进 展 与 评 述

生物金属位点的配位化学模拟

COORDINATION CHEMISTRY AND THE MODELLING OF METALLOBIOSITES *

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Introduction

Although traditionally biology, and hence life, has been regarded as organic life is inorganic too. In order to read this you are continually sending messages throughout your nervous system by using the changes in electrical flux caused by the movememt of sodium and potassium ions across cell membranes. More importantly whilst you are reading you are also breathing, taking in dioxygen from the atmosphere and using it for the essential process of respiration through its interaction with the iron atoms present in haemoglobin and myoblobin. The iron is coordinated by the nitrogen atoms of a porphyrin ring, one of nature's macrocycles, and so the value of biocoordination chemistry is immediately obvious. Without it you would have no facility to read at all.

Other species use other metals to transport dioxygen. In molluscs and arthropods copper is used and it is on the biocoordination chemistry of copper that we will now focus in this presentation.

1. Copper Biosites

i. 'Blue' oxidases

The copper(Ⅱ) atoms present in copper metalloproteins have been classified according to their spectroscopic properties: type−1, as found in 'blue' copper proteins such as plastocyanin, azurin and stellacyanin, contains a mononuclear copper biosite with a distorted tetrahedral N_2S_2−donor set and has high absorption in the visible region ($\varepsilon > 3000 M^{-1}cm^{-1}$ at 600nm) and an EPR spectrum with $A_{\parallel} < 95 \times 10^{-4}cm^{-1}$; type−2, or normal, is present in all multicopper 'blue' oxidases and displays spectroscopic properties similar to those found for typical mononuclear Cu(Ⅱ) complexes, i. e. a broad unresolved band near to 700nm in the UV−visi-

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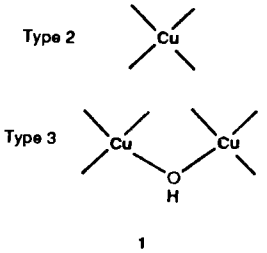
ble spectrum and an EPR spectrum typical of small molecule copper(Ⅱ) complexes ($A_{\parallel} > 140 \times 10^{-4}$ cm^{-1}); type–3, which has a strong absorption in the near U. V. region ($\lambda_{max} = 330nm$) and no EPR signal, is believed to consist of a pair of antiferromagnetically coupled copper (Ⅱ) ions[1].

Copper proteins are also known that contain more than one of the above types of copper biosite. Ascorbate oxidase, laccase and ceruloplasmin constitute a group of multicopper enzymes, known as 'blue' oxidases, which catalyse the one–electron oxidation of the substrate with concomitant four electron reduction of dioxygen to water[2]. Laccase (M～65,000) is the simplest member of this family and contains four copper(Ⅱ) atoms (one type–1, one type–2 and two type–3)[1]; dimeric ascorbate oxidase contains eight copper(Ⅱ) atoms and it was suggested that it was a dimer of two identical laccase–like sub–units[3]. Cumulative spectroscopic and azide bonding studies on *Rhus vernicifera* laccase led to the proposal that the type–2 and type–3 centres defined a trinuclear copper cluster site(1)[4].

The long absence of crystallographic information concerning this type of site was redressed with the publication of an X–ray diffraction study of oxidised ascorbate oxidase from green Zucchini[5]. Two crystal forms were analysed, one a dimer (M～ 140,000) and one a tetramer (M～280,000). Each sub–unit has four copper atoms present bound as mononuclear and trinuclear species. The mononuclear copper, bound to two histidines, one cysteine and one methionine ligand, is of type–1, resembling plastocyanin, and is isolated from the trinuclear site by *ca.* 15Å. The trinuclear site(2) may be subdivided into a type–2 copper and a pair of type–3 copper atoms held in an approximately isosceles triangular array. The type–2 copper, 3.9Å from one type–3 copper and 4.0Å from the second, is coordinated to two histidine ligands and an oxygen atom from water or hydroxide. The type–3 coppers are each coordinated by three histidine ligands and form a trigonal prism with an intermetallic separation of 3.4Å [although the structure has recently been further refined[6] leading to metal separations of 3.66, 3.78 and 3.68Å in subunit A and 3.69, 3.90 and 3.73Å in subunit B. The original report has been adhered to as this provided the crystallographic information on which our first generation model was based]. The X–ray data indicates the existence of an oxo–, or hydroxo– bridging ligand and so the identification of the trinuclear site thus provides confirmation of the earlier proposals from solution studies.



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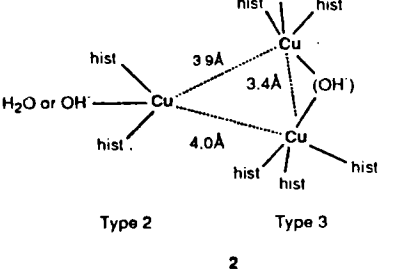

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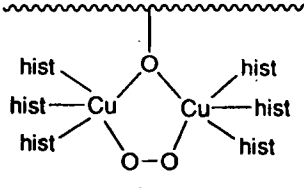
ii. Haemocyanins

Haemocyanin is a multisub-unit protein found in the haemolymph of several species of the phyla Mollusca and Arthropoda which reversibly binds di-oxygen and acts as a di-oxygen transport protein. Collective evidence from vibrational, magnetic, chemical and electronic data have suggested that the metallo-biosite in oxyhaemocyanin (3) requires a type-3 dinuclear Cu(Ⅱ) centre with both endogenous and exogenous bridges present in order to mediate the strong antiferromagnetic coupling which leads to diamagnetism[7]. The nature of the purported endogenous bridge is not yet finally established but EXAFS data and charge transfer bands at 345 and 570nm in the electronic spectrum suggested that it arose from an oxygen atom with hydroxide, alkoxide, phenoxide and carboxylate all being listed as contenders for this bridging role. The absence of enhanced tyrosine vibrations in resonance Raman experiments indicated that there is no conserved tyrosine present and so a phenoxide bridge is unlikely[8]. EXAFS studies show the presence of terminal N or O donor atoms of which two per Cu(Ⅱ) are imidazole N atoms and that the Cu···Cu separation is *ca.* 3.6Å; the Cu(Ⅱ) atoms are in approximately tetragonal environments. Resonance Raman studies with isotopically substituted di-oxygen have shown that it is symmetrically bound to the dinuclear centre through both oxygen atoms to provide the exogenous bridge. The Cu···Cu distance separation of *ca.* 3.6Å upon oxygenation indicates that this peroxide is probably bound in a *cis*-μ-1,2 mode[9].

The size of the protein has hampered attempts to elucidate the structure of the oxy-form crystallographically but the structure of the colourless deoxy-form from *Panulirus interruptus*(spiny lobster) has been reported[10] and shows that each Cu(Ⅰ) ion is co-ordinated by three histidine residues with an inter-copper distance of *ca.* 3.7Å (4). There is no endogenous or exogenous bridging group and the nearest tyrosine is *ca.* 10.6Å away. The structure determination of deoxyhaemocyanin has now clearly shown that a tyrosine bridge is unlikely, however it is plausible that a bridging water or hydroxide is present in oxyhaemocyanin, and that the necessary free water molecule near to the biosite has not yet been identified on the deoxyhaemocyanin electron density maps.



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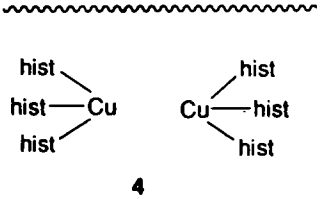

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2. The Modelling of Metallobiosites

For several decades single crystal X-ray diffraction has served as the final arbiter for biological structure determination. However, good crystals are often difficult to prepare and the resolution of the structure determination is often limited. The structure, when solved, will only give detailed information about one particular form of the metalloprotein, and it may not be

easy to infer from this the nature of any structural changes which might occur on conversion to another form by processes such as the binding of substrates. As a consequence many inorganic chemist become involved in inorganic biochemistry by attempting to devise small molecule models the properties of which can be compared with those of the metalloprotein and hence provide a model compound.

The properties of a metal in a metalloprotein are dependent upon its chemical environment, that is the number of co-ordinated ligands, the nature of their donor groups and the coordination geometry at the metal. These structural factors affect the spectroscopic, electrochemical, and, in multinuclear systems, the magnetic characteristics of the metallobiosite. Comparison of a range of model compounds having slightly different metal environments builds up an understanding of the effects of these structural factors on the physico-chemical properties of the models and this can then be used in the deduction of structural features of the metalloprotein under study.

