Model Complexes for Ligand Probes of Met-hemocyanin and Met-tyrosinase Derivatives. Structure of a Novel 1,1-Azido Bridged Binuclear Copper(II) Complex

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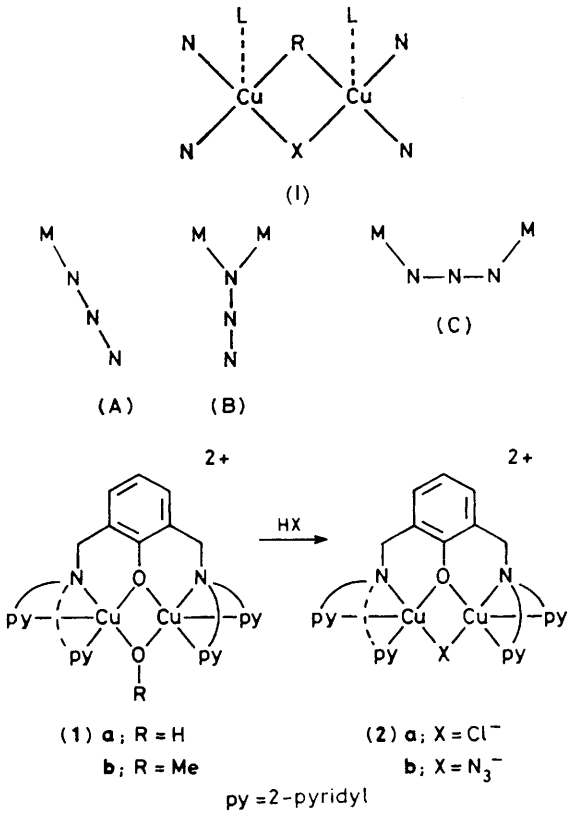
Addition of HX ($X = N_3^-$, Cl^-) to a hydroxy-bridged binuclear copper(II) complex gives X-bridged complexes with an environment analogous to protein binuclear sites; the structure of the azide derivative shows a novel μ-1,1-bridging mode, suggesting this as a possibility in the biological systems.

From extensive spectroscopic and chemical investigations,[1-3] a detailed picture of the active sites of the copper proteins hemocyanin (arthropod and mollusc dioxygen carrier) and tyrosinase (mono-oxygenase which hydroxylates mono-phenols) has emerged. Both these proteins contain similar binuclear copper active sites which in the reduced state are proposed to contain two[2] or three[3] co-ordination by imidazole to copper(I). Upon oxygenation, a bicopper(II) complex is formed in which two tetragonal copper(II) ions separated by 3.6 Å are bridged by an endogenous R group (thought to be a phenolate from a tyrosine moiety) and the exogenous μ-1,2-peroxo ligand X derived from dioxygen [structure (I)].

In establishing the nature of the active site, investigations have relied heavily upon spectroscopic studies of the products of binding and interactions of small molecules such as acetate, chloride, azide, *etc.*, in chemically modified hemocyanin or



tyrosinase derivatives.[1,4,5] Details such as Cu · · · Cu distances in the active sites, differences amongst mollusc and arthropod hemocyanins, and electron delocalization in half-met (Cu^{II}–Cu^{I} derivatives) have been determined from derivatives in which these ligands may act as Cu–Cu bridging groups X. However, because of the limited resolution obtainable on studies of the oversized and low symmetry proteins, such conclusions must in large part be based on detailed studies of binuclear copper model systems containing these ligands. This is due to the unique spectroscopic and structural properties conferred by a dicopper centre.[6]

Azide ligand binding to protein binuclear copper centres has played a key role in bioinorganic studies. This is because of the occurrence of strong charge-transfer absorptions in the visible region, accessibility by i.r. and e.s.r. spectroscopic techniques and its variable mode of binding.[7] Terminal (A),[8–10] 1,1-bridging (B),[10–12] and 1,3 bridging (C)[8–10,13] are all known in copper chemistry, and both modes (A) and (C) have been proposed to occur in met-hemocyanin derivatives.[1,4]

Because of the importance of azide co-ordination in protein studies, we and others have sought to examine its co-ordination properties in relevant model systems. Here, we report the synthesis and structural characterization of a novel μ-1,1-azido bridged binuclear Cu^{II} complex. This is the first such compound also containing a μ-phenolate bridge having tetragonally co-ordinated Cu^{II} ions, thus mimicking structural and co-ordination aspects of the protein active centres.

We recently reported the synthesis and characterization of a binuclear copper complex containing three-co-ordinate Cu^{I} ions with amino and pyridine nitrogen donor atoms. Addition of dioxygen to this complex results in the hydroxylation of the *meta*-xylyl binucleating ligand producing phenolate-bridged, tetragonally co-ordinated, binuclear Cu^{II} complexes (**1**).[14] Reaction of complex (**1**) with HX (X = azide, chloride) gives high yields of the X-bridged complexes (**2**), which have been characterized by *X*-ray crystallography.

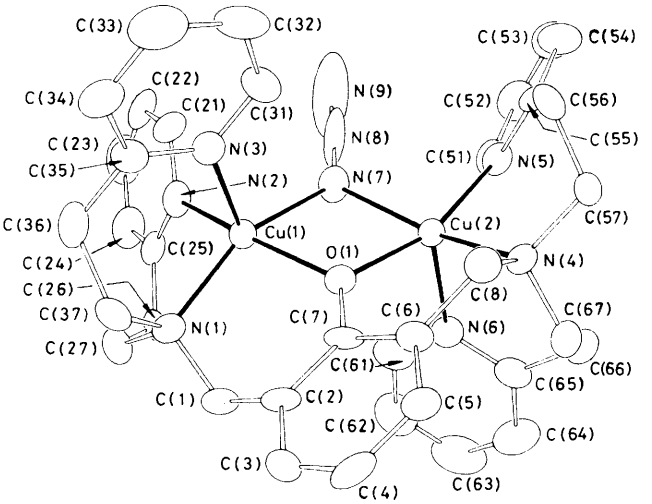


Figure 1. ORTEP diagram of the $\{Cu_2(C_{36}H_{39}N_9O)\}$ dication of (**2b**) showing the atom labelling scheme. Selected bond lengths (Å) and angles (°) are Cu(1) · · · Cu(2), 3.185(3); Cu(1)–O(1), 1.963(8); Cu(1)–N(1), 2.057(10); Cu(1)–N(2), 1.985(10); Cu(1)–N(3), 2.204-(10), Cu(1)–N(7), 2.024(12), Cu(2)–O(1), 1.976(8); Cu(2)–N(7), 2.028(12); N(7)–N(8), 1.12(2); N(8)–N(9), 1.15(3); ∠O(1)–Cu(1)–N(7), 74.4(4); O(1)–Cu(2)–N(7), 74.0(4); O(1)–Cu(1)–N(1), 94.1-(3); O(1)–Cu(1)–N(2), 158.9(4); O(1)–Cu(1)–N(3), 96.0(3); N(1)–Cu(1)–N(2), 94.9(4); N(1)–Cu(1)–N(3), 97.5(4); N(1)–Cu(1)–N(7), 155.6(5); N(2)–Cu(1)–N(3), 101.7(4); N(2)–Cu(1)–N(7), 89.9(4); N(3)–Cu(1)–N(7), 104.9(4); Cu(1)–N(7)–N(8), 130.5(12); Cu(2)–N(7)–N(8), 125.1(11).

