An Explanation for the Observed Stoichiometry of Carbon Monoxide Binding to Hemocyanin

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Hemocyanin (Hc)[1] is the protein responsible for oxygen transport in many species of the mollusca and arthropoda phyla. Specroscopic evidence[2-4] has established that the active site contains two copper centers, bridged in the oxygenated form by a dioxygen molecule and by an oxygen-atom donor. Stronger support for the latter comes from model studies that suggest either an endogenous alkoxy group[5] or an exogenous hydroxide ion[6] as the bridging moiety in the protein. EXAFS data have been used to probe further the ligation of copper; those studies have demonstrated the presence of two histidyl-imidazole ligands[7] and possibly an additional donor[8] in the coordination sphere. Noteworthy is the possibility that each copper in deoxy-Hc is only two-coordinate.[7b]

Frequently overlooked in probing the structure of the protein is the characteristic reaction of deoxy-Hc with carbon monoxide.[9] It is puzzling that the protein binds only 1 CO/2 Cu even though the two copper centers are apparently identical in the deoxy form.[10] The paucity of characterized two-coordinate Cu(I) complexes having N-donor ligands has precluded a comprehensive study of their reactions. We report here only the second structurally characterized complex of two-coordinate Cu(I) having heterocyclic nitrogen donors and its *lack* of reactivity toward CO in the absence of additional ligands. The latter observation provides corroboration for bidentate protein coordination to copper in deoxyhemocyanin as well as possible reasons for the known stoichiometry of CO binding to the protein.

The ligand α,α'-bis(3,5-dimethylpyrazoyl)-*m*-xylene (**1**) was prepared by the reaction of the corresponding dibromide with the anion of 3,5-dimethylpyrazole in DMF. Slow evaporation of an acetