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FUNCTIONAL MODELLING OF HEMOCYANIN, TYROSINASE AND CATECHOL OXIDASE

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Many enzymes and proteins have copper at their active sites that play key roles in biology. An important goal of bioinorganic chemistry is the development of small inorganic coordination complexes that reproduce structural, spectroscopic features and functional aspects in a manner similar to their natural counterparts. Such studies help to elucidate active site structures responsible for carrying out specific biological reactions and to provide a molecular level understanding of functioning of proteins/enzymes. Such efforts led to a triumph in biomimetic chemistry because the true O_2 binding mode occurring in oxy-hemocyanin, had not been considered as a possibility until a synthetic analogue revealed the actual Cu_2-O_2 coordination. Comparisons of chemical and spectroscopic properties of tyrosinase and its derivatives with those of hemocyanin, whose crystal structures in both deoxy and oxy forms have been determined, establish a close similarity of the active sites structures in these two proteins. To provide an overview of the activities in this field, the current information available for the dicopper-containing bio-sites and selected results on synthetic modelling of relevant proteins/ enzymes hemocyanin, tyrosinase and catechol oxidase are described in this article.

Key Words: Dicopper Proteins/Enzymes; Hemocyanin; Tyrosinase; Catechol Oxidase; Oxygen Transport; Monooxygenase Activity; Oxidase Activity; Synthetic Models; Magnetism.

Introduction

Copper is one of the transition elements frequently found at the active site of proteins. The copper-containing enzymes and proteins constitute an important class of biologically active compounds. The biological functions of copper proteins/enzymes include electron transfer, dioxygen transport, oxygenation, oxidation, reduction and disproportionation[1-10].

Reactions that copper proteins carry out have long interested inorganic chemists. Copper is an important element in oxidation catalysts for laboratory and industrial use[8,11-15]. Interest in the copper-dioxygen complexes[16-21] stems from the diverse occurrence of copper proteins which function as highly efficient biooxidation catalysts. Copper-dioxygen adducts are suggested as key reaction intermediates in these enzymatic reactions. The differentiation in the function of these proteins is attributed primarily to the coordination structure of the copper-dioxygen intermediate formed in the protein matrices, depending on the ligand donors, the geometry, and the coordination mode of the dioxygen. However, the correlation between these structural factors and the function/catalysis of the proteins/enzymes remains to be elucidated.

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Importance of Inorganic Model Chemistry

Intuitively, one can anticipate that the behaviour of metal ions in proteins cannot be vastly different from that governed by the fundamental chemistry of the particular metal. *The synthetic analog approach*, the primary focus of this article, is based on the premise that the chemistry of the metal-binding site ("active site") is dependent, for the most part, on the immediate coordination environment of the metal ion[22-25]. For most metalloproteins, the immediate coordination environment consists of donors from the side-chains of amino acids. Sometimes, a prosthetic group (e.g., a porphyrin ring) completes the coordination sphere of the metal ion. Thus it could be generalized that the "metallobiomolecules" are highly elaborated coordination complexes whose metal-containing sites (coordination units), comprising one or more metal atoms and their ligands, are usually the loci of electron transfer, binding of exogenous molecules and catalysis. Two major factors control the properties of metal ions in biological systems: (i) the stereochemistry of the metal site and the nature of the ligands attached to the metal and (ii) the protein environment, which plays a crucial role in controlling the reactivity of the metal site. In some cases the protein can force metal ions into unusual geometries; the protein environment may be the determining factor

controlling the activity of the increasing number of functionally distinct metalloproteins that have essentially identical metal centres.

From the aforesaid discussion, it is understandable that inorganic chemists can contribute considerably to the understanding of the structural, electronic and mechanistic aspects of metal ions in metalloproteins, by synthesizing small coordination compounds, which mimic the specific properties of those metal sites. These synthetic models are usually intended to serve as stereochemical and electronic analogs of these sites and have the substantial advantage of being amenable to characterization at a very high level of detail. Simultaneous attainment of biological structure and function in a synthetic system has proven more difficult. The problem becomes more demanding when catalysis is involved.

Interest in elucidating or mimicking the physico-chemical properties of metalloproteins led to and still spurring activity in the synthesis of numerous interesting coordination complexes. However, there has been an increased recent emphasis upon functional modelling of proteins. While the structural and spectroscopic modelling of metalloprotein active sites is an important and ongoing endeavour, the realization that coordination chemists can and should make significant contributions to reactivity studies and mechanism has become apparent. The value of models for metalloproteins will always be relative. One of the difficulties encountered in simulating a biosite is that, as time passes, the objective may change with advancing knowledge. If the structure of the metal ion environment in the metalloprotein is unknown, the objective may be to reproduce some property of the system in a similar model coordination compound, but when the structure of the biosite is known, then the complex of such a nature that it reproduces, as far as possible, the known structure. A different emphasis is obtained when the action of the metal in the protein is reproduced by a model compound and the mechanism of a particular reaction is elucidated or partially explained. The purpose of models is not necessary to duplicate natural properties but to sharpen or focus certain questions. The goal is to elucidate fundamental aspects of structure, spectroscopy, magnetic and electronic structure, reactivity and chemical mechanism. A synergistic approach to the study of metalloenzymes can and has yielded crucial information because synthetic analogs can be used to investigate the effects of systematic variations in coordination chemistry, ligation, local environment and other factors, often providing insights that cannot be easily attained from protein studies (Fig. 1)[24,25]. Reproducing complex biological reactivity within a simple synthetic molecule is a challenging endeavour with both intellectual and aesthetic goals.

Scope of the Review

Hemocyanin and tyrosinase[1-21], binuclear copper protein/enzyme with a similar active-site structure yet very different reactivity, exemplify the multidisciplinary nature of bioinorganic enzymology[26-29]. Crystallography allows the elucidation of overall atomic structures and provides a framework for detailed investigations into mechanism. Details of the electronic structure of active sites are determined through spectroscopy. Many metalloenzymes have spectroscopic features that are indicative of unusual electronic structures. Synthesis of model complexes allows investigators to probe the intrinsic reactivity of the active site structure, systematically perturb the site, and use high-resolution investigative techniques without the inherent difficulties and limitations often associated with a protein matrix. The first successful synthesis of a stable bridged m-h^2:h^2-peroxo binuclear copper centre, as found in oxy-hemocyanin, was reported by Kitajima *et al.* (see below).

The interaction and subsequent reactivity of dioxygen with copper ions is of great interest due to the importance of dioxygen binding and/or activating proteins in biological systems and the utility of copper compounds in oxidative synthetic reactions.

We endeavoured to understand the structure and function of copper proteins involved in copper(I)/O_2 interactions, by studying inorganic models, i.e., synthetically derived copper(I) complexes, and their O_2 reactivity. Such biomimetic approaches can lead to fundamental insights into the copper-based chemistry. One might also envision the development of reagents or catalysts for use in practical oxidation processes[11-15].

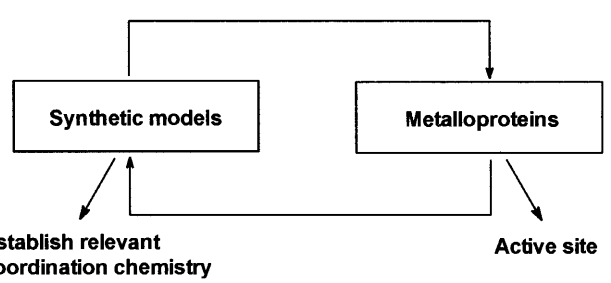


Fig. 1 The synergistic relationship between studies involving metalloprotein biochemistry and inorganic modelling[24].

The interplay between model and protein biophysical studies has provided considerable insight into Cu-O_2 chemistry occurring in O_2 carrier protein hemocyanin and tyrosinase activity. Solomon and co-workers have demonstrated that the electronic structure of oxy-Hc and oxytyrosinase (oxy-Tyr) is closely similar[4,5].

It is the purpose of this article to highlight recent advances in bioinorganic model studies on dicopper-containing proteins/enzymes *hemocyanin*, *tyrosinase* and *catechol oxidase*. These proteins contain electronically coupled binuclear copper active sites. Therefore, from this background model studies on suitably ligand-bridged dicopper(II) complexes include temperature-dependent magnetic susceptibility measurements to extract information on the extent of magnetic exchange interactions. Hence, in this article a portion is dedicated to highlight the results from author's laboratory on such systems.

Hemocyanin and Synthetic Models

Hemocyanins are very large dioxygen transporting proteins that function in the hemolymph of invertebrates belonging to several species of the phyla of Mollusca and Arthropoda. The active site has a dinuclear copper centre to which dioxygen is bound as peroxide and the two copper ions are divalent in the dioxygen binding state (so-called oxy-hemocyanin, oxy-Hc) (Fig. 2). Oxy-Hc is EPR silent and in fact, diamagnetic at room temperature, due to a very strong antiferromagnetic exchange coupling ($-2J > 600$ cm^{-1}) between the two Cu(II) ions. Furthermore, instead of d-d bands normally observed at 600-700 nm for Cu(II) complexes, oxy-Hc exhibits two intense bands at ca. 350 nm (~20 000/2Cu) and ca. 580 (~1000), both attributable to O_2^{2-} ® Cu(II) ligand-to-metal charge-transfer (LMCT) transitions. The deoxy state possesses three-coordinate cuprous ions (Cu—Cu: 4.6 Å) which bind O_2 to give a peroxo-dicopper(II) oxy form, the coordination sphere of copper(II) is best described as distorted square pyramidal, t = 0.10 (for an idealized square pyramidal geometry t = 0, while for an idealized trigonal bipyramidal arrangement t = 1)[30], Cu—Cu: 3.6 Å and u(O-O) ~ 750 cm^{-1} (resonance Raman). The coupled binuclear site in oxygenated arthropod hemocyanin has been structurally characterized (Fig. 2)[31]. Details of the bound peroxide are based on the structure of the m-h^2:h^2-peroxo complex prepared by Kitajima *et al.* (see below).

In the copper-containing bio-sites histidine (imidazole) is most commonly observed amino acid side chain as ligands. In model studies of Cu^I and Cu^{II} complexes containing bidentate/tridentate/tetradentate ligating groups, emphasis has therefore been placed on the coordination properties of aromatic nitrogen-containing donors[32]. In this section results of selected synthetic model studies on hemocyanin have been highlighted.

During the course of modelling copper-dioxygen chemistry Kitajima *et al.* first reported the synthesis of a μ-peroxo dinuclear complex with 3,5-dimethyl-substituted tris(pyrazolyl)borate ligand, which showed remarkable physicochemical similarities to oxy-Hc and oxy-Tyr. Using 3,5-di-isopropyl-substituted terminal ligand they provided the first structural proof of the existence of a μ-h^2:h^2 peroxo dicopper(II) core (complex **1**) [copper geometry: t = 0.03; Cu-Cu: 3.560 Å] and reported detailed characterization properties[33], which eventually led to the structural characterization of oxy-Hc[32]. Tolman and co-workers discovered[34] a novel phenomenon that when copper(I) complex of 1,4,7-triisopropyl-triazacyclononane oxygenated at -78 °C, there exists an equilibrium between the two oxygenated species $[Cu^{II}_2(\mu\text{-}h^2\text{:}h^2\text{-}O_2)]^{2+}$ [side-on peroxodicopper(II)] and $[Cu^{III}_2(\mu\text{-}O)_2]^{2+}$ [bis(μ-oxo)dicopper(III)] (Fig. 3) depending on the solvent chosen, with CH_2Cl_2 favouring the former species and THF favouring the latter. They provided first structural proof of a bis(μ-oxo)dicopper(III) core (complex **2**). Using a 10-membered macrocycle, 1,4,7-tris(isopropyl)-1,4,7-triazacyclodecane, Alvarez and Tolman and their co-workers provided X-ray structural evidence[35] of a side-on peroxodicopper(II) core (copper geometry:

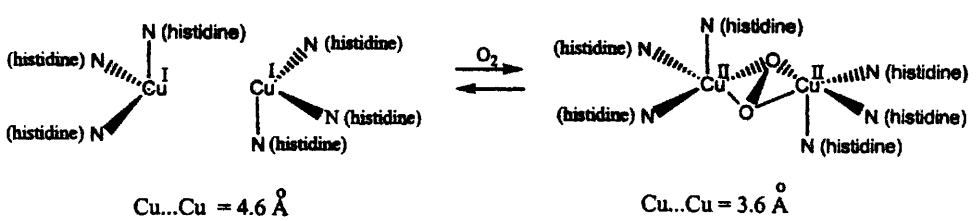
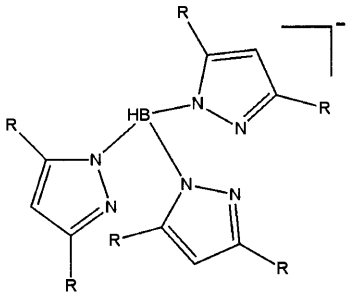


Fig. 2 X-ray structures of hemocyanin (a) in deoxy state and (b) in oxy state.

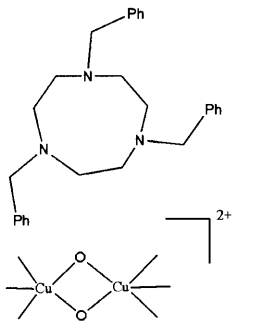
t = 0.30; Cu-Cu: 3.5 Å) (complex **3**). Using a fluorinated tris(pyrazolyl)borate ligand Gorun and co-workers[36] very successfully isolated and structurally characterized a dinuclear oxygenated complex **4**, revealing that the crystal contains about 80% μ-h^2:h^2 peroxo complex (core structure: *cf.* complex **1**) and 20% bis(μ-hydroxo) complex [core structure: *cf.* complex **12** (see below)]. In a major breakthrough, using a strapped tridentate ligand Kodera *et al.* reported[37] reversible O_2-binding of a room temperature stable μ-h^2:h^2 peroxo dicopper(II) complex **5** (core structure: *cf.* complex **1**; stereochemistry at the copper centre: square pyramidal; Cu-Cu: 3.477 Å).

Karlin's group provided the first structurally characterized (-90 °C) example of a trans-(μ-1,2-peroxo)dicopper(II) core (complex **6**) (Cu-Cu: 4.359 Å)[38], supported by two tripodal ligands. The system exhibited reversible binding of dioxygen. However, it should be noted that the active site of hemocyanin and tyrosinase, in their oxy form, do not have this type of peroxo linkage. Very interestingly, Krebs's group[39] reported a thermally stable peroxocopper(II) complex (complex **7**) (Cu-Cu: 2.994 and 3.030 Å; $2J$ = -510 cm^{-1}). Recently Meyer and co-workers reported a novel example of unusual μ_4-peroxo coordination (complex **8**)[40]. The schematic representations of these complexes, along with the structures of the ligands present, are shown below.

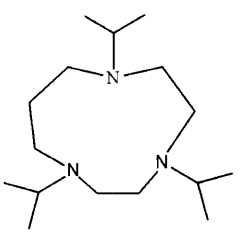
It is expected that targeted research on hemocyanin modelling would provide many more interesting synthetic models to sharpen our knowledge of understanding of the functioning of hemocyanin.



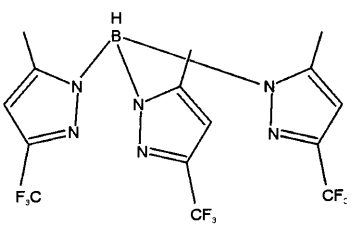
[Cu(L^{iPr})]$_2$(O)$_2$.6CH$_2$Cl$_2$ (**1**)



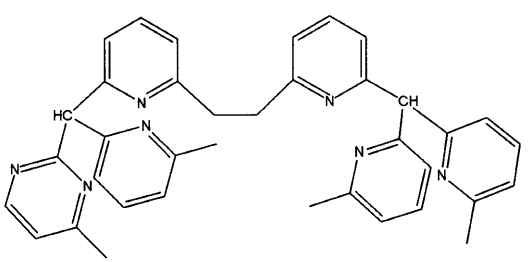
[(L)$_2$Cu$_2$(O)$_2$][SbF$_6$]$_2$.7Me$_2$CO 2MeCN (**2**)



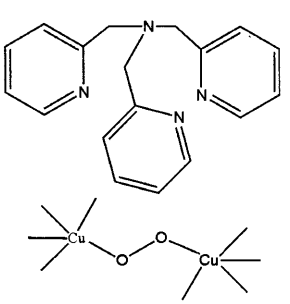
[(L)$_2$Cu$_2$(O)$_2$][BPh$_4$]$_2$.xsolvate (**3**)



[Cu(L)]$_2$O$_2$(**4**)



[(L)Cu$_2$O$_2$][PF$_6$]$_2$.3CH$_2$Cl$_2$ (**5**)



[(L)$_2$Cu$_2$(O)$_2$][PF$_6$]$_2$.5Et$_2$O (**6**)

Tyrosinase and Synthetic Models

All of us are aware of the fact that when potatoes, apples, bananas, sweet potatoes or mushrooms are injured they turn brown. This is due to the conversion of tyrosine to the pigment melanin, by the sequence of reactions shown in Fig. 4. The same process causes skin tanning, following exposure to ultraviolet radiation. The enzymatic reactions are catalysed by tyrosinase,

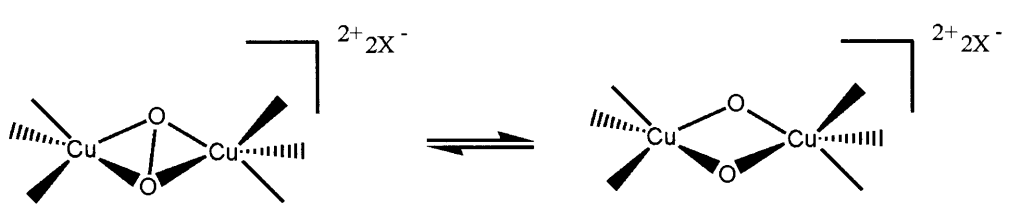


Fig. 3 Interconversions between isomeric μ-h^2:h^2-peroxo-dicopper(II) and bis(μ -oxo)dicopper(III) cores.

a copper-containing enzyme. The enzyme is present in the interior of the plant material and since the reaction requires molecular oxygen, the pigmentation does not occur until the interior is exposed. Tyrosinase[1-21] catalyses (i) the *o*-hydroxylation of monophenols to *o*-diphenols and the further oxidation of these to *o*-diquinones (Fig. 5). These quinones undergo further enzymatic and nonenzymatic reactions that lead to polymeric pigmented material (Fig. 4). In animals, these reactions give skin, eyes and hair their distinctive pigmentation. In order to deduce the structures and mechanism of action of the protein-active sites, a major focus of research has utilised the biomimetic approach.

The activation of molecular oxygen by copper plays a vital role in synthetically useful stoichiometric and catalytic oxidative conversions of organic molecules and in biological systems. Based on the reactivity of molecular oxygen with dicopper(I) complexes of designed ligands, considerable progress has been made in the chemical modelling of tyrosinase. The X-ray structural characterization of tyrosinase in either form (deoxy- and oxy-) has not been achieved so far. However, it has been realized that the active site of tyrosinase apparently has greater accessibility to exogenous ligands, including substrate molecules, by comparison with the active site in hemocyanin. The similarity of the oxy-states of Hc and Tyr point to the probable close relationship between the binding of dioxygen in both and the ability to activate it for incorporation into organic substrates in the latter. In this section, results of selected synthetic model studies on tyrosinase have been highlighted, emphasizing systems developed in author's laboratory.

Karlin *et al.* reported[41,42] the first chemical model (Fig. 6) (complex **9** and complex **10**) of tyrosinase (intramolecular ligand hydroxylation of a tailor-made *m*-xylyl unit) consisting of a ligand that provides two tridentate bis[2-(2-pyridylethyl)amine] donor units to each copper ion. The reactive dicopper(II)-peroxo complex (as present in oxy-Hc/oxy-Tyr) could not be isolated, but its occurrence as an intermediate during aromatic ring hydroxylation was proved spectroscopically in a detailed kinetic study. As a part of this investigation this group has provided examples of a large number of systems showing stoichiometric aromatic ring hydroxylation[32].

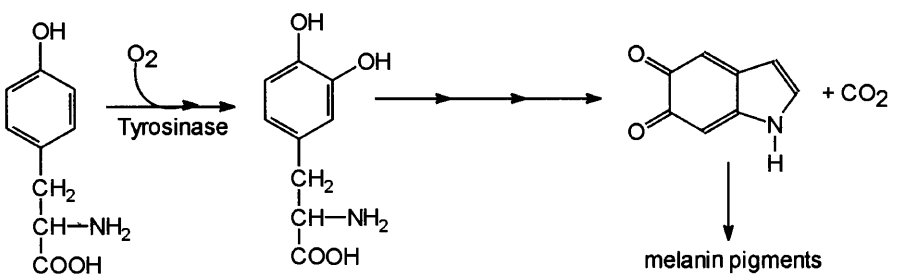
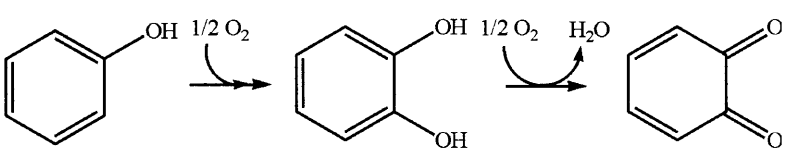
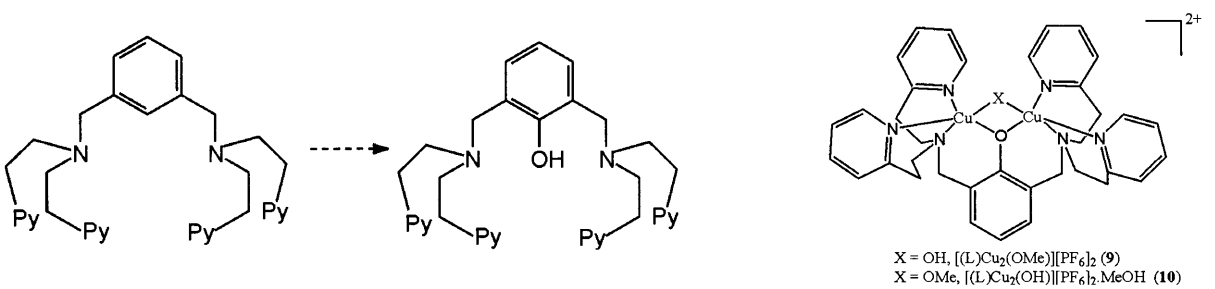


Fig. 4 The role of tyrosinase in tyrosine metabolism in mammalian cells.



(tyrosinase) (tyrosinase and catechol oxidase)

Fig. 5 Reaction pathway of the oxygenation and oxidation catalysed by tyrosinase and catechol oxidase.

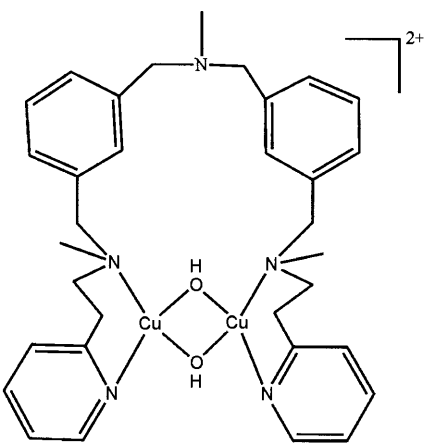


X = OH, $[(L)Cu_2(OMe)][PF_6]_2$ (**9**)
X = OMe, $[(L)Cu_2(OH)][PF_6]_2.MeOH$ (**10**)

Fig. 6 Chemical model of Karlin *et al.*[41,42]

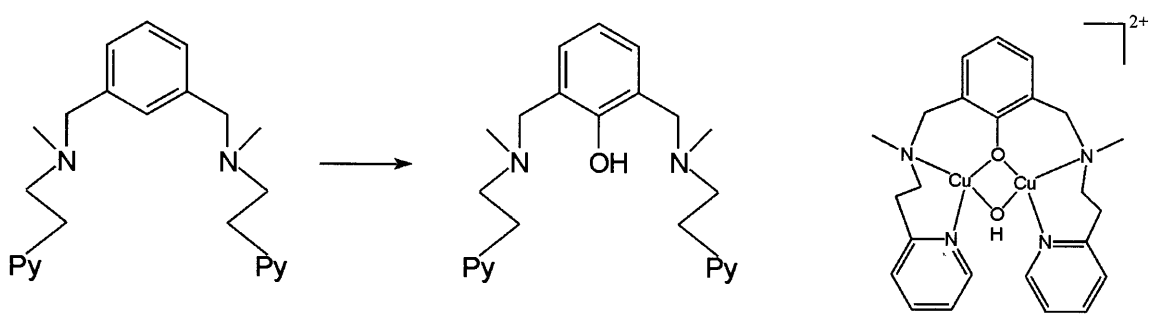
Interestingly, when 1-pyrazolyl or 2-imidazolyl donor groups fully or partially replace the 2-pyridyl ligands hydroxylation does not occur. However, Schiff base ligands (imine moiety) providing three or even only two nitrogen donors are used hydroxylation takes place. It should be emphasized here that non-Schiff base ligands are the ones which should be used to model histidine side chain of protein. In order to complete the missing link between the Schiff base and non-Schiff base families, we designed a new ligand system (see below).

To gain more insight into the reactivity of the binuclear copper(I) complexes with dioxygen and in the dependency of the arene hydroxylation on ligand topology, we initiated a program to systematically investigate tyrosinase-like monooxygenase activity using new *m*-xylyl-based dinucleating ligand systems of the open-chain type or of the macrocycle type, capable of providing only two N-coordinations to each copper site. The chemical system which we investigated involved the dinucleating ligand, *m*-XYL-H, in which two bidentate units (aliphatic amine and pyridyl nitrogen) are connected by a *m*-xylyl group. X-ray analysis of the final product (complex **11**) revealed incorporation of two oxygen atoms into the complex [presumably originating from molecular oxygen via peroxo-copper intermediates as documented by Karlin *et al.* (vide supra)]: one into the aryl-hydrogen bond and the other into the hydroxyl bridge (Fig. 7)[43-45]. With the synthesis of an interesting series of *m*-xylyl-based ligands[44], capable of providing only two nitrogen coordinations to each copper centre, the oxygenation of the dicopper(I) complexes have been systematically investigated. Rapid decomposition to aromatic ring-hydroxylated product even at -80 °C implies that the energy barrier for conversion of intermediate dicopper(II)-peroxo to such species is small. Our studies pinpoint (i) the effect of ligand structure, i.e., a six-membered chelate-ring forming ligand gives rise to aromatic ring hydroxylation whereas a five-membered chelate ring-forming ligand (-CH_2- spacer instead of –CH_2CH_2- spacer present in complex **11**) gives only irreversible oxidation of dicopper(I) precursor complex (ligand remained unchanged) and (ii) the effect of appropriate positioning (geometry) of the xylyl ring of the ligand, i.e., compared to our ligand present in complex **11**, the failure of the ligand present in complex **12** to undergo hydroxylation reaction [dihydroxo-bridged dicopper(II) complex **12**].



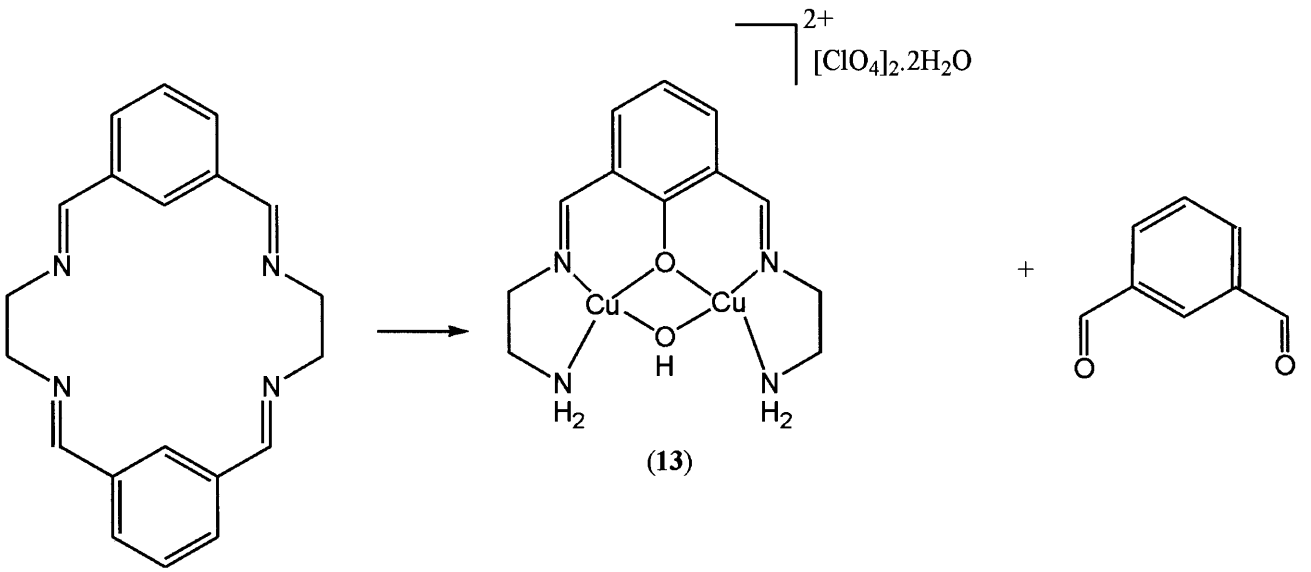
$[(L)Cu_2(OH)_2][ClO_4]_2$ (**12**)

As in an enzyme active site, the peroxo group (a highly reactive intermediate formed due to reaction between dicopper(I) complex of the chosen ligand and O_2; *cf.* Fig. 2) is located in a highly favourable proximity to the xylyl ligand substrate and facile hydroxylation occurs by electrophilic attack on the arene substrate p system. These xylyl hydroxylation model systems serve as a functional mimic for tyrosinase, revealing how a dicopper(I) centre can activate O_2 for hydrocarbon oxidation under mild conditions. We demonstrated a similar xylyl-ring hydroxylation reaction using a macrocyclic ligand system, as well (Fig. 8)[46]. Aromatic



$[(L)Cu_2(OH)][ClO_4]_2$(**11**)

Fig. 7 Chemical model of tyrosinase developed in author's laboratory.



(13)

Fig. 8 Model tyrosinase-like activity with a macrocyclic ligand system.

hydroxylation in one of the rings has been observed with concomitant partial hydrolysis of the macrocycle. The final dicopper complex **13** has a m-phenoxo m-hydroxo bridged structure as evidenced from spectroscopic and magnetic studies.

After being successful in bringing about stoichiometric aromatic ring hydroxylation (chemical modelling of tyrosinase) we turned our interest towards catalytic externally added substrate oxidation chemistry. For this purpose we designed a new ligand system, *m*-XYL-F. The only difference between this and *m*-XYL-H is that the C-H bond which is cleaved in the reaction of Cu(I) complex of *m*-XYL-H with O_2 is replaced by a C-F moiety. In other words, a fluorine atom is placed in the position that is hydroxylated. We were successful in demonstrating catalytic oxidations of hindered phenols, as that observed for mushroom tyrosinase (Table 1)[47].

Here also it is expected that targeted research on chemical modelling of tyrosinase would provide many more interesting synthetic models to sharpen our knowledge of understanding of the functioning of tyrosinase (monooxygenase activity).

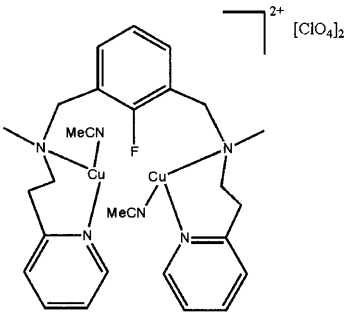
Catechol Oxidase and Synthetic Models

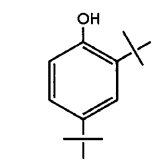
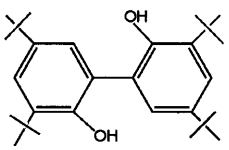
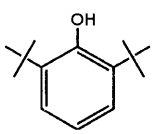
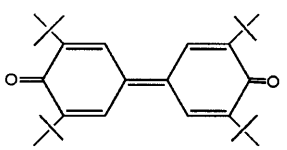
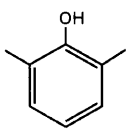
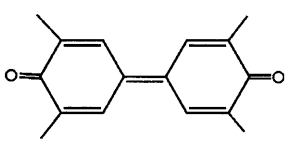
The ubiquitous plant enzyme catechol oxidases[9], in contrast to tyrosinases, catalyse exclusively the oxidation of catechols to the corresponding *o*-quinone by molecular oxygen, without acting on monophenols (Fig. 5). Thus catechol oxidase lacks hydroxylase activity. The resulting highly reactive quinones auto-polymerize to form brown polyphenolic catechol melanins, a process thought to protect the damaged plant from pathogens or insects. The enzyme contains an antiferromagnetically coupled (EPR silent) dicopper centre. Three-dimensional X-ray crystal structural analysis of catechol oxidase[9], from sweet potato, in the resting Cu(II)-Cu(II) state, the reduced Cu(I)-Cu(I) form, in complex with the inhibitor have been achieved. Both copper centres have three histidine ligands. In the oxidized catechol oxidase structure the two Cu(II) ions are 2.9 Å apart. In addition to the six histidine ligands a bridging hydroxide ion, completes the four-coordinate trigonal pyramidal coordination sphere for each Cu(II) ion. Mechanism of cresolase and catecholase activity of tyrosinase and/or catechol oxidase is presented in Fig. 9.

A large number of complexes with phenoxo- and alkoxo-bridged complexes have been examined for catecholase activity (aerial oxidation of 3,5-di-*tert*-butylcatechol). Recently we have shown[48] that the phenoxo-/hydroxo-bridged dicopper(II) complex (complex **11**) is an efficient catalyst for catecholase activity (Fig. 10)[48]. We believe that for the complex **11** the reaction occurs with a first stoichiometric fast phase followed by a slower reaction. In the second phase dicopper(I) species formed due to initial reaction between catechol and copper(II) has to react with molecular oxygen to generate transient copper(II)-peroxy species which allows the catalytic cycle to operate (*cf.* Fig. 9).

Table I

Catalytic oxidation of hindered phenols by dicopper(I) complex (shown below) and dixygen.[a]



Substrate	Product	yield (%)	Reaction time (hrs.)	Turnover number
		91	6	5
		85 (nil)[d]	6 (60)	5 (50)
		86 (96)[e]	6 (9)	10 (50)

[a] Yields and turnover numbers in parentheses are for mushroom tyrosinase.

