} chromophore include the abnormally low ν(O–O) wavenumber (*ca.* 750 cm^{-1}) and a characteristic band at *ca.* 450 nm detected only in the CD spectrum. In the generally accepted scenario, the existence of an endogenous bridging ligand, responsible for the strong magnetic interaction, has been suggested. Based on this interpretation, dioxygen is believed to be bound at the dicopper site in oxyhaemocyanin in a *cis*-co-ordination mode as illustrated in Scheme 1.

As a most plausible candidate for the endogenous bridging ligand, a phenoxide from tyrosine was proposed, yet this cannot be the case since the 3.2 Å resolution X-ray analysis of the reduced state haemocyanin (deoxyhaemocyanin) from a lobster established that there is no amino acid residue within 6 Å of the dicopper site where each copper(I) ion is co-ordinated to three histidines.[14] Thus, another small ligand, most probably hydroxide, is currently accepted as being the endogenous bridging ligand.

Given the co-ordination mode of the peroxide as *cis*-μ-1,2, the characteristic two intense absorption bands observed for oxyhaemocyanin can be interpreted qualitatively. When peroxide bridges between two copper(II) ions in this fashion, the π^* orbital of the peroxide splits depending upon the orientation of

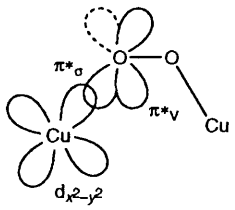


Fig. 2 Orientation of π* orbital of peroxide with respect to copper(II)

the π* lobe towards the plane comprised of the two copper(II) ions and the peroxide (see Fig. 2). Thus, π^*_σ is oriented along the Cu–O bond, while π^*_v is perpendicular. The $O_2^{2-} \longrightarrow Cu^{II}$ l.m.c.t. band is thus predicted to split into two which are referred to as the $O_2^{2-}(\pi^*_\sigma) \longrightarrow Cu^{II}$ and $O_2^{2-}(\pi^*_v) \longrightarrow Cu^{II}$ l.m.c.t. bands, respectively.

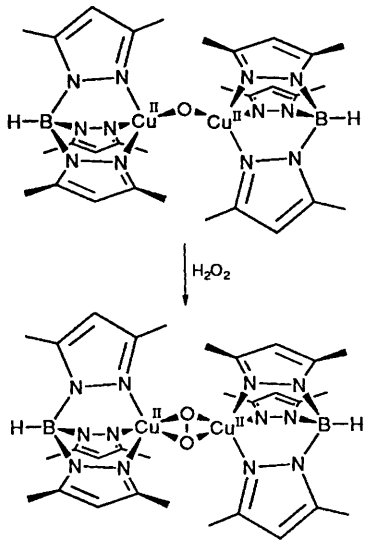
Support for the *cis*-μ-1,2 co-ordination of the peroxide with an endogenous ligand lies mainly in the detailed spectroscopic studies performed by Solomon[15] on met-azide haemocyanin, which is prepared by azide ion treatment of oxyhaemocyanin. Because met-azide haemocyanin is EPR silent as well as oxyhaemocyanin, it was suggested that simple ligand displacement between the peroxide and the azide occurs with the preservation of the endogenous briding ligand, which causes the strongly antiferromagnetic property.[15] The azide ion has a π^{nb} highest occupied molecular orbital (HOMO) which is very similar to the π* valence level of peroxide. In analogy to peroxide, π^{nb} splits into $\pi^{nb}{}_\sigma$ and $\pi^{nb}{}_v$ when an azide bridges between two copper(II) ions. Whereas the $\pi^{nb}{}_v \longrightarrow Cu^{II}$ l.m.c.t. band is too weak to be observed, the $\pi^{nb}{}_\sigma \longrightarrow Cu^{II}$ transition appears as an intense optical band, which splits further into two bands when the azide bridging mode is *cis*-μ-1,3, as a result of the transition dipole coupling.[15] A detailed comparison of the absorption spectrum of met-azide haemocyanin with those of a series of dicopper azide model complexes led to the conclusion that the azide ion in met-azide haemocyanin adopts a *cis*-μ-1,3 co-ordination mode with an endogenous bridging ligand, most likely hydroxide.[16] Since the azide ion favours the same co-ordination mode as peroxide, this strongly supports the hypothesis that the peroxide co-ordination mode in oxyhaemocyanin is *cis*-μ-1,2 as indicated in Scheme 1. The possibility that the *trans*-μ-1,2 co-ordination mode is adopted is highly unlikely because the properties of a model complex, $[\{Cu(tpa)\}_2(O_2)]^{2+}$ {tpa = tris[(2-pyridyl)methyl]amine}, whose structure has been determined by X-ray crystallography, do not fit with the characteristics known for oxyhaemocyanin [Cu • • • Cu separation, 4.36 Å; UV/VIS bands at 440 (ε 2000), 525 (11 500), 590 (7600), 1035 nm (160 $dm^3\ mol^{-1}\ cm^{-1}$); ν(O–O) at 834 cm^{-1}].[17]

Tyrosinase is a monooxygenase that catalyses the oxidation of tyrosine to the corresponding benzoquinone, which is an indispensable oxidation step for melanin biosynthesis. The active site of tyrosinase also consists of a pair of copper ions and the peroxide binding state is known to exhibit extremely similar characteristics to those of oxyhaemocyanin. Hence, it is reasonable to suggest that the peroxide co-ordination mode in tyrosinase is the same as that in oxyhaemocyanin.

Synthesis of the μ-η^2:η^2-Peroxo Dicopper Complex

The striking magnetic and spectral characteristics of oxyhaemocyanin have fascinated many inorganic chemists, and the synthesis and characterization of a μ-peroxo dicopper(II) complex modelling the active site of oxyhaemocyanin has been a long-standing challenge in bioinorganic chemistry.[1c,18] Despite extensive efforts, however, no complex which can closely mimic the physicochemical characteristics of oxyhaemocyanin had been synthesised.

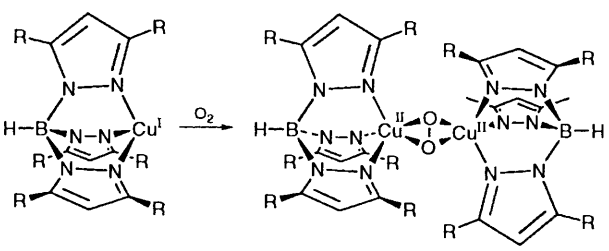
However in 1988, we reported[19] the preparation of a μ-peroxo dicopper complex from the reaction between a μ-oxo complex of the tripodal nitrogen ligand $HB(3,5\text{-}Me_2C_3HN_2)_3$



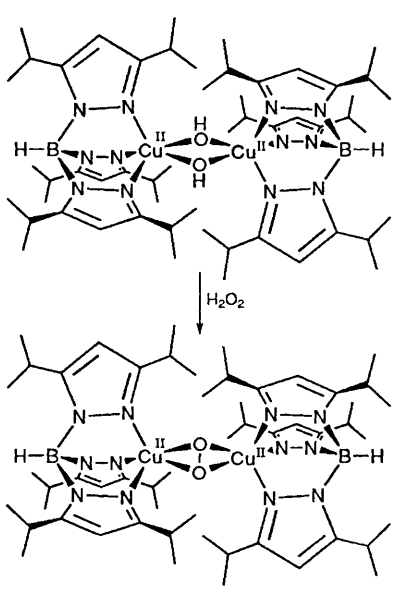
(1)

and H_2O_2 as shown in equation (1). The complex bears many similarities to those of oxyhaemocyanin in its magnetism, electronic and Raman spectroscopic characterisation as summarized in Table 1. While its crystal structure was not determined, field-desorption MS supported a dinuclear structure bridged by one peroxide and thus the complex was formulated as $[\{Cu[HB(3,5\text{-}Me_2C_3HN_2)_3]\}_2(O_2)]$ **1**.[19]

Subsequently, with two other more hindered hydrotris(pyrazolyl)borate ligands employed to enhance the stability and crystallinity, the analogous μ-peroxo complexes $[\{Cu[HB(3,5\text{-}R_2C_3HN_2)_3]\}_2(O_2)]$ (R = Ph **2** or Pr^i **3**) were prepared either by direct dioxygen addition to a monomeric copper(I) precursor [equation (2)] or by H_2O_2 treatment of a di-μ-hydroxo dicopper(II) complex [equation (3)].[8,10b] Again, both complexes



(2)



(3)

Table 1 Physicochemical properties of μ-η^2:η^2-peroxo complexes and oxyhaemocyanin and oxytyrosinase

Complex	Magnetic property	λ/nm (ε/dm^3 mol^{-1} cm^{-1})	ν(O–O)/cm^{-1}	Cu · · · Cu/Å
[{Cu[HB(3,5-$Me_2C_3HN_2)_3$]}$_2$(O_2)]	Diamagnetic	530 (840), 338 (20 800)	731	—
[{Cu[HB(3,5-$Ph_2C_3HN_2)_3$]}$_2$(O_2)]	Diamagnetic	542 (1040), 355 (18 000)	759	—
[{Cu[HB(3,5-$Pr^i_2C_3HN_2)_3$]}$_2$(O_2)]	Diamagnetic	551 (790), 349 (21 000)	741	3.56
Oxyhaemocyanin	Diamagnetic	580 (1000), 340 (20 000)	744–752	3.5–3.7
Oxytyrosinase	Diamagnetic	600 (1200), 345 (18 000)	755	*ca.* 3.6

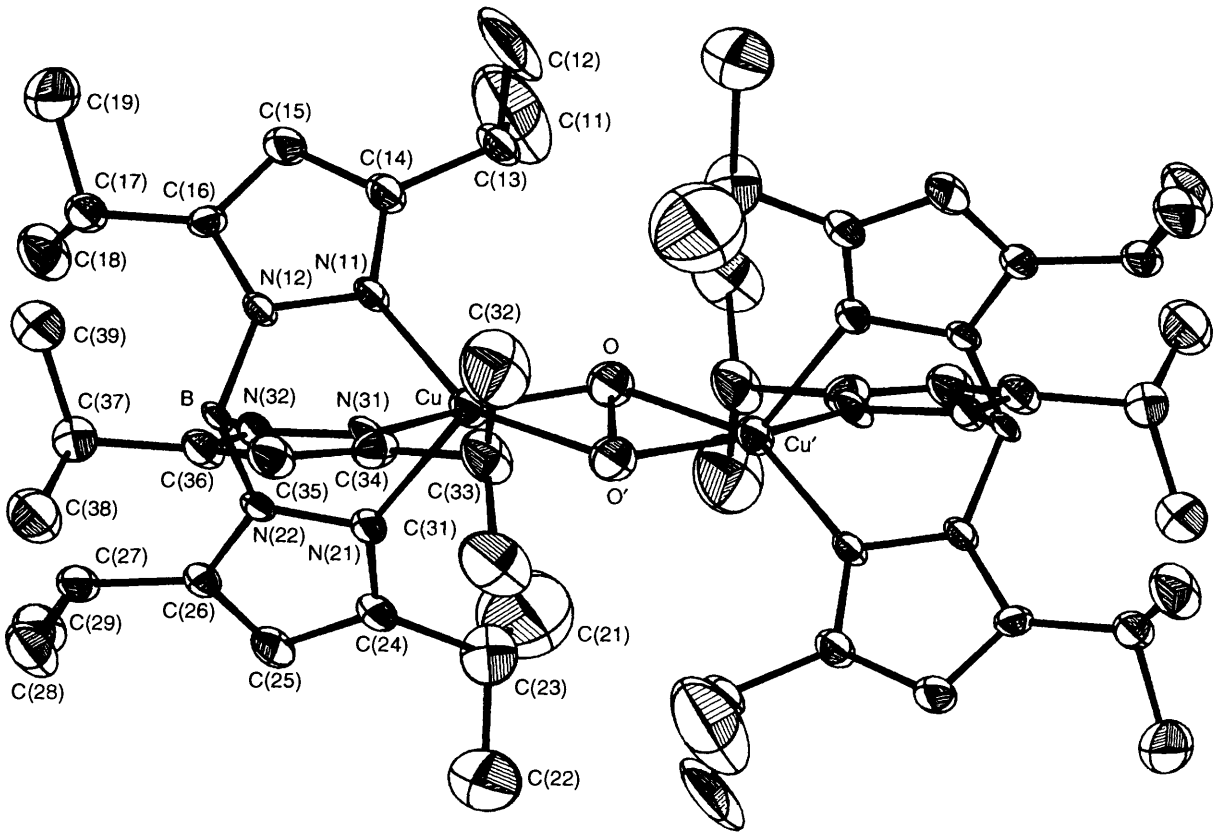


Fig. 3 Molecular structure of [{Cu[HB(3,5-$Pr^i_2C_3HN_2)_3$]}$_2$(O_2)] **3**

closely resemble oxyhaemocyanin in their physicochemical characteristics (Table 1). Complexes **1**–**3** are diamagnetic, as proved by ^{1}H NMR spectroscopy and the Evans method. The variable-temperature magnetic susceptibilities of **2** determined by SQUID indicated that $-2J$ is > 800 cm^{-1}.[20] All the complexes also exhibit two characteristic intense absorption bands at *ca.* 345 (ε *ca.* 20 000) and *ca.* 540 nm (*ca.* 1000 dm^3 mol^{-1} cm^{-1}). These features are very close to those of oxyhaemocyanin not only qualitatively but also quantitatively. In addition, the tailing feature of the blue-purple band suggests the overlapping of a d–d band at *ca.* 650 nm, indicative of a tetragonal geometry around copper as known for oxyhaemocyanin. Moreover, the ν(O–O) wavenumbers of these complexes are unusually low (transition-metal peroxo complexes usually give values in the range 800–900 cm^{-1}).[1b] The detailed resonance-Raman study with mixed labelled dioxygen clearly established that the co-ordination of peroxide in these complexes is symmetric as in oxyhaemocyanin. These remarkable similarities suggested strongly that the $N_3Cu(O_2)CuN_3$ chromophore in **1**–**3** is essentially identical to that in oxyhaemocyanin.[10]

Molecular Structure of [{Cu[HB(3,5-$Pr^i_2C_3HN_2)_3$]}$_2$(O_2)] 3

Slow recrystallization of **3** from CH_2Cl_2 at low temperature gave solvated crystals of **3**·6CH_2Cl_2 suitable for X-ray diffraction. Whereas the quality of the data collected was not excellent because of the facile loss of the solvent of crystallization, the crystal-structure determination of **3**·6CH_2Cl_2 definitely established the novel co-ordination mode of the peroxide as planar μ-η^2:η^2.[8,10b] The molecular structure of **3** is presented in Fig. 3. The molecule sits on a crystallographically imposed centre of symmetry. Thus, the two copper(II) ions and the peroxide comprise one plane, in which two pyrazolyl nitrogens [N(11) and N(31)] bind tightly to each copper as evidenced in the expanded view of the $N_3Cu(O_2)CuN_3$ moiety shown in Fig. 4. The other bond distance, Cu–N(21), is distinctly elongated, indicating that the co-ordination geometry of the copper is best described as square pyramidal with N(21) as an apical ligand, although it is very distorted. The Cu · · · Cu separation is 3.56 Å, which is considerably shorter than that found in the *trans*-μ-1,2-peroxo dicopper(II) complex reported by Karlin and co-workers (4.36 Å)[17] but very close to the *ca.* 3.6 Å estimated for oxyhaemocyanin and oxytyrosinase on the basis of extended X-ray absorption-fine structure (EXAFS) analysis.[21,22]

Two examples of planar μ-η^2:η^2-peroxo complexes of lanthanum and uranium are known and very recently a bent μ-η^2:η^2 co-ordination mode for peroxide was found in a vanadium complex.[3,4,9] The driving force substantiating the

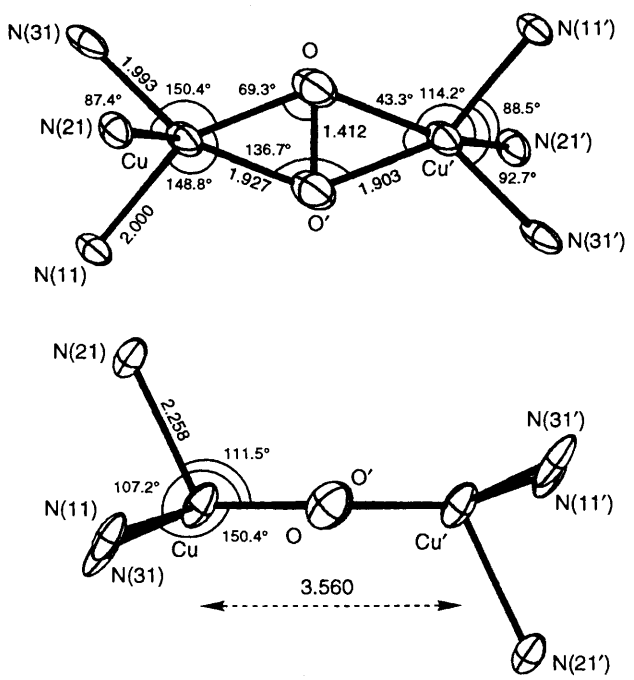


Fig. 4 Expanded view of $N_3Cu(O_2)CuN_3$ moiety in **3**; distances in Å (reproduced with permission from The American Chemical Society[10b])

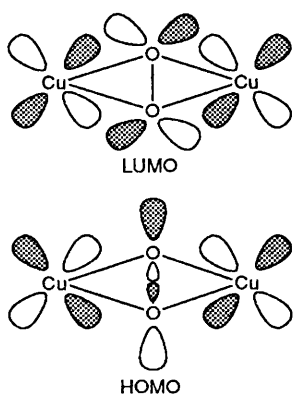
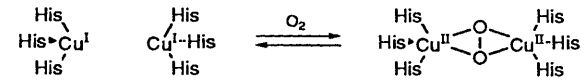