Hill[11] has introduced two useful definitions for model compounds of metalloproteins, *speculative models* and *corroborative models*. *Speculative models* are prepared when the structure of the microenvironment of the metallobiosite is unknown and the objective is to reproduce some physico-chemical property of the system in a simpler complex. When the structure of the metallobiosite is known a *corroborative model* can be prepared. This is usually a low molecular weight complex in which the environment of the metal is reproduced as accurately as possible. This then helps determine whether the observed properties of the metal in the protein are dominated by the first co-ordination sphere and can give insights into the relationship between structural features of the metallobiosite and its properties. A third type of model is the *functional model* in which the actual functioning of the metallobiosite is reproduced. This type of modelling is deemed to be the most difficult and it is still correct to say that it has not yet been satisfactorily achieved. This may be related to the concept of the *entatic state*[12], namely that the active metal site of an enzyme is in a geometry approaching that of the transition state of the appropriate reaction and so is uniquely adapted for catalytic action.

The nature of type-3 copper sites provides a challenge to the synthetic chemist and makes them prime targets for modelling studies. In order to do this a versatile ligand system, having the potential for modification in order to incorporate metal atoms such that the requisite site properties are present, must be designed. Necessary design features include the use of nitrogen donor atoms and donor sets capable of holding the copper atoms in the correct coordination environment and at the correct intermetallic separation, and the presence of available bridging groups. Fine tuning of the ligand system by systematic variance of these features can then be used to develop the spatial features of the model. The advent of dinucleating ligands, which hold two metals within the same ligand framework, opened the door to a wide range of dinuclear complexes and to interest in their application as small molecule models for bimetallobiosites.

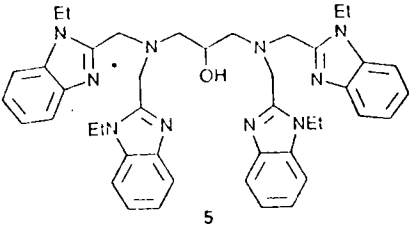
To date most research in this area has concentrated on the oxygen transport protein haemocyanin and the polyfunctional oxidase tyrosinase. We report here the synthesis and

characterisation of a first-generation model for the trinuclear copper site found in ascorbate oxidase.

3. Models for the Biosites

i. The endogenous bridge era.

Our early work concerning the use of dinucleating Schiff base macrocyclic ligands in the modelling of copper biosites was based on the premise that there was a protein-based endogenous bridge present in oxyhaemocyanin. Several model systems were extant in the literature and one of these was the polypodal ligand (5), derived by Reed *et als.*[13], which gave the dinuclear Cu^{II}-azido complex schematically depicted as (6). The physico-chemical properties of this complex [Cu···Cu separation (from the X-ray crystal structure), 3.62Å; diamagnetic; charge transfer band, 364nm] compare closely with those found for *met*-azidohaemocyanin and it was concluded that as "the popularity of phenoxide may be at odds with the absence of enhanced tyrosine vibrational modes in resonance Raman studies of oxyhaemocyanin.... alkoxide from serine or threonine now becomes a particularly viable candidate" for the EXAFS-predicted endogenous bridge.



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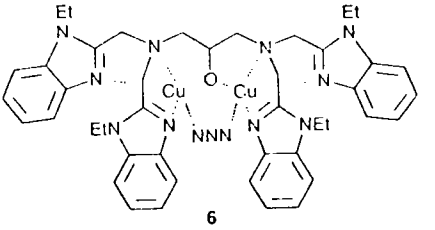

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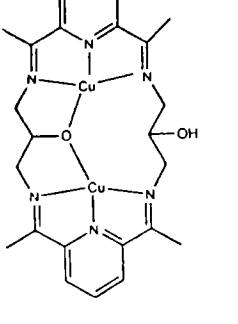
Our system was derived from the barium-templated cyclocondensation of 2,6-diacetylpyridine and 1,3-diamino-2-propanol followed by transmetallation with copper to give the dinuclear complex (7), the crystal structure of which was obtained[14]. This revealed a Cu..Cu separation of 3.64Å, and provided the first example of a structurally-characterised copper dimer with a single alkoxo-bridge. Electrochemical studies on the complex indicated that structural changes occurred during the reduction-oxidation cycle that were not incompatible with a bridge-breaking and -making process. If there is no endogenous bridge present in deoxyhaemocyanin, but there is such a bridge, even if it is hydroxide, in oxyhaemocyanin then a bridge- making and -breaking process must occur on reaction of the dicopper site with dioxygen. The complex (7) was therefore viewed as a speculative model for the haemocyanin site.