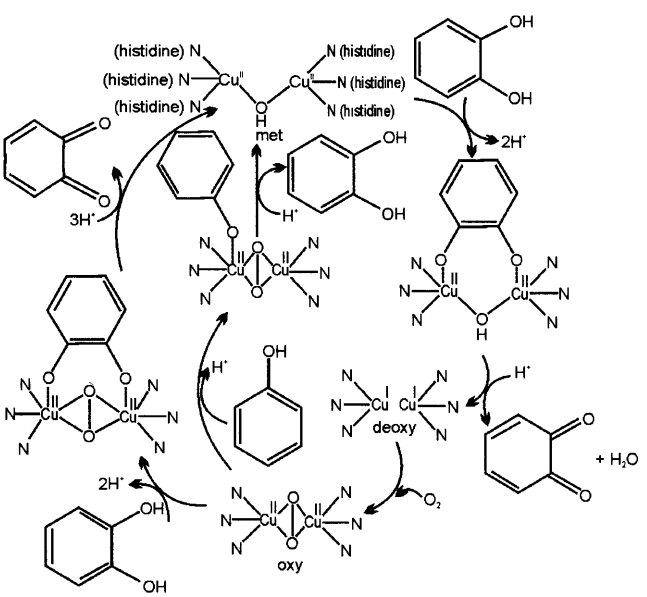


Fig. 9 Mechanism of cresolase and catecholase activity of tyrosinase and /or catechol oxidase[9]

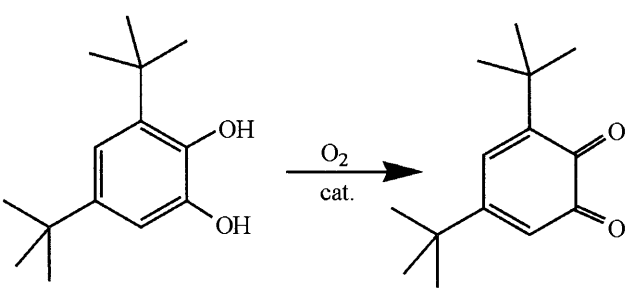


Fig. 10 Model catechol oxidase reaction.

Magnetism

The dicopper structural units have been shown (vide supra) to be important in biological reactions involving molecular oxygen including oxygen transport, oxygen activation in the copper monooxygenase tyrosinase and in catechol oxidase. Therefore, bimetallic coordination complexes containing two copper ions in close proximity have been under extensive study. Examination of model systems is necessary to help elucidate the nature and

mechanism of action of the copper containing active sites. In addition, model systems are of interest in studies of metal-metal interactions.

When two copper(II) centres with unpaired electrons interact, the result may be a parallel 'ferromagnetic' (- -) or an antiparallel 'antiferromagnetic' (- ⁻) coupling of the electron spins (Fig. 11).[27] If the orbital interaction is small, e.g., because of orthogonal arrangement of p or d orbitals, Hund's rule requiring maximal multiplicity in order to avoid the spin-pairing energy favours a parallel spin-spin coupled situation. The more frequent case, however, is the antiparallel (antiferromagnetic) coupling in which the energy gain from possibly only indirect orbital interactions ('super exchange') compensates for the spin pairing.

As part of our own investigations into the chemistry of binuclear Cu(I) and Cu(II) complexes, we have initiated studies utilizing a number of ligand systems, where two bidentate/tridentate donor groups are separated by a wide variety of spacers. Dicopper(II) complexes with endogenous bridging phenolate ligands are of ongoing interest because of their relevance to tyrosinase and catechol oxidase and/or due to their interesting magnetic properties.

a. Phenoxo-/hydroxo- and Bis(hydroxo)bridged Dicopper(II) Complexes

The structurally characterized complexes (complex **11** and complex **12**) gave us an unique opportunity to study the magnetic behaviour of two dicopper(II) complexes with phenoxo-/hydroxo- (complex **11**) and bis(hydroxo)-(complex **12**)-bridging having closely similar terminal ligation. Therefore, there is a possibility to arrive at a magneto-structural trend. The detailed temperature-dependent magnetic studies of complex **11** and complex **12** in the solid state (Faraday method) were undertaken to elucidate the extent of magnetic exchange interaction in these systems. In the case of complex **11**, the value of m_{eff}/Cu at 300 K was found to be 1.13 m_B. A plot of molar susceptibility (per dimer) versus temperature is illustrated in Fig. 12[44]. The behaviour is typical of a strongly antiferromagnetically coupled system. A sharp rise in c_M at low temperatures indicates the presence of small amount of paramagnetic impurity. The observed magnetic susceptibility data were fitted to the modified Bleaney-Bowers eq. (1) by allowing for the presence of monomeric impurity, where r is the mole-fraction of the non-coupled copper(II) impurity. In this expression, N, g, k have their usual meaning; $2J$ is the energy difference between the singlet and triplet states; c_M is the molar susceptibilty per dimer.

$$c_M = 2Nb^2g^2/3kT[1+1/3\exp(-2J/kT)]^{-1}(1-r) + Nb^2g^2r/2kT + 2Na \quad \ldots(1)$$

The best fit data, obtained by a nonlinear least-squares fitting procedure, obtained using eq. (1), are presented in Table II. The large negative value of J indicates a strong coupling between the two copper centres, expected for this type of systems. In the related system of Karlin *et al.* even stronger coupling was observed ($2J$ = -600 cm^{-1}). The behaviour of complex **12** (Fig. 12) is closely similar to that of complex **11**[44]. The extent of antiferromagnetic exchange coupling is comparable to closely related dihydroxo-bridged

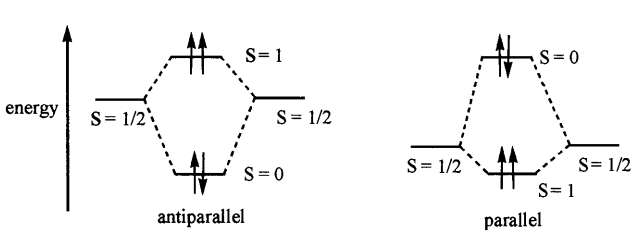


Fig. 11 Spin-spin coupling and magnetism.

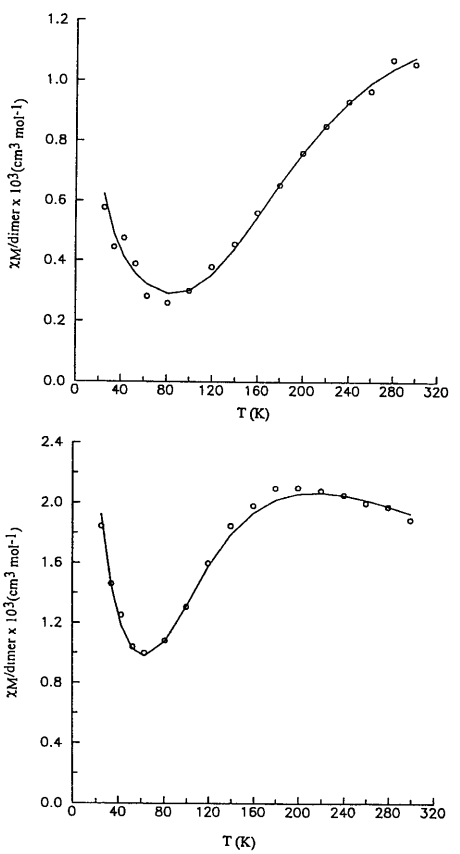


Fig. 12 Plots of molar susceptibility (c_M per dimer) versus temperature for polycrystalline samples of complex **11** (upper plot) and complex **12** (lower plot). The solid lines result from a least-squares fit of the susceptibility data to eq. (1).

Table II

Temperature-Dependent Magnetism Data for Ligand-bridged Dicopper(II) Complexes[a]

Complex No.	Bridging Type	2J, cm^{-1}	g	r	Ref.
11	phenoxo-/hydroxo-	-440	2.05	0.016	44
12	bis(hydroxo)-	-258	2.13	0.054	44
13	phenoxo-/hydroxo- (macro)	-458	2.13	0.042	46
14	diphenoxo-	-1204	2.00 (fixed)	0.042	49
15	alkoxo-/pyrazolato-	-550	2.10 (fixed)	0.004	50
16	alkoxo-/azido-	-366	2.10 (fixed)	0.061	50
17	alkoxo-/acetate-	-112	2.10 (fixed)	0.070	50

[a] Temperature-Independent-Paramagnetism (TIP): 120 x 10^{-6} cm^3 mol^{-1}

Table III

X-ray Structural Metric Parameters for phenoxo-/hydroxo- and bis(hydroxo)- bridged Dicopper(II) Complexes[a]

	Complex XXX	Complex XXX
Average Cu-N(py) distance, Å	2.003(6)	1.997(8)
Average Cu-N(am) distance, Å	2.017(7)	2.045(8)
Average Cu-O(phenoxo) distance, Å	1.961(5)	
Average Cu-O(hydroxo) distance, Å	1.913(5)	1.925
Average Cu-O(phenoxo)-Cu angle, deg	99.8(2)	
Average Cu-O(hydroxo)-Cu angle, deg	96.6(3)	102.3(4)
Cu...Cu, Å	2.999(1)	3.004(2)
Torsion angle of Cu_2O_2 unit, deg	~7	~12
Average angles between the planes N-Cu-N and O-Cu-O (deg)	~6	~2

complexes. This result reveals that in complex **12** the extent of coupling is much reduced than that in complex **11**. The pronounced decrease in coupling in going from complex **11** (phenoxo-/hydroxo-bridge) to complex **12** (dihydroxo bridge) for a closely similar ligand environment is worth noticing.

It is well documented now that the type and magnitude of magnetic exchange interactions in dinuclear complexes depend on the bridge identity, the Cu—Cu distance, the bond angles at the bridging atoms, the dihedral angle between the planes containing the copper(II) ions, the metal-bridging ligand bond lengths and the metal ion stereochemistry. A closer look at Table III reveals that[43,44], in both the complexes, the Cu—Cu distance, the stereochemistry around each Cu centre, and the average Cu-OR-Cu bond angle at the bridging atoms are comparable. The metal-bridging ligand bond lengths are slightly longer for complex **11** than for complex **12**. The fact is that the Cu_2O_2 unit in complex **12** deviates more from planarity than that of complex **11**. This difference can provide a reasonable explanation to the difference in the magnetic behaviour of the two complexes. It can be suggested that the lesser coupling in complex **12** is attributable to the fact that two copper(II) ions do not lie in one plane. When the metal ion moves into the plane, overlap between the metal-based $d_{x^2-y^2}$ orbitals and the oxygen-based sp^2 hybrid orbitals is increased. Since this s framework represents the dominant pathway for the superexchange mechanism, the enhanced overlap should result in an increase in the antiferromagnetic interaction. Temperature-dependent magnetic studies on complex **13** reveal[46] results closely similar to that observed for complex **11** (Table II).

b. Diphenoxo-bridged Dicopper(II) Complexes

During investigation on the reactivity of a m-peroxo-bridged copper(II) complex of tris(3,5-dimethylpyrazolyl) hydridoborate with externally added phenolic substrate 2,6-dimethylphenol, Kitajima *et al.* proposed a diphenoxo-bridged copper(II) intermediate[11]. As the active site of tyrosinase is quite open, we felt that synthesis and structural characterization of diphenoxo-bridged copper(II) complexes with only two pyrazole coordination at terminals would be a valuable complement to Kitajima's work. We reported the synthesis and characterization of di-m-phenoxo- bridged

copper(II) complex with 4-methyl-2,6-bis(pyrazol-1-ylmethyl)phenol, complex **14**). Structural analysis revealed that each copper(II) centre is square pyramidal (t = 0.06) with two bridging phenoxide oxygens and two terminal pyrazole nitrogens in the equatorial plane and a perchlorate oxygen atom axially coordinated. Variable-temperature magnetic susceptibility measurements and analyses by using eq. (1) revealed that the dicopper(II) centers are strongly antiferromagnetically coupled [singlet-triplet energy separation, $2J$ (in cm^{-1}): -1204] (Fig. 13) (Table II)[49].

c. Alkoxo-bridged Complexes

Using a new dinucleating ligand, 1,3-bis[*N*-methyl-*N*-(2-pyridylethyl)amino]propan-2-ol, in its deprotonated form, three new binuclear copper(II) complexes $[Cu_2(L)(m\text{-}X)(ClO_4)(H_2O)](ClO_4).H_2O$ [X = $C_3H_3N_2^-$ (pyrazolate) (complex **15**), N_3^- (complex **16**) and $MeCO_2^-$ (complex **17**)] have been synthesized[50]. The crystal structure of pyrazolate bound compound was determined and revealed that the complex contains endogenous bridging alkoxide ligand and exogenous bridging pyrazolate ligand. A water molecule is coordinated to a copper(II) centre and is hydrogen-bonded to the alkoxide oxygen atom. A perchlorate ion is "semi-co-ordinated" to another copper(II) centre as a monodentate ligand and is hydrogen-bonded to second water molecule. The coordination geometry around both copper centres resembles a distorted square pyramid (t = 0.24/0.07). The magnetic properties of the present complexes are clearly of interest owing to the presence of an invariant alkoxide bridge and variable exogenous bridge. Therefore they were subjected to magnetic susceptibility measurements. The m_{eff}/Cu values (at 300 K) are 0.96, 1.31 and 1.73 m_B for **15**, **16** and **17**, respectively. The best-fit parameters obtained using eq. (1) are in Table II. The strong antiferromagnetic coupling observed for the pyrazolate complex is due to (i) the planarity of the Cu(1)-O(1)-Cu(2)-N(5)-N(6) unit and (ii) an enhanced Cu-OR-Cu bridge angle of ~126° (*cf.* X-ray structure). As before, we note that small changes in the co-ordination geometry at the copper(II) ions can bring about a profound effect on the extent of antiferromagnetic exchange coupling.

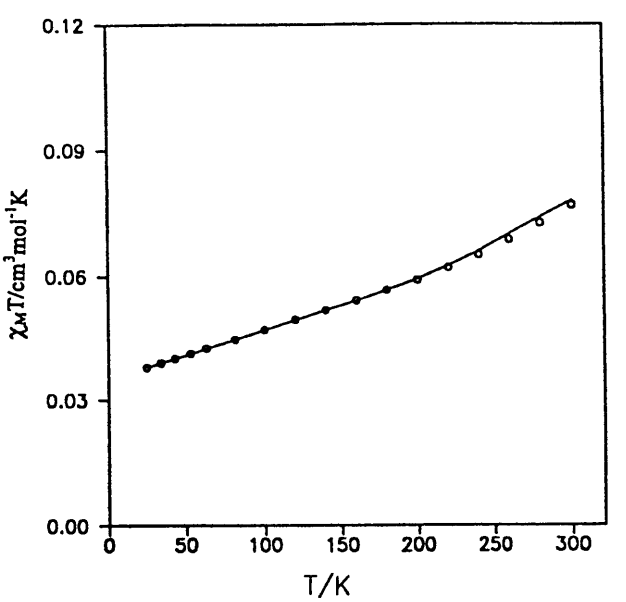
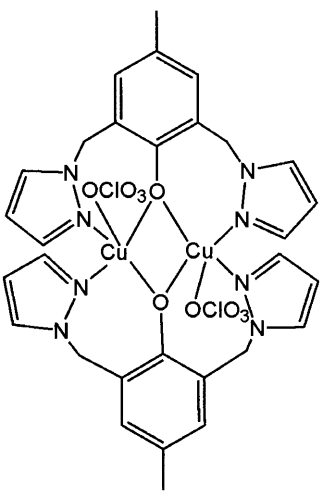
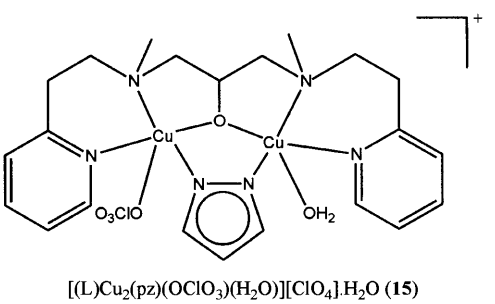


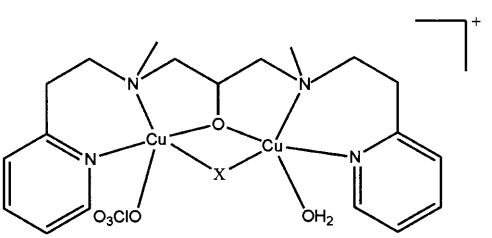
Fig. 13 Plot of $c_M T$ (per dimer) versus Temperature for polycrystalline sample of complex **14**.



[{Cu(L)}$(OClO_3)$]$_2$ (**14**)



[(L)Cu_2(pz)$(OClO_3)(H_2O)$][ClO_4].H_2O (**15**)



[(L)$Cu_2(N_3)(OClO_3)(H_2O)$][ClO_4].H_2O (**16**)
[(L)$Cu_2(O_2CMe)(OClO_3)(H_2O)$][$ClO_4$].$H_2O$ (**17**)

Based on the X-ray structure of **15** and closely similar spectral properties of **15** – **17** [*cf.* (i) IR spectra of **15** – **17**, (ii) identical d-d band position of **15** and **16** (the copper(II) centres in **17** might have slightly more trigonal bipyramidal component, compared to **15** and **16**)] we could assume that the present complexes have predominantly square-based copper(II) centres. According to theoretical predictions made by Hoffmann and co-workers, strong antiferromagnetic interactions arise in any dimer when the energy separation between the highest d-like molecular orbitals, *viz.* symmetric (f_s) and antisymmetric (f_a) combinations of magnetic orbitals is large, irrespective of which combination of the orbitals is lower in energy. In the case of an alkoxide bridge the intervening oxygen p_x orbital interacts with the f_a orbital and exerts no effect on it (Fig. 14). It is well documented that in a dicopper complex with two different bridging groups (endogenous alkoxide and exogenous X groups), the bridging groups act 'complementary' or 'countercomplementary' in inducing antiferromagnetic interaction depending on the symmetries of their HOMO's which interact with metal d-like oribtals. Based on the above considerations, we provide here a rationale of the observed magnetic behaviour of **15** – **17** (Fig. 14)[50].

For the pyrazolate complex **15** both bridging ligands provide orbital interaction pathways that act in concert (bridging ligand orbital complementarity) to increase the energy of f_a. For **16** the energy of f_a orbital is lowered, because the highest occupied orbital of an azide ion in the m-1,1-mode does not have the correct symmetry (in the antibonding sense) to interact with f_a orbital. For **17** the acetate bridge has no net overlap with the f_a orbitals; therefore, no net change in energy of f_a is expected with this orbital combination. However, the energy of f_s orbital is raised, because the highest occupied orbital of an acetate ion has the correct symmetry (in the antibonding sense) to interact with f_s orbital (not shown in Fig. 14). Thus effectively the gap between f_a and f_s decreases (countercomplementary) in **16** and **17**, thereby diminishing the antiferromagnetic interaction. Even though m-1,1-N_3^- bridge exerts a ferromagnetic contribution, which in the present case does not entirely compensate the strong antiferromagnetic contribution due to the alkoxo-bridge. Thus within the endogenous alkoxo bridge, with variation in the exogenous bridging groups the order of the strength of the exchange interaction is **15** > **16** > **17**.

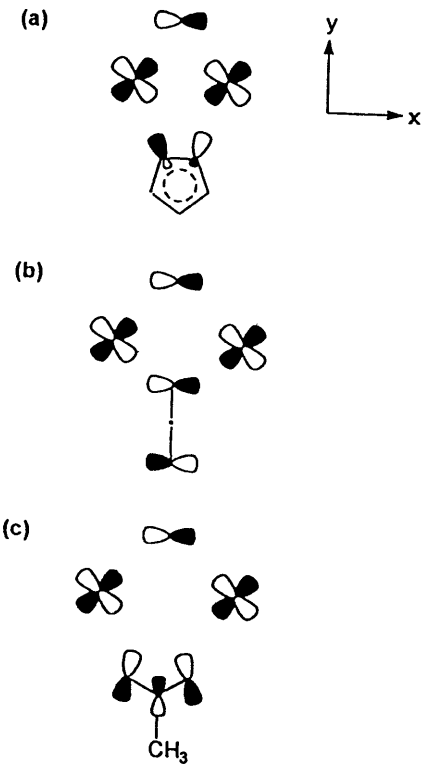


Fig. 14 Bridging ligand orbital interactions with $d_{x^2-y^2}$ orbitals of copper for various dibridged systems: (a) complementarity of alkoxide/pyrazolate, (b) countercomplementarity of alkoxide/1,1-azide and (c) countercomplementarity of alkoxide/sacetate. Only the antisymmetric combination of copper magnetic orbitals is illustrated.

Conclusion

Much effort has been devoted over the last 20 years in designing dinuclear copper complexes in order to mimic the structure and the magnetic and spectral properties of hemocyanin and tyrosinase. Kitajima's model complex provided a solid impetus to the structural and spectroscopic properties of hemocyanin. Despite much effort, faithful examples of functional models of hemocyanin are rare. As far as the chemical modelling of tyrosinase is concerned a few good functional models are now available. However, these systems function in a stoichiometric manner. New synthetic models are sought for to demonstrate aromatic hydroxylation of externally added phenols. Apart from Kitajima's contribution our result towards such a goal has been highlighted here. In recent years efforts are on to find an accurate electronic structural and efficient functional model of catechol oxidase. More model studies are necessary to sharpen our knowledge about this fascinating copper-dioxygen chemistry.

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