Fig. 5 HOMO and LUMO orbitals of $[\{Cu(NH_3)_2\}_2(O_2)]^{2+}$

unusual co-ordination modes in these complexes is attributable to the high oxophilicity of these elements. However, this interpretation cannot be applied to complex **3**, since copper(II) does not have such a particular preference. Rather, it seems more likely that the μ-η^2:η^2 structure found in **3** (or **1** and **2**) is associated with the strong peculiarity of copper(II) to favour a square-pyramidal (tetragonal) structure over a tetrahedral one. Were the co-ordination mode of the peroxide *trans*-μ-1,2 or *cis*-μ-1,2 for **3**, the copper would adopt a tetrahedral geometry but it is not preferred (a tetragonal-type geometry with a N_3O ligand arrangement is not allowed with hindered tripodal facial capping ligands).

MO Interpretation of the Unusual Properties of the μ-η^2:η^2-Peroxo Complex

The striking characteristics of μ-η^2:η^2-peroxo dinuclear copper(II) complex as well as oxyhaemocyanin are (*a*) the enormously strong antiferromagnetic coupling, (*b*) the unusually low frequency of ν(O–O) and (*c*) the intense absorption bands at *ca.* 350 and 540–580 nm. These unique features have been interpreted on the basis of SCF-Xα-SW calculations performed for the model $[\{Cu(NH_3)_2\}_2(O_2)]^{2+}$.[23] The HOMO and LUMO orbitals derived from the calculations are shown in Fig. 5. The strong antibonding nature between $d_{x^2-y^2}$ and O_2 π^*_σ is



Scheme 2

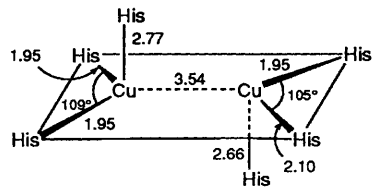


Fig. 6 Schematic view of the $N_3Cu\cdots CuN_3$ moiety in deoxyhaemocyanin; distances in Å (reproduced with permission from The American Chemical Society[10b])

evident. This causes a HOMO–LUMO gap as great as 5660 cm^{-1}, which accounts for the diamagnetism of the μ-η^2:η^2-peroxo complex. In the HOMO there is a considerable interaction between the Cu $d_{x^2-y^2}$ and $O_2^{2-}\sigma^*$ orbitals. Since the $O_2^{2-}\sigma^*$ is an antibonding orbital, this interaction weakens the O–O bond, which is responsible for the unusually low ν(O–O) frequency. Based on the calculations the bands at 350 and 540–580 nm are reasonably assigned to $O_2^{2-}\pi^*_\sigma \longrightarrow Cu^{II}$ and $O_2^{2-}\pi^*_v \longrightarrow Cu^{II}$ l.m.c.t. bands, respectively.

Possibility of μ-η^2:η^2-Peroxide Binding in Oxyhaemocyanin

Although a considerable number of μ-peroxo dinuclear copper(II) complexes has been reported recently, only **1**–**3** can mimic all the characteristic spectral features of oxyhaemocyanin. This implies that the $Cu(O_2^{2-})Cu$ chromophore in oxyhaemocyanin is structurally, and consequently, spectroscopically very unique. The remarkable similarities of the μ-η^2:η^2-peroxo complexes **1**–**3** to oxyhaemocyanin in their physicochemical characteristics, including their Cu $\cdots$ Cu separation, magnetic properties, UV/VIS and Raman spectra thus lend strong support to a new possibility that the peroxide co-ordinates in oxyhaemocyanin in the μ-η^2:η^2 mode without an additional bridging ligand. As described already, the co-ordination mode of the peroxide in oxyhaemocyanin has been generally believed to be *cis*-μ-1,2 with an endogenous bridging ligand, most likely hydroxide. However, the existence of the additional bridging ligand has not been proved experimentally yet, despite many spectroscopic investigations. Given the co-ordination mode of the peroxide as μ-η^2:η^2, it is not necessary to consider the hypothetical presence of an additional ligand and thus the binding of dioxygen at the dicopper site is interpreted by the simple addition of dioxygen to deoxyhaemocyanin shown in Scheme 2.

A structural comparison of the $N_3Cu(O_2)CuN_3$ moiety in **3** and the crystal structure of deoxyhaemocyanin supports the new binding model favourably. Each copper(I) ion in deoxyhaemocyanin is surrounded by three histidine nitrogen atoms from the protein chains, with a Cu $\cdots$ Cu separation of 3.54 Å.[14] The co-ordination of the two copper(I) ions are similar, consisting of two short Cu–N bonds and the other distinctly elongated Cu–N bond. This is illustrated in Fig. 6. It is noteworthy that the four nitrogens and the two copper(I) ions sit approximately on the same plane and the two apical nitrogen atoms relate to each other in a *trans* configuration. These features of the co-ordination environment are very close to those found in the $N_3Cu(O_2)CuN_3$ moiety of **3** (see Fig. 4), except for the longer $Cu–N_{apical}$ distance. Accordingly, dioxygen would fit in the planar $N_2Cu–CuN_2$ frame of deoxyhaemocyanin without a serious structural change, resulting in the peroxide binding in the μ-η^2:η^2 mode.

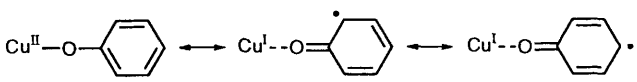
Very recently, Magnus[24] finally succeeded in determining the

2.4 Å resolution structure of oxyhaemocyanin from a limulus, which confirms that the peroxide bridges between the two copper(II) ions in a planar μ-$\eta^2:\eta^2$ co-ordination mode and not *cis*-μ-1,2 with an additional bridging ligand. The overall structure of the $N_3Cu(O_2)CuN_3$ chromophore is very similar to that found in **3**.[24]

Reactivity of the μ-$\eta^2:\eta^2$-Peroxo Complex

The understanding of the general reactivity of the μ-$\eta^2:\eta^2$-peroxo complex should be useful in gaining insight into the catalytic mechanism of tyrosinase. With this in mind, the reactivity of **1** toward a variety of substrates, particularly phenols, was explored in detail.[25] Complex **1** is not thermally very stable, and thus spontaneously decomposes to the μ-oxo dicopper(II) complex $[\{Cu[HB(3,5\text{-}Me_2C_3HN_2)_3]\}_2O]$ with the evolution of dioxygen at room temperature. This spontaneous decomposition is explained in terms of a mechanism which involves O–O bond homolysis of the peroxide as illustrated in Fig. 7. The Cu^{II}–O$^{\bullet}$ radical generated by the O–O bond homolysis couples with a copper(I) complex which is in equilibrium with the peroxo complex at room temperature in solution. Support for this mechanism stems from the observation that the addition of PPh_3 or CO to a solution of the μ-$\eta^2:\eta^2$-peroxo complex results in instantaneous reductive ligand displacement, affording the corresponding copper(I) complexes $[Cu(PPh_3)\{HB(3,5\text{-}Me_2C_3HN_2)_3\}]$ and $[Cu(CO)\{HB(3,5\text{-}Me_2C_3HN_2)_3\}]$ respectively, with the evolution of dioxygen. The first-order dependence of the rate of consumption of **1** with respect to the concentration of **1** in the spontaneous decomposition is also in accord with the mechanism, where the O–O bond homolysis is the rate-determining step.[25]

The anaerobic reactions of phenols and the μ-$\eta^2:\eta^2$-peroxo complex **1** solely gave diphenoquinones but not benzoquinones, whereas under aerobic conditions, benzoquinones were also formed but as minor products.[25] The formation of diphenoquinones as major products strongly suggests a free-radical type reaction mechanism *via* a phenoxo radical as a key reaction intermediate. In fact, kinetic experiments indicated that there are two distinct reaction pathways responsible for the formation of a phenoxo radical (see Fig. 8).[25] One reaction step is the H$^{\bullet}$ radical abstraction from a phenol by the Cu^{II}–O$^{\bullet}$ radical generated by O–O bond homolysis. The other is the acid–base reaction of **1** with a phenol resulting in the formation of a phenoxo copper(II) complex initially, followed by the Cu–O homolysis to afford a phenoxo radical. This reaction is competitive with the H$^{\bullet}$ abstraction of a phenol, and faster when the concentration of the phenol is high or the temperature low. The diphenoquinones are formed by the oxidative coupling of the phenoxo radical, the mechanism of which is well established as a classical type radical reaction. The formation of benzoquinones, on the other hand, is ascribed to the direct addition of dioxygen to the phenoxo intermediate to form a peroxy phenoxo radical. Here, it should be emphasized that the *ortho* (or *para*) position of a phenoxide co-ordinated to a copper(II) ion is activated and accessible to radical addition because of the resonance structure shown below.



Although the phenoxo complex could not be isolated in the reaction system with **1**, the low-temperature reaction of **3** and a phenol such as *p*-fluorophenol successfully gave the phenoxo complex $[Cu(OC_6H_4F\text{-}p)\{HB(3,5\text{-}Pr^i_2C_3HN_2)_3\}]$, which was isolated and structurally characterized by X-ray crystallography.[26] These facts reasonably suggest the involvement of a phenoxo copper(II) species as a key reaction intermediate in the catalysis of tyrosinase as well. In this hypothetical mechanism, the μ-$\eta^2:\eta^2$-peroxide plays a role as a base to react with acidic phenol to give a phenoxo copper(II) intermediate in which the *ortho* position is activated and susceptible to a radical addition (in tyrosine the *para* position is substituted). Because tyrosinase has a dinuclear copper site and only one phenol molecule can be

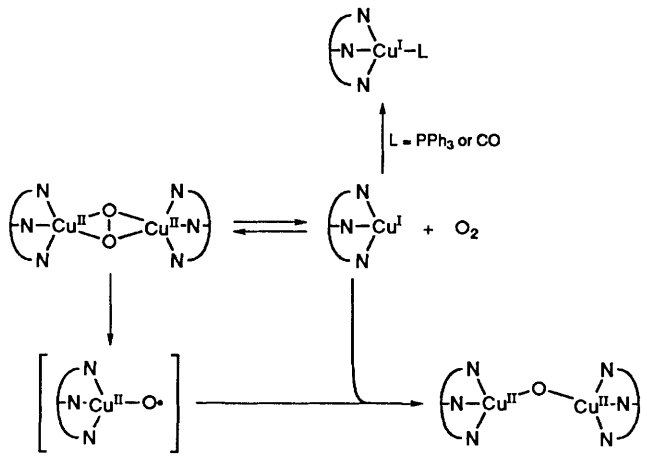
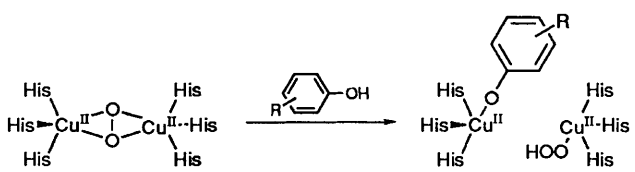


Fig. 7 Mechanism of spontaneous decomposition of the μ-$\eta^2:\eta^2$-peroxo complex **1**



Scheme 3

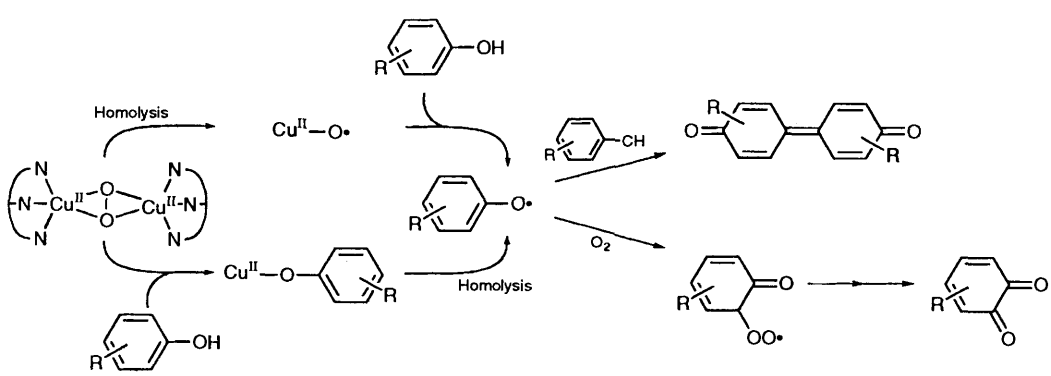


Fig. 8 Mechanism of formation of benzoquinones and diphenoquinones from peroxo complex **1** with phenols

inserted into the active site, the formation of the phenoxo intermediate [if it is terminally bound on one-side to a copper(II) ion] also causes the formation of a hydroperoxo copper(II) intermediate (Scheme 3). In general, the hydroperoxo complex is more reactive than the corresponding peroxo complex; thus dioxygen is activated at the dicopper site with formation of the phenoxo copper(*ii*) intermediate. The subsequent oxygen-transfer reaction presumably proceeds *via* a concerted radical-type mechanism.

Karlin and co-workers[27] have reported on the intramolecular arene hydroxylations supposed to occur *via* a bent μ-η^2:η^2-peroxo intermediate and they proposed an electrophilic direct oxygen transfer reaction of the peroxide into a CH bond of the arene ring. This interpretation together with a theoretical prediction that the μ-η^2:η^2-peroxide is more electrophilic than those of *cis*-μ-1,2- or *trans*-μ-1,2-peroxide,[23] may raise another possibility that the phenol oxidation by tyrosinase proceeds *via* a similar electrophilic oxygen insertion from the μ-η^2:η^2-peroxide directly. However, there is no conclusive experimental evidence to identify the structure of the intermediate as a μ-η^2:η^2-peroxide, and thus the mechanism proposed by Karlin and co-workers is still speculative.

