

Copper biosites: the merits of models

David E. Fenton

Department of Chemistry, The University, Sheffield S3 7HF, U.K.

Abstract - The role of dinuclear copper complexes as speculative models for the oxygen carrying site in haemocyanins is discussed.

INTRODUCTION

"Many inorganic chemists first become involved in inorganic biochemistry in attempts to model some feature of a metalloprotein or metalloenzyme, the purpose being to reproduce some feature of the structure or the function, and then allow comparison with related systems and ease experimental or theoretical investigations. The work cannot be separated from the chemistry of the element in question, for example, the chemistry of copper in a metalloprotein differs in detail, but not in kind, from the rest of copper chemistry." (ref.1).

What is meant by model? A naive question but maybe necessary as there is a school of thought which suggests that a model is only a model if it works. The Oxford English dictionary is quite clear (ref.2).

MODEL: Representation in three dimensions of proposed structure etc., especially on a smaller scale (WORKING model); simplified description of system etc. to assist calculations and predictions.
WORKING MODEL: of machine etc., able to operate, though on reduced scale.

Hill introduced two useful definitions, the SPECULATIVE MODEL, where the structure of the microenvironment of the metal ion in the biological system is unknown and the objective is to reproduce some property (spectroscopic) of the system in a simpler complex, and the CORROBORATIVE MODEL where the structure of the metallobiosite is known and so the synthesis of a corroborative system may occur (ref.1). To these one might add the FUNCTIONAL MODEL whereby the mode of action of the metallobiosite is also reproduced. It is important throughout to "define one's objectives" with care and to perhaps bear in mind a couplet written by Robert Frost (ref.3),

'A theory, if you hold it hard enough
and long enough, gets rated as a creed.'

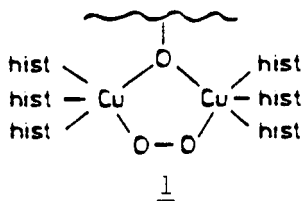
THE CONCEPT OF MODELLING SITES

Essentially one is attempting to imitate aspects of the metalloprotein such as spectral properties, structural features and reactivity and to use the information gained to develop and correlate the structure and function of the biomolecule. The chemistry of an ion depends on its immediate surroundings and so the closer the correspondence between the environment in a model system and that in the biomolecule the greater the resemblance between the chemistries involved.

THE SITE IN QUESTION

Oxy- and met-haemocyanins

Haemocyanin is the multisubunit dioxygen-carrying protein found in the haemolymph of several species of the phyla Mollusca and Arthropoda. Collective evidence from vibrational, magnetic, chemical and electronic data suggest that the site requires a dinuclear copper(II) centre with both endogenous and exogenous bridges present in order to mediate strong anti-ferromagnetic coupling leading to diamagnetism (1) (ref.4). The exogenous bridge is peroxide in oxy-haemocyanin and azide, acetate etc., in met-haemocyanins. The nature of the endogenous bridge is not yet established



but is suggested as being derived from an oxygen atom (EXAFS data and 345 and 570nm bands in the electronic spectrum). Hydroxide, alkoxide, phenoxide and carboxylate have all been listed as contenders for this bridging role, however the absence of enhanced tyrosine vibrations in resonance Raman experiments (ref.5) together with homologous sequencing studies (ref.6) indicate that there is no conserved tyrosine present and so a phenoxide bridge is unlikely. EXAFS studies show the presence of terminal nitrogen or oxygen donor atoms of which two per copper are imidazole nitrogen atoms from histidine residues and that the Cu...Cu separation is ca. 3.6Å. The copper atoms are in approximately tetragonal environments (ref.7).

Deoxyhaemocyanin

The site contains two copper(I) atoms and so is essentially spectroscopically invisible. EXAFS gave an ambiguous result as although two imidazoles per copper with a Cu...Cu separation of ca. 3.5Å are indicated it is not possible to state whether there is an endogenous bridge, a single ligand leading to one two-coordinate and one three-coordinate copper(I) or no endogenous ligand at all (ref.7). Carbon monoxide uptake experiments show that only one carbon monoxide binds per copper pair and this reinforced the thought that there are two coordination environments as carbon monoxide would be expected to bind more readily to a three-coordinate copper(I) atom (ref.8). The X-ray crystal structure of haemocyanin derived from the spiny lobster *Panulirus Interruptus* (Fig.1) shows three histidines per copper atom with a Cu...Cu separation of ca. 3.7Å. There is NO bridging ligand and the nearest tyrosine is ca. 10.6Å distant (ref.9).

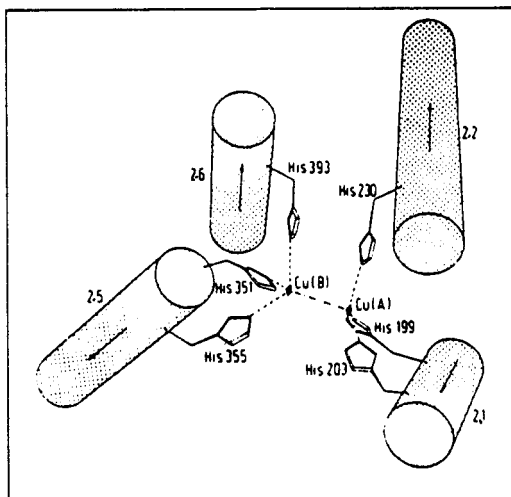


Fig. 1. The dicopper(I) site in deoxyhaemocyanin (reproduced from Rev.Quim.Port., 27, 42-44,(1985) with permission).

AN APPROACH TO SITE MODELLING

The need to simulate a homodinuclear site is clear. In order to do this a versatile ligand system with potential for modification to be able to accommodate metals in such a way that the required site properties can be introduced must be designed. Necessary design features include the use of requisite ligand donors, the presence of appropriate donor sets and the presence of available bridging groups (endogenous or exogenous). Fine-tuning of the ligand system may then be achieved by the systematic variance of one or more pertinent features such as the ligand donor type, the ligand donor arrangement, the coordination geometry at the metal and the inter-metallic separation in order to develop the spatial features of the model.

SPECULATIVE MODELS FOR HAEMOCYANINS

Subsequent to Robson's initial studies on dinucleating Schiff base ligands derived from 2,6-diformyl-4-methylphenol (ref.10) there has been much interest in the use of such compounds as small molecule models for dicopper-biosites (ref.11). The relative rigidity of the ligand framework, imposed by the diiminophenolic head-units limits the Cu...Cu separation to ca.3.0Å as compared to ca.3.6Å in the biosites and so attempts to define more flexible systems have been made. The nature of the endogenous bridge was conjectural and so models incorporating alkoxy-(serine, threonine), aryloxy-(tyrosine) or hydroxy- groups were assembled and studied; representative examples are depicted below (Fig.2).

