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Copper(II) Hemocyanin Models

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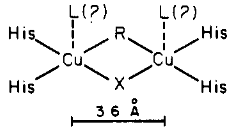
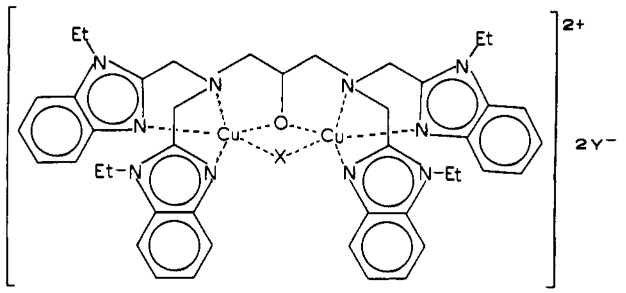


The structural picture of the active site of hemocyanin emerging from recent chemical and spectroscopic studies has set the stage for a detailed model compound approach. For oxy- and methemocyanin the collective evidence from vibrational,[1,2] magnetic,[3] chemical,[4] and electronic[1,4,5] data requires a dinuclear copper(II) site having endogeneous and exogenous bridging ligands which mediate strong antiferromagnetism resulting in diamagnetism ($-J > 550$ cm^{-1}). The exogenous bridge X is O_2^{2-} in oxyhemocyanin and is N_3^-, OAc^-, etc., in methemocyanin derivatives. The endogeneous light atom bridge R is unknown although there are frequent suggestions of an O-atom donor because of the 425-nm absorption in the UV-VIS spectrum.[4] Moreover, bridging ligands such as hydroxide, phenoxide, or carboxylate are well-known to mediate strong antiferromagnetic coupling. The popularity of phenoxide may be at odds with the absence of enhanced tyrosine vibrational modes in resonance Raman studies of oxyhemocyanin.[1,2] For the remaining ligands, EXAFS data point to two[6] or three[7] N- or O-atom donors, two of which must be imidazole from histidine.[6] The Cu⋯Cu separation of oxyhemocyanin is close to 3.6 Å.[6,7] Approximate tetragonal stereochemistry about copper is inferred from electronic spectroscopy.[4] We report herein the synthesis and structural characterization of a dinuclear copper(II) complex which mimics these structural features.

A wide variety of new chelating benzimidazole ligands is accessible by condensation of 1,2-diaminobenzene with carboxylic acid functionalities.[8,9] For the present study, condensation of 2-hydroxy-1,3-diaminopropanetetraacetic acid with 4 equiv of 1,2-diaminobenzene followed by N-alkylation with bromoethane provided a good yield (>70%) of L-Et, an excellent binucleating ligand for copper(II). Treatment with CuY_2 ($Y = BF_4^-$, ClO_4^-) followed by an anionic ligand X (N_3^-, OAc^-, pyrazolate, $HCOO^-$, NO_2^-, etc.) gives isolable green or blue crystalline materials[10] of the following structure.



The deprotonation of the ligand to give the alkoxide bridge is spontaneous.

While the acetate complex ($X = OAc^-$, $Y = ClO_4^-$) is essentially spin free ($\mu^{25} = 1.83\ \mu_B$ per Cu), most derivatives ($X = NO_2^-$, pyrazolate, etc.) have reduced magnetic moments indicative of antiferromagnetic coupling.[11] The azide complex, however ($X = N_3^-$, $Y = BF_4^-$), is diamagnetic at room temperature. Since fully diamagnetic copper(II) dimers are rare,[12,13] and since azidomethemocyanin is diamagnetic,[14] we decided to structurally characterize this azide complex by X-ray crystallography.[15] $[Cu_2(L\text{-}Et)(N_3)][BF_4]_2$ crystallizes from acetonitrile–ether as green prisms, and the structure of the discrete dispositive cation is shown in Figure 1. Each copper atom is coordinated to a tertiary amine and two *N*-ethylbenzimidazoles. The bridging alkoxide and the 1,3-azide bridge complete five-coordination. There is a C_2 axis passing through the alkoxide C3–O2 bond and the central N7 atom of the azide. However, the azide is not coplanar with the Cu–O–Cu plane (dihedral angle = 7.0°) (see Figure 2). The Cu⋯Cu separation is 3.615 (3) Å. Such a close approach of the metal atoms in a 1,3-μ-azido complex is quite unexpected. All previously reported 1,3-μ-azido complexes have Cu⋯Cu separations ≥5 Å.[12,16,17] Figure 2 shows the inner coordination sphere, illustrating that the copper stereochemistry is quite removed from an ideal geometry. It can be viewed as a distorted tetragonal pyramid with the longer (dashed) copper–benzimidazole bond as the apical bond [Cu–N4 = 2.11 (1) Å]. A similar distance has been observed for apical copper–imidazole in tetragonal copper(II)[18] and, as expected, is significantly longer than the basal benzimidazole bond [Cu–N2 = 1.99 (1) Å].[19] Such tetragonality is probably very important in dictating a $(d_{x^2-y^2})^1$ ground state and optimizing superexchange via the bridging ligands to give diamagnetism. By contrast, the paramagnetic acetate complex $[Cu_2(L\text{-}Et)(OAc)][ClO_4]_2$ has a more nearly trigonal-bipyramidal stereochemistry about copper and a likely $(d_{z^2})^1$ ground state.[9,20] Although bridging azide is known