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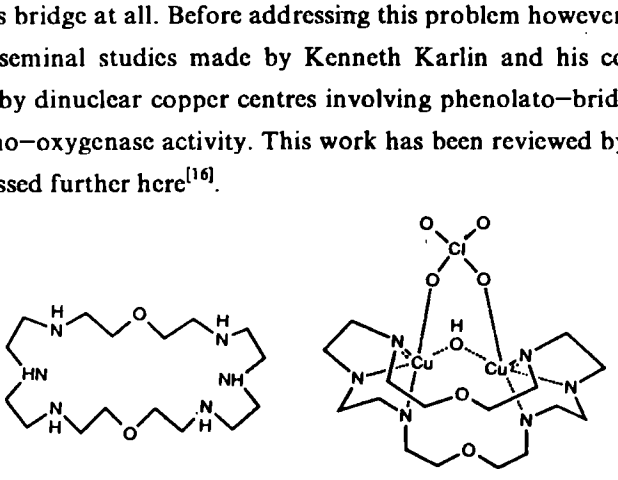
With the benefit of hindsight offered by the structure of deoxyhaemocyanin it is now accepted that phenolate (from tyrosine) and alkoxide (from serine or threonine) cannot act as the endogenous bridge and so one of the most cogent comments arising from the early modelling

studies was Lippard's interpretation[15] of his results from complex (8) which stated that "the stability and ubiquity of the Cu_2OH^{3+} unit suggest that the 'endogenous' protein bridging ligand might simply be the hydroxide anion ". It is now necessary to ask if there is any requirement for an endogenous bridge at all. Before addressing this problem however it is important to draw attention to the seminal studies made by Kenneth Karlin and his co-workers on the activation of dioxygen by dinuclear copper centres involving phenolato-bridges and on model systems for copper mono-oxygenase activity. This work has been reviewed by the investigators and so will not be discussed further here[16].



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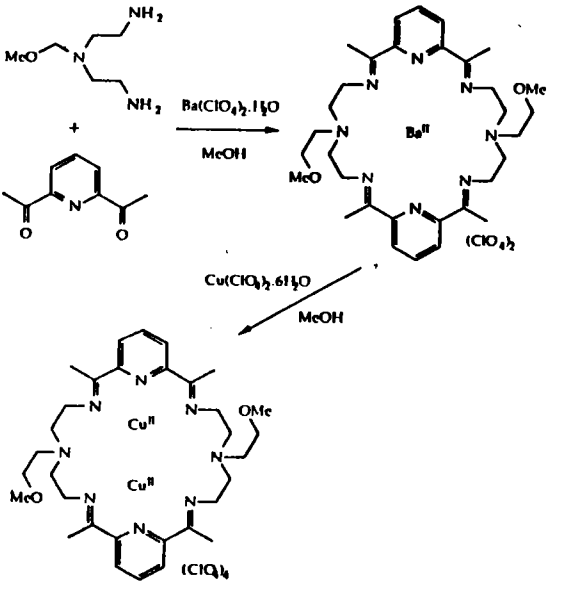
ii The post-endogenous bridge era.

During the course of studies on ligands capable of providing endogenous bridges it became apparent from the structures of several mononuclear barium complexes of functionalised tetraimine Schiff base macrocycles that the macrocyclic ligands had folded to present molecular clefts into which the metal ions coordinated[17]. This mode of metal incorporation is not dissimilar to that of metalloproteins in which the requisite metal is bound in a pocket or cleft produced by the conformational arrangement of the protein. Our objective then became the synthesis of flexible macrocycles capable of generating clefts for metal coordination without the presence of a ligand-based endogenous bridge. In order to do this a series of bibracchial, or doubly pendant armed, macrocycles were synthesised from 2,6-diacetylpyridine and a range of N, N-bis(3-aminopropyl)-and N, N-bis(2-aminoethyl)-alkylamines using barium or silver(Ⅰ) templates[18]. The resulting mononuclear barium complexes and dinuclear silver(Ⅰ) complexes were found to have the required conformation and in the latter cases the metals were separated by distances ranging from 2.9->6.0Å depending on the nature of the donor groups in the pendant arms and on the length of the carbon atom chains present in the lateral diamine derived spacers.

Transmetallation of the barium complex of the macrocycle derived from N, N-bis(2-aminoethyl)-2-methoxyethylamine readily gave a dinuclear copper(Ⅱ) complex. The X-ray crystal structure of which showed that the cleft conformation had been destroyed (Scheme 1 and Figure 1)[19]. As the primary objective at that time was to recover a complex in which the cleft conformation was retained and which could be used as an endogenous bridge-less haemocyanin model it was obviously necessary to rethink. It was decided that reduction of the imine bonds in the ligands would give a more flexible and more stable

ligand system and this was achieved by reductive demetallation of the disilver complexes using sodium borohydride. Reaction of the reduced metal-free macrocycles with copper(Ⅱ) then gave complexes of stoichiometry $Cu_3L(OR)_2(ClO_4)_4$, nH_2O[20].