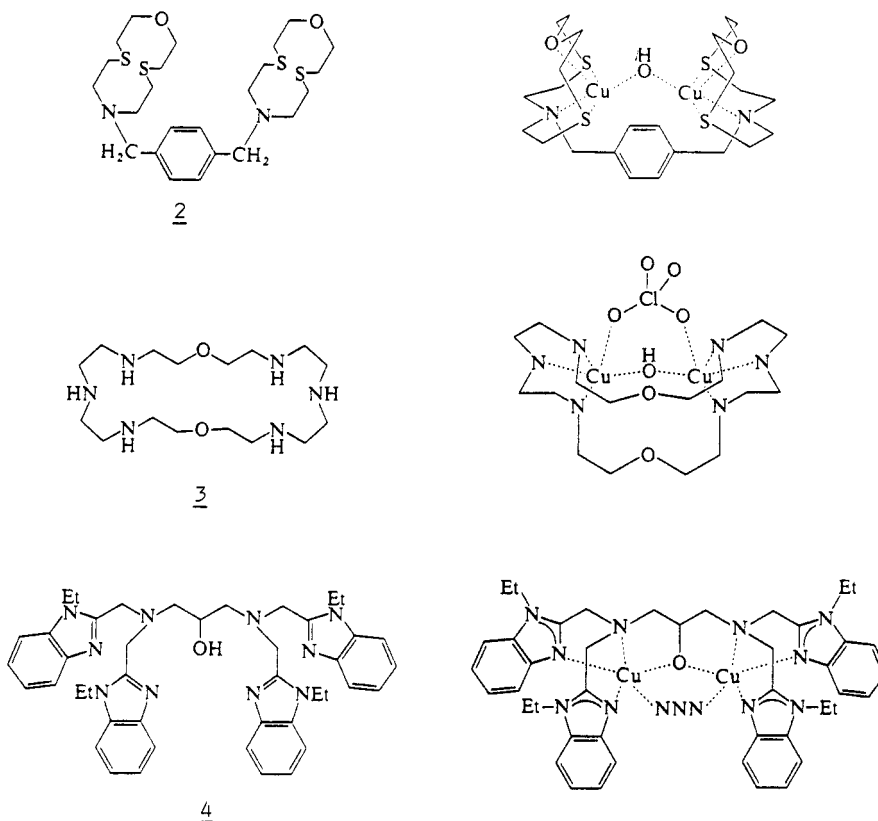
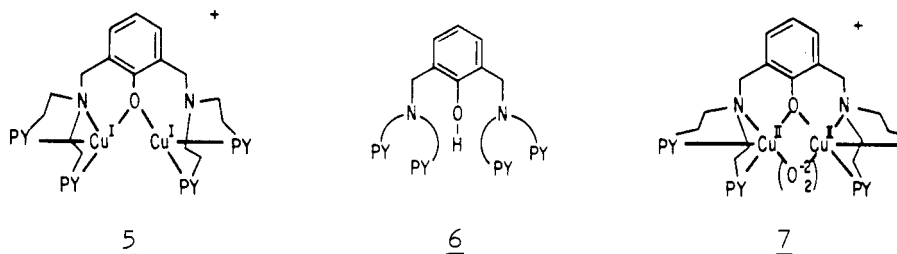


Fig. 2. The dinucleating ligands 2, 3 and 4 together with schematic representations of the corresponding dicopper(II) complexes.

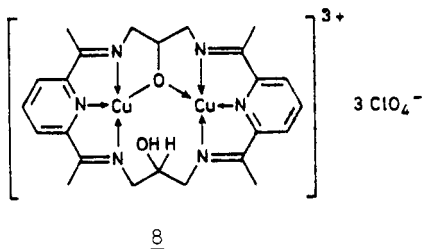
These compounds were found to parallel the physico-chemical properties (e.g. the strong antiferromagnetic coupling, the Cu...Cu separation of ca. 3.5Å) of the dicopper(II) site sufficiently closely that as well as being considered to provide supporting evidence for an aminoacid-based endogenous bridge the comment was made that ,from the complex of (3), "the stability and ubiquity of the Cu₂OH unit suggest that the 'endogenous' protein bridging ligand might simply be the hydroxide ion" (ref.13).

Viable physical models for the biosite are therefore accessible but, with the advantage of hindsight offered by the structure of deoxyhaemocyanin, should the alkoxy- or aryloxy-endogenous bridge be viewed as a necessary component of a model system? If it is regarded simply as a suppositional model for a putative one-atom bridge then it can be used as a device to enable constraints to be imposed on the Cu...Cu separation such that the use of flexible ligands which include such a bridge make the requirement for a second exogenous bridge more explicit. Such one-atom endogenous bridges have been found to support second one-atom bridges with Cu...Cu separations of ca. 3.0Å but when the second bridge is a two-atom system then the Cu...Cu separation is increased and approaches 3.5Å (ref.15). This information can then be offered as support for the proposition that the peroxide binds to the dicopper(II) unit in oxyhaemocyanin in a 1,2-bridging mode.

A most influential study has been made using the dicopper(I) complex (5) of the ligand (6) which has present an endogenous phenoxy-bridge and a Cu...Cu separation of ca. 3.6Å (ref.16). This system will react with dioxygen below -50° to give a peroxy-bridged dicopper(II) complex (7). The application of a vacuum whilst warming solutions of (7) leads to the removal of dioxygen and the regeneration of (5). Here the function of the biosite has been reproduced in part. It is also of interest to note that the ligand itself is prepared from the copper-mediated hydroxylation of its arene precursor and so this reaction is itself considered to be a model system for copper mono-oxygenase activity (ref.17).