The chloro-bridged dication (**2a**) was isolated by the dropwise addition of 1.5 equiv. of aqueous 0.1 M HCl to an acetone–dimethoxypropane solution of (**1a**) (PF_6^- salt). The brown solution obtained was stirred for 24 h and a 3-fold excess of $NaBPh_4$ in acetone was added. Precipitation with diethyl ether, followed by recrystallization from dimethylformamide–ether, gave a 60% yield of brown crystalline material.† Complex (**2b**) was isolated by dropwise addition of 1.5 equiv. of Me_3SiN_3 in MeOH to a suspension of (**1a**) in MeOH–dimethoxypropane. Ether was added to the green-brown solution to precipitate the product. Recrystallization from CH_2Cl_2–Et_2O afforded *X*-ray quality crystals of (**2b**).†

Crystal data: $[Cu_2C_{36}H_{39}N_6O(N_3)](PF_6)_2$, triclinic, space group $P\bar{1}$, $a = 9.583(1)$, $b = 10.123(2)$, $c = 23.758(4)$ Å, $\alpha = 87.19(1)$, $\beta = 88.83(1)$, $\gamma = 84.85(1)°$, $U = 2292.5(6)$ Å^3, and $Z = 2$. The positional parameters of the copper atoms were determined by the Patterson method. The remaining atoms were located on difference Fourier maps. A total of 3077 unique reflections were refined to $R = 0.075$ and $R_w = 0.073$. (Mo-K_α, $\bar{\lambda} = 0.71069$ Å).‡

The structure of the dication of (**2b**) is shown in Figure 1. The novel feature of the structure is the μ-1,1-bridging azide ligand which has replaced the bridging hydroxide group of (**1a**). The co-ordination is very similar to that found in the OH or OMe bridged complexes (**1**) with little variation in bond angles or distances. The geometry around each Cu^{II} ion is distorted square pyramidal, with one pyridine N, the amino N, the phenolate O(1), and the azide N(7) in the basal positions.

† Satisfactory C, H, and N analyses were obtained for compounds (**2a**) and (**2b**). The structure of (**2a**) is analogous to that of (**2b**), and will be reported elsewhere. For (**2a**), λ_{max} 375 (ϵ 2100), 430 (1560), and 660 nm (250).

‡ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

The other pyridine nitrogen atoms, N(3) or N(6), occupy the apical positions, and are related by an approximate two-fold symmetry axis passing through the O(1)–N(7) vector. The azide ligand is nearly linear with $\angle$N(7)–N(8)–N(9) = 174(2)°; N(7)–N(8) = 1.12(2) and N(8)–N(9) = 1.15(3) Å. The Cu(1) · · · Cu(2) distance is 3.185(3) Å, *ca.* 0.1 Å longer than in (**1b**).

The azide complex (**2b**) exhibits ligand-to-metal charge transfer (L.M.C.T.) band maxima at 368 (ϵ 2600 l mol^{-1} cm^{-1}) and 462 nm (ϵ 3310), and a d–d absorption at 655 nm (ϵ 440). The 368 nm band is assigned to a Cu^{II}–phenolate L.M.C.T. absorption since it is also observed in compounds (**1**) and other phenolate-bridged copper complexes.[15] Thus, the new, relatively low energy band at 462 nm is ascribed to the azide-to-copper C.T. transition. Absorptions ranging from 380 to 500 nm have been assigned to azide-to-Cu^{II} C.T. transitions in azido derivatives of various hemocyanins.[4] I.r. bands due to azide ligation are observed for (**2b**), a strong absorption, ν_{asym} (N_3^-), occurs at 2068 and ν_{sym} (N_3^-) is found at 1280 cm^{-1}. Complexes of type A[7] and C[8,12,13a] also show similar absorptions; thus, azide i.r. absorptions appear *not* to be diagnostic of the mode of co-ordination.[7,12,13a]

Azide binding as a probe for binuclear copper centres in metalloproteins has proved to be very important and will continue to be so in the elucidation of the structure of the binuclear site (type III) in 'blue' copper oxidases (*e.g.* laccase[16]) and on distinguishing structure and reactivity differences among the hemocyanins.[4,6] The present results direct attention to the possible occurrence of 1,1-azido bridging (concurrent with OR bridging) in binuclear Cu centres and consequent structural features such as Cu · · · Cu $<$ 3.2 Å. In addition, the resemblance of the i.r. and visible absorption properties of (**2b**) (co-ordination mode B) with those of type A or C point to the dangers of structural conclusions based on these criteria. Further investigations on the detailed spectroscopy of azide co-ordination (and magnetic properties) are needed.