Scheme 1

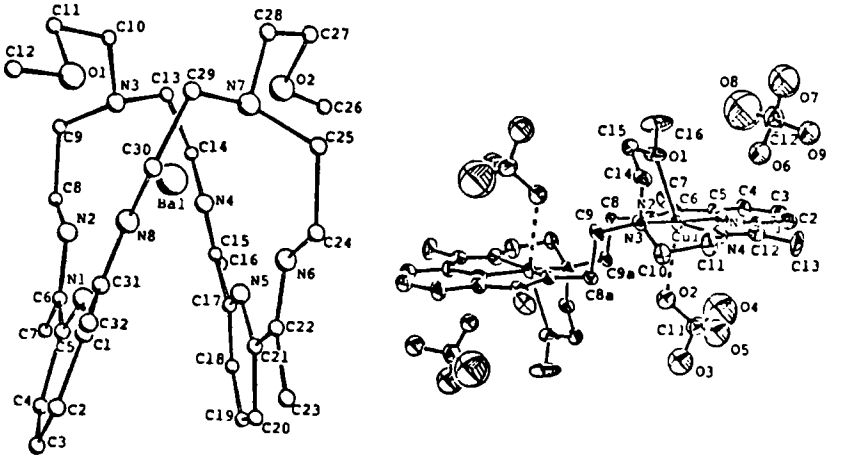
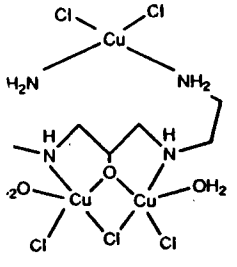


Figure 1 Molecular structures of the cations from the mononuclear barium and dinuclear copper(Ⅱ) complexes showing the opening up of the molecular cleft

In related work with the acyclic ligand 1, 11-diamino-6-hydroxy-4, 8-diazaundecane we had prepared a trinuclear complex $Cu_3LCl_5 \cdot 2H_2O$ and had assigned, from the F. A. B m.s

results and in the absence of suitable crystals for X-ray analysis[21], the formulation (9). It was noted that this compound displayed the basic features of the trinuclear site determined for ascorbate oxidase. Both of the above species have present a dinucleating centre which can be directly attached to a third coordinating site via donor atoms sited in pendant arms. Consequently it was possible to conceive that suitable modification of the pendant-armed macrocycles would lead to a model for the trinuclear copper site in ascorbate oxidase.



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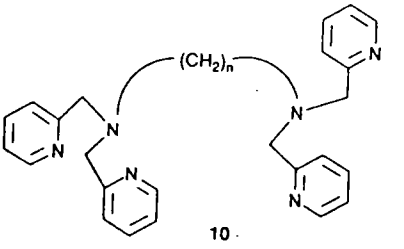

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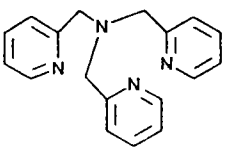
Before discussing the trinuclear model it would be appropriate to comment briefly on post-endogenous bridge models for haemocyanin. If no endogenous bridge is required then a model compound must be considered in which the unsupported Cu···Cu separation is *ca.* 3.6Å. A range of polypodal ligands in which the spacer groups between the podands have no bridging properties have been devised[22-24]; the podands used have been derived from pyridine, imidazoles, pyrazoles or amines. A constant feature of the dinuclear Cu^{II} complexes of these ligands is an 8.0Å, or greater, intermetallic separation; the small molecule model does not have the natural constraints imposed on it that the protein can offer the biosite and so there is a distinct synthetic challenge in this area. The dicopper(Ⅰ) complexes of the ligands (10) react reversibly with dioxygen and can be directly interconverted through several cycles with relatively little ligand decomposition[22]. This reinforces the concept that an endogenous bridge is not a pre-requisite for reversible dioxygen binding. The dicopper(Ⅱ) species formed in the experiments are believed to be peroxo-species and are e. p. r. -silent in frozen solution which suggests that a single μ-peroxo-bridge can promote the strong antiferromagnetism found in oxyhaemocyanin. A similar observation has been made for singly bridged dinuclear Cu^{II} complexes derived from two separate tridentate tris-(3, 5-dimethylpyrazolyl) borate tripodal ligands[25] thus promoting the thought that a dinucleating ligand may not be crucial to the modelling process. Recent publications with mononucleating ligands have provided exciting results concerning the possible nature of any bridge within oxyhaemocyanin.