If the endogenous bridge is derived from the water present in the protein structure then it is required that a bridge-making and -breaking process occurs on reaction of the dicopper unit with dioxygen. Electrochemical studies on the complex (8) indicate structural changes during the oxidation reduction reaction that are not incompatible with such a process (ref.18).



The structure of the dicopper(II) complex bears resemblance to that proposed for metaquahaemocyanin (Fig.3).

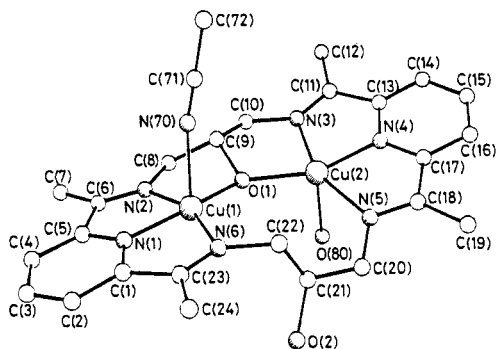
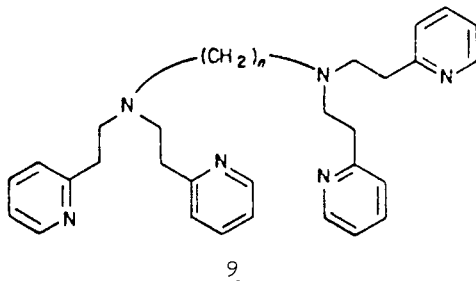


Fig. 3. The molecular structure of the dicopper(II) complex of 8 (reproduced with permission from reference 18).

If no endogenous bridge is required then a model compound for deoxy-haemocyanin must be devised in which the 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 (refs.19-21); the podands present are derived from pyridine, imidazoles, pyrazoles or amines. A constant feature of the dinuclear copper(II) complexes of these compounds is a long Cu...Cu separation of >8.0Å. 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 ligands (9) do however react reversibly with dioxygen and can



be directly interconverted through several cycles with relatively little decomposition to alternative dioxygen pickup (at -80°) and release (through warming under vacuum) (ref.19). This indicates that the presence of an endogenous bridge is not necessarily a prerequisite for a dicopper system to bind dioxygen reversibly. The dicopper(II) species is likely to be peroxo-bridged and preliminary results in frozen solutions show that this species is epr-silent. This suggests that a single peroxo-bridge is sufficient to promote the strong antiferromagnetic coupling displayed in oxyhaemocyanin. A similar result has also been obtained for a singly peroxo-bridged dinuclear copper(II) complex derived from two separated tris-pyrazoylborate tripodal ligands (ref.22).

CONCLUSIONS

The value of models will always be questioned - particularly, by their very definition, that of speculative models. One of the difficulties encountered in simulating a biosite is that as time passes the target may change with advancing knowledge. This is evidenced here in that the crystal structure of one form of the biosite is now available and has clearly enhanced our understanding of that site. Whilst much interesting chemistry has been made available during the search for models for the dicopper site in haemocyanins there are still many advances to be made in the simulation of the biosite. Of particular need are systems which will function at room temperature and in aqueous media. These will eventually arrive. Even if they do not the contribution of the models should not be judged in isolation as the 'spin-off' to other areas is also of value. For example the study of magnetic coupling, oxidation catalysis and basic ligand design have all benefitted substantially from work carried out in the above area.

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ADDENDUM

Shortly after the submission of this article Karlin et al. reported the crystal structure and characterisation of a reversible oxygen-binding system. By using the tripodal ligand tris[2-pyridyl]methylamine they were able to demonstrate the ability of mononuclear copper(I) complexes to reversibly bind dioxygen without the presence of an endogenous bridge. Furthermore the dicopper(II)peroxo-complex formed was e.p.r.-silent showing that the trans-1,2-bridging peroxide ligand is capable of mediating a strong antiferromagnetic coupling. The complex is not a precise model for oxyhaemocyanin as the Cu...Cu separation is 4.36Å and there are also differences in the coordination geometry and the electronic spectra. (R.R.Jacobson, Z.Tykelar, A.Farooq, K.D.Karlin, S.Liu and J.Zubieta, J.Amer.Chem.Soc., 110, 3690-3692, (1988).)