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(15) $[Cu_2(L\text{-}Et)(N_3)][BF_4]_2$: monoclinic; $a = 19.08$, $b = 23.89$, $c = 13.23$ Å; $\beta = 116.21°$; $Z = 4$; space group $C2/m$; Mo Kα radiation; 1631 independent nonzero [$I > 2\sigma(I)$] reflections with 2θ between 3.5 and 45°; $R = 9.5\%$. Disorder in the BF_4^- and ethyl groups mars the precision of the structure, but the essential features described here are undoubtedly correct. A redetermination at low temperature is planned.

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(19) If the present structure is viewed as a distorted trigonal bipyramid with N1–Cu–N6 as the axis, unreasonably large distortions of the "equatorial" ligand angles from 120° must be tolerated [N2–Cu–O2 = 143.8 (4); N2–Cu–N4 = 98.6 (5); N4–Cu Also, the Cu–N2 and Cu–N4 bonds to benzimidazole are quite unequal [1.99 (1) and 2.11 (1) Å, respectively). By contrast, the acetate derivative has equatorial ligand angles of 119.4, 113.5 and 121.6° and the copper–benzimidazole bond lengths are equal within 1 SD.[9]

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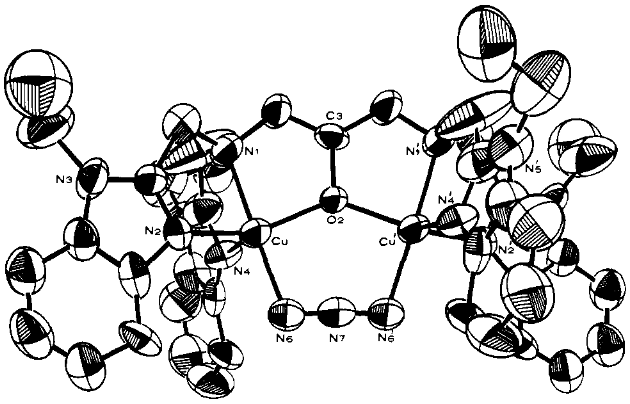

Figure 1. ORTEP plot (50%) of the cation $[Cu_2(L\text{-}Et)(N_3)]^{2+}$. A crystallographic C_2 axis passes through atoms N7, O2, and C3. Selected distances (Å): Cu-N1 = 2.06 (1); Cu-N2 = 1.99 (1); Cu-N4 = 2.11 (1); Cu-N6 = 2.04 (1), Cu-O2 = 1.94 (1); Cu$\cdots$Cu = 3.615 (3); N6-N7 = 1.15 (2). Selected bond angles (deg): O2-Cu-N2 = 143.8 (4); O2-Cu-N6 = 92.3 (5); O2-Cu-N1 = 85.9 (5); O2-Cu-N4 = 114.2 (4); N2-Cu-N6 = 98.8 (5); N2-Cu-N1 = 83.4 (5); N2-Cu-N4 = 98.6 (5); N6-Cu-N1 = 177.8 (5); N6-Cu-N4 = 97.1 (5); N1-Cu-N4 = 82.6 (5); Cu-N6-N7 = 106 (1); Cu-O-Cu = 136.9 (6).

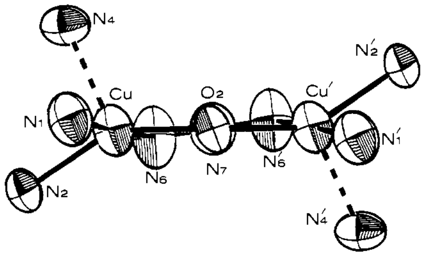

Figure 2. ORTEP plot (50%) of the inner coordination sphere viewed down the C_2 axis showing the approximate tetragonal-pyramidal stereochemistry about copper. The longer, axial bond to benzimidazole is identified by the dashed bond. The displacements of the basal donor atoms from the least-squares mean plane of N1, N2, N6, and O2 are as follows: N1, +0.32; N2, −0.29; N6, +0.27; O2, −0.30; Cu, 0.29 Å.

as a mediator of antiferromagnetic coupling,[12,16] we suspect that the alkoxide oxygen atom with its large Cu-O-Cu angle [136.9 (6)°] provides the major superexchange pathway for spin pairing. μ-Monohydroxo bridged copper(II) dimers have recently been shown to have similarly large Cu-O-Cu angles (132.2-143.7 Å) and strong antiferromagnetic coupling ($-J$ = 161-500 cm^{-1}).[21,22,24] The Cu-O-Cu angle in the acetate complex is 130.6°, but, since the alkoxide takes an equatorial site in a $(d_{z^2})^1$ trigonal-bipyramidal ground state, the unpaired electrons do not lie in orbitals collinear with the Cu-O bonds.

The structural data and diamagnetism of the azide complex make it a useful model for both oxy- and methemocyanin derivatives. In azidomethemocyanin it has been anticipated that a 1,3-μ-azido bridge might easily force the copper atoms >5 Å apart.[5] This is probably the case in ESR-active "dimer" methemocyanin,[5] but the present complex shows that a stable 1,3-μ-azido bridge could be accommodated with a 3.6-Å Cu$\cdots$Cu separation in azidomethemocyanin. Spectroscopically, the azide complex shows considerable similarity to azidomethemocyanin. In acetonitrile solution there is a strong UV maximum at 364 (414 sh) nm (ϵ 2380 M^{-1} cm^{-1}), which is not present in the acetate complex. A VIS band is present at 695 nm (ϵ 195). By comparison to the bands of very similar energy in azidomethemocyanin (*Busycon canaliculatum*) at 360 ($\epsilon \sim 1500$) and 710 nm ($\epsilon \sim 200$),[5] they are assigned to azide LMCT and d-d, respectively. Like other μ-1,3-azido copper (II) complexes,[12,16] the present complex has an intense IR band in the region 2020-2040 cm^{-1} assignable to $\nu_{\text{asym}}N_3$ (2020 cm^{-1}, Nujol mull). This narrow range indicates that $\nu_{\text{asym}}N_3$ is not particularly diagnostic of the detailed structure of the 1,3-azido bridge. Since $\nu_{\text{asym}}N_3$ in azidomethemocyanin[14] is at 2042 cm^{-1}, it is not unreasonable to suggest that the present complex and azidomethemocyanin have similar bridge dimensions. The Cu$\cdots$Cu separation of 3.61 Å in the azide complex is equal to average of the EXAFS determinations (3.55 and 3.67 Å) for two oxyhemocyanins.[6,7] Moreover, the metal-ligand bond lengths (see Figure 1 caption) are very close to those of the most recent EXAFS study of oxyhemocyanin (Cu-N$_{\text{His}}$ = 2.01; Cu-O,R = 1.92 Å).[6] A further detail of possible similarity is the C_2 twist of the azide group with respect to the Cu-O-Cu plane (see Figure 2). A similar C_2 twist of the bridging peroxide is a previously favored,[1] but not required,[4] structural feature of oxyhemocyanin. Alkoxide from serine or threonine now becomes a particularly viable candidate for the endogenous bridging ligand R.[23]

Many of the structural features of copper(II) hemocyanin appear to be intrinsic to the coordination chemistry of copper(II) with an appropriate ligand. This augurs well for the model compound approach, and the substitution experiments suggested by the visualized replacement of N_3^- by O_2^{2-} are under investigation.

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[20] If both azide and acetate structures are considered to be trigonal bipyramidal[19] and if both complexes are assumed to have d_{z^2} ground states, then the rationale for spin pairing in the azide but not the acetate would require azide to be a markedly superior mediator of antiferromagnetic coupling. While it is presently not possible to dispute this, to our knowledge, there are no examples of diamagnetic trigonal-bipyramidal copper(II) dimers. Thus, we favor the tetragonality rationale presented in the text.

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Competitive Inhibitor Binding to the Binuclear Copper Active Site in Tyrosinase

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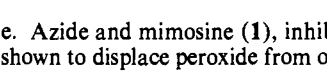
Recently derivatives of the binuclear copper active site of tyrosinase (monophenol,dihydroxyphenylalanine:oxygen oxidoreductase, EC 1.14.18.1) have been made which parallel those generated for hemocyanin and which exhibit chemical and spectral properties indicative of quite similar active sites. The resting form of tyrosinase is the EPR nondetectable met [Cu(II)Cu(II)] derivative, which can be converted to the oxy derivative by ligand



binding of peroxide. Azide and mimosine (**1**), inhibitors of tyrosinase, have been shown to displace peroxide from oxytyrosinase

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