Using the tetradentate tripodal ligand tris[2-pyridinyl] methyl-amine (11) Karlin and his coworkers have shown unambiguously that Cu^{I} complexes can reversibly bind dioxygen without the presence of an endogenous bridge[26]. The dinuclear Cu^{II}-peroxide species formed is e. p. r. -silent and the X-ray structure of this compound shows the presence of a *trans*-1, 2-bridging peroxide structure sustaining a Cu···Cu separation of 4.36Å. Whilst this is not a

precise model for the biosite it does eliminate the likelihood of a *trans*–1, 2–bridging peroxide at the biosite. More recently Karlin has shown that it is possible to reversibly react dioxygen with copper(I) complexes of 1, 2–dimethylimidazole[27].



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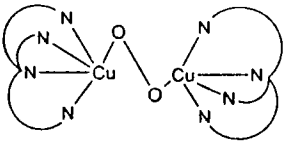

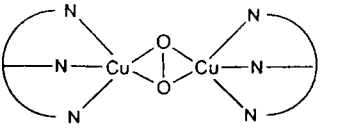

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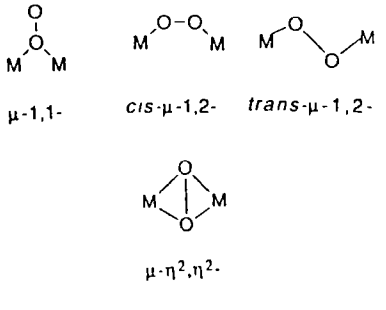
The strong antiferromagnetic coupling present in a μ–peroxo tris–pyrazolylborate complex has been referred to earlier[25]. The X–ray crystal structure of the complex derived from tris–(3, 5–di–isopropylpyrazolyl) borate, [Cu(HB–(3, 5–*i*Pr$_2$pz)$_3$)]$_2$[O$_2$], (12)], has been carried out and reveals a novel μ–η^2 : η^2 bridging coordination of the peroxide[28]. The Cu···Cu separation is 3.56Å, significantly shorter than that found in the complex derived from (11) where the *trans*–μ–1, 2–mode was found, and consistent with the EXAFS–derived distance of 3.58–3.60 Å for oxyhaemocyanin. The complex is diamagnetic and so the close physico–chemical comparisons between it and oxyhaemocyanin led to the postulation that, in the absence of a second bridging species, a peroxide could be bound in this novel way at a type–3 bimetallobiosite.

Binding modes for peroxide are depicted in Figure 2. A *cis*–μ–1, 2–bridging mode has generally been proposed for the peroxide in haemocyanin on the basis of magnetic and spectroscopic measurements but there has been no direct evidence for this binding mode. From model studies it has been noted that one–atom endogenous bridges support a second one–atom exogenous bridge with Cu··· Cu separations of *ca* 3.0Å whereas when the second bridge is composed of two–atoms the separation is *ca* 3.6Å[29]. Consequently a μ–1, 1–mode for the peroxide binding has been disregarded and until the publication of the above work a μ–1, 2–mode was regarded as more compatible with the known intermetallic separation. Last year the crystal structure of oxyhaemocyanin was finally solved and the presence of the μ–η^2 : η^2 bridging coordination of the peroxide was confirmed[30]. This therefore provides a success story for modelling studies.



μ-1,1- *cis*-μ-1,2- *trans*-μ-1,2-

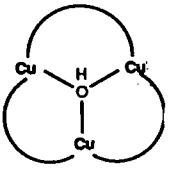
μ-η^2,η^2-

Figure 2 Binding modes of peroxide

4. The Trinuclear Site Model

Synthetic analogues for the trinuclear site in ascorbate oxidase and the related 'blue'

oxidases are scarce. There are numerous examples in the literature of hydroxo–bridged triangulo–copper(Ⅱ) complexes, depicted schematically in (13)[31]. They are mostly based on equilateral triangles of copper atoms with intermetallic distances close to 3.0Å and supported by at least one μ_3–hydroxo bridge; there is a single complex derived from a polytopic macrocyclic ligand which has a double μ_3–hydroxo bridge[32]. One hexanuclear copper(Ⅱ) complex, derived from a polypodal ligand (14, X=N_3), has been reported[33] in which there are two approximately isosceles triangular arrays of copper(Ⅱ) atoms present, each having type–3–like pairs of copper atoms having 3.11Å separation and supported by an endogenous phenoxo–bridge derived from the ligand; the third copper is distant from the pair by 7.78 and 7.46Å respectively.



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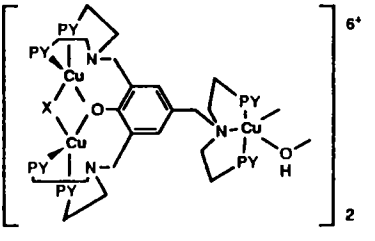
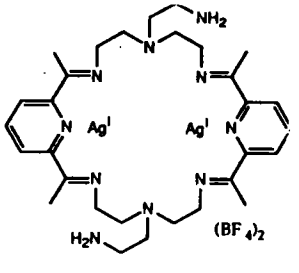


14 X =OH, N_3 ; PY = 2-pyridyl

In our attempt to prepare a synthetic analogue for trinuclear site in ascorbate oxidase the disilver diprimary amine pendant armed macrocyclic complex (15) was first prepared *via* the silver(Ⅰ) templated [2+2] cyclocondensation of *tris*(2–aminoethyl) amine (*tren*) and 2, 6–diacetylpyridine[34]. The structure of the dication shows the ligand to have the cleft conformation with the silver ions bound in the diimino pyridyl head units of the macrocycle and separated by 3.17Å. Functionalisation of (15) in order to introduce a strong exo–macrocyclic copper binding site was achieved through reaction with salicylaldehyde to yield the dinuclear silver(Ⅰ) complex (16). Addition of one equivalent of copper(Ⅱ) acetate and two equivalents of copper(Ⅱ) tetrafluoroborate to a refluxing solution of (16) in methanol / acetonitrile effected transmetallation; addition of an excess of sodium perchlorate then led, on cooling, to the isolation of dark green crystals analysis of which indicated the formation of the tricopper(Ⅱ) hydroxo species $Cu_3(OH)(L)(ClO_4)_3 \cdot 3H_2O$, (17).



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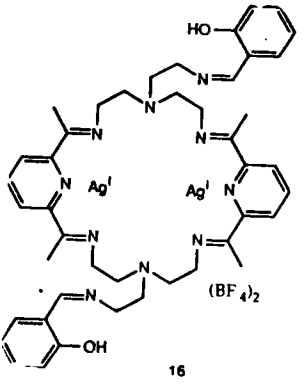

16

X-ray crystallography confirmed the presence of a discrete trinuclear copper species bound within the macrocyclic framework (Figure 3)[35]. The cluster is comprised of a μ_2-hydroxo bridged pair, Cu(1) and Cu(2), and a non-bridged copper atom Cu(3). The two metal ions of the dinuclear moiety are separated by 3.6Å with a Cu(1)-OH(1)-Cu(2) angle of 137.8°. A scalene triangular array is completed by the third copper atom Cu(3) with Cu(1)-Cu(3) and Cu(2)-Cu(3) distances of 5.9Å and 4.9Å respectively.

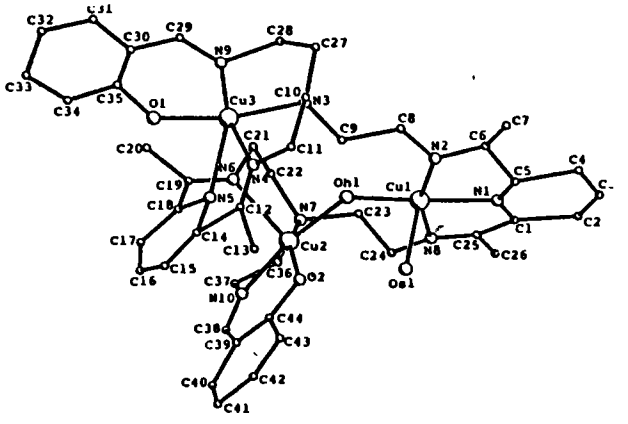

Figure 3 Molecular structure of the cation from the trinuclear copper(Ⅱ) complex (17) showing the opening up of the molecular cleft