There have been several other proposals that direct arene hydroxylations proceed *via* a μ-peroxo intermediate, possibly a μ-η^2:η^2 complex, on the basis of the intramolecular ligand hydroxylations similar to Karlin's reaction.[28] However, again, in none of these systems, has the definite identification of the intermediate been completed yet. Moreover, controversy arises from the fact that all these systems are ineffective for oxygen-transfer reactions of substrates added externally. Thus, the hypothesis that the μ-η^2:η^2-peroxo dicopper complex is very electrophilic and will incorporate oxygen into a CH bond of a substrate directly remains to be proved.

Consequently, there are two possible reaction mechanisms for tyrosinase in which a μ-η^2:η^2-peroxo species is a key intermediate: one is the mechanism involving initial formation of a phenoxo hydroperoxo intermediate followed by a radical-type oxidation; the other is direct electrophilic insertion into a CH bond of tyrosine. Clearly, more mechanistic studies, not only on synthetic models but also on tyrosinase itself, are required to lead to a conclusive catalytic mechanism for tyrosinase.

Possibility of Involvement of μ-η^2:η^2-Peroxide in Other Biological Systems

Although the presence of μ-η^2:η^2-peroxide was definitely established for **3** by X-ray crystallography, the discussion above suggests that this co-ordination mode is not necessarily exceptionally unusual. There is a consensus that the oxophilic metal elements can adopt such a structure. In the case of a late transition-metal complex, the preference of the central metal for a particular co-ordination geometry can become a driving force. For the copper complex described here, the strong preference of copper(II) for a tetragonal (including square-pyramidal five-co-ordination) over a tetrahedral co-ordination structure is the key factor substantiating the μ-η^2:η^2 structure. Accordingly, the μ-η^2:η^2-peroxo dicopper compound should be formed with other ligands, provided, like tris(pyrazolyl)borates, they have a rigid facial tripodal geometry so as not to allow the tetragonal geometry. Possibly, Karlin's complex described already adopts such a co-ordination mode as proposed. Although no solid characterization has been completed, several other μ-peroxo dicopper complexes reported recently may possess the same structure on the basis of the structure of the ligand and the similarity in the UV/VIS spectra to those of **1**–**3** and oxyhaemocyanin.[29]

The potential ubiquitous presence of the μ-η^2:η^2-peroxide raises a new possibility that another biological system may utilize this particular co-ordination mode to bind or activate dioxygen. Blue oxidases such as laccase and ascorbate oxidase are known to contain a dinuclear copper site which is strongly magnetically coupled. Based on spectroscopic studies on laccase, it was proposed that the dicopper site in blue oxidase is distinctive from that in haemocyanin and tyrosinase; it was suggested that an additional monomeric copper ion was positioned in close proximity to the dicopper site. This was established recently by the X-ray analysis of ascorbate oxidase.[30] Two copper ions which are strongly magnetically coupled are bridged by a hydroxide with a Cu · · · Cu distance of 3.7 Å. The Cu · · · Cu distance between one of the paired copper ions and the third copper ion is approximately 3.7–3.8 Å.[30b] Since there is a hydroxide ion between the magnetically coupled pair of copper ions, it seems unlikely that dioxygen is bound to this site in a μ-η^2:η^2 manner, yet it may be possible that dioxygen is bridged in this mode between the one of the paired copper ions and the monomeric copper ion. This possibility was discussed in detail based on the crystal structure.[30b] If the μ-η^2:η^2-peroxo species is formed in these proteins, then the spectroscopic properties should verify their presence easily as the spectral feature of the μ-η^2:η^2 peroxide is so very characteristic. Very recently, a peroxide-like intermediate of laccase was reported, but its absorption spectrum is significantly different from those of oxyhaemocyanin and complexes **1**–**3**.[31]

Several other metalloproteins which contain dinuclear metal sites are known.[32] It is of interest that most of these proteins participate in the metabolism of dioxygen. These include several non-haem iron proteins (haemerythrin, methane monooxygenase, ribonucleotide reductase) and manganese proteins (manganese ribonucleotide reductase, manganese catalase, manganese protein of the oxygen evolving centre in photosynthetic system II). Apart from haemerythrin which binds dioxygen to one iron as hydroperoxide, how dioxygen (peroxide) co-ordinates in the proteins remains to be elucidated. The μ-η^2:η^2 structure should therefore be taken into account in considering the co-ordination mode of the peroxide at the dinuclear metal site in these proteins.

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