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References

1 E. I. Solomon, in 'Metal Ions in Biology.' vol. 3, ed. T. H. Spiro, Wiley-Interscience, New York, 1981, pp. 41—108.
2 J. M. Brown, L. Powers, B. Kincaid, J. A. Larrabee, and T. H. Spiro, *J. Am. Chem. Soc.*, 1980, **102**, 4210.
3 (a) M. S. Co, K. O. Hodgson, T. K. Eccles, and R. Lontie, *J. Am. Chem. Soc.*, 1981, **103**, 984; (b) M. S. Co and K. O. Hodgson, *ibid.*, 1981, **103**, 3201.
4 R. S. Himmelwright, N. C. Eickman, C. D. LuBien, and E. I. Solomon, *J. Am. Chem. Soc.*, 1980, **102**, 5378.
5 (a) R. S. Himmelwright, N. C. Eickman, C. D. LuBien, K. Lerch, and E. I. Solomon, *J. Am. Chem. Soc.*, 1980, **102**, 7339; (b) M. E. Winkler, K. Lerch, and E. I. Solomon, *ibid.*, 1981, **103**, 7001.
6 'Copper Coordination Chemistry: Biochemical and Inorganic Perspectives,' eds. K. D. Karlin and J. Zubieta, Adenine Press, Albany, New York, 1983, in the press.
7 Z. Dori and R. F. Ziolo, *Chem. Rev.*, 1973, **73**, 247.
8 Y. Agnus, R. Louis, and R. Weiss, *J. Am. Chem. Soc.*, 1979, **101**, 3381.
9 M. G. B. Drew, M. McCann, and S. M. Nelson, *J. Chem. Soc., Chem. Commun.*, 1979, 481.
10 J. Comarmond, P. Plumeré, J.-M. Lehn, Y. Agnus, R. Louis, R. Weiss, O. Kahn, and I. Morgenstern-Badarau, *J. Am. Chem. Soc.*, 1982, **104**, 6330.
11 J. Pickardt, *Z. Naturforsch., Teil B*, 1982, **37**, 110.
12 M. G. B. Drew, J. Nelson, F. Esho, V. McKee, and S. M. Nelson, *J. Chem. Soc., Dalton Trans.*, 1982, 1837.
13 (a) V. McKee, J. V. Dagdigian, R. Bau, and C. A. Reed, *J. Am. Chem. Soc.*, 1981, **103**, 7000; (b) T. R. Felthouse and D. N. Hendrickson, *Inorg. Chem.*, 1978, **17**, 444; (c) R. F. Ziolo, A. P. Gaughan, Z. Dori, C. G. Pierpoint, and R. Eisenberg, *ibid.*, 1971, **10**, 1289.
14 (a) K. D. Karlin, Y. Gultneh, J. P. Hutchinson, and J. Zubieta, *J. Am. Chem. Soc.*, 1982, **104**, 5240; (b) K. D. Karlin, P. L. Dahlstrom, S. N. Cozzette, P. M. Scensny, and J. Zubieta, *J. Chem. Soc., Chem. Commun.*, 1981, 881.
15 E. W. Ainscough, A. H. Bingham, A. M. Brodie, J. M. Husbands, and J. E. Plowman, *J. Chem. Soc., Dalton Trans.*, 1981, 1701.
16 D. J. Spira, M. E. Winkler, and E. I. Solomon, *Biochem. Biophys. Res. Commun.*, 1982, **107**, 721.