The coordination geometries around the copper atoms of the hydroxo bridged pair, Cu(1) and Cu(2), may be described as distorted square based pyramidal. The basal donors of the former are provided by the nitrogen atoms, N(1), N(2) and N(8), of one pyridine-diimine unit and the bridging hydroxide OH(1). The axial site is filled by the oxygen atom Os(1) of a water molecule. The other copper atom, Cu(2), of the dinuclear moiety is coordinated by the donor atoms, O(1) and N(10), of a salicylaldimine pendant arm, a tertiary amino nitrogen N(7) of the macrocyclic ring, the bridging hydroxide OH(1) and one of the imine nitrogen atoms N(6) of the second pyridine-diimine unit. The source of the hydroxide ion is most likely to be water present in the reaction medium, originating either from the hydration sphere of the copper(Ⅱ) salts employed in the transmetallation or alternatively from the solvent itself, with the two copper(Ⅱ) atoms acting in concert as a super acid pair to promote the generation of a nucleophile. The presence of the hydroxo-bridge reinforces the statement by Lippard[15] that the endogenous bridging protein ligand proposed for type-3 biosites might simply be the hydroxide anion itself, generated from accompanying water molecules.

The third copper atom Cu(3) also has a distorted square pyramidal coordination environment derived from the donors, O(1) and N(9), of the second pendant arm, a tertiary amino nitrogen N(3), together with the remaining imine nitrogen N(4) from the second macrocyclic head unit and a pyridyl nitrogen N(5). The coordination mode of the pyridyl nitrogen N(5) to the copper ion Cu(3) is unusual since the copper atom lies 1.17Å out of the plane of the

pyridine ring; thus the nitrogen lone pair does not point directly at the metal ion.

The trinuclear site in ascorbate oxidase may be subdivided into a type–3 pair of copper atoms, separated by *ca* 3.4Å, with six histidine ligands arranged in a trigonal antiprismatic configuration[5] this structural feature being similar to that found in deoxyhaemocyanin. The copper atoms are bridged by an oxo– or hydroxo– ligand. The remaining type–2 copper has two histidines, an oxygen derived ligand, possibly OH^- or H_2O, and the fourth coordination site has been attributed to the pair of copper atoms which are 3.9 and 4.0Å distant from it. There are therefore significant differences between the coordination geometries found in (17) and the metallobiosite as shown schematically in figure 4; the detection of a hydroxo–bridge in (17) does however give credence to the probability that the bridge in ascorbate oxidase is of the same type.

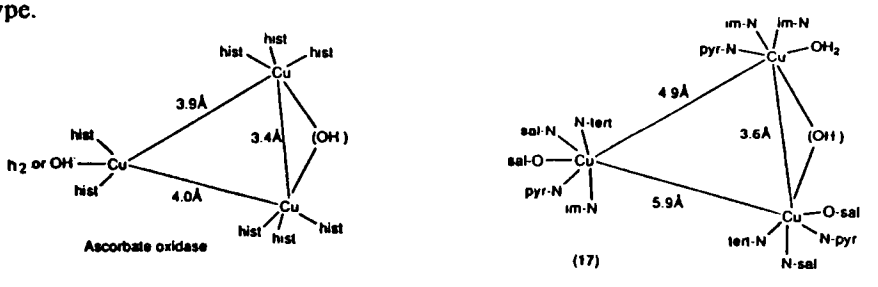

Figure 4 A comparison of the trinuclear arrays in ascorbate oxidase and (17)

Magnetic susceptibility measurements carried out on (17) in the temperature range 5 to 300K are consistent with a system composed of an antiferromagnetically coupled copper(Ⅱ) pair and a magnetically independent third copper(Ⅱ) ion. The macrocyclic complex therefore reproduces qualitatively features of the oxidase active site and the observed $2J$ value of $-192cm^{-1}$ indicates the presence of a moderate antiferromagnetic interaction within the dinuclear moiety, albeit weaker than that of the protein type–3 site which is diamagnetic even at room temperature.

Conclusion

The trinuclear copper(Ⅱ) can not be claimed to provide a precise replication of the ascorbate oxidase cluster; the coordination spheres of the metal ions differ both in terms of the nature and the geometric arrangement of the donor atoms. The small molecule derived cleft has a greater degree of conformational freedom than the more highly defined proteinaceous clefts and so the need to design in features to constrain this mobility is apparent. The trinuclear copper(Ⅱ) complex does however serve as a first generation model for a type–3 oxidase site in that it reproduces features of the biosite to a greater extent than any synthetic complex previously reported.

Coordination chemistry therefore has an important part to play in our attempts to simulate and understand the roles of metals at metallobiosites. It is however essential to remember that the model is a small molecule and so is not in ant way constrined by the immediacy of a protein

environment and the accompanying subtle roles that the folded protein chains may play in governing the nature of the biosite. Therefore whilst a model may accurately reproduce the physico–chemical properties of a site it may not be able to respond in a functional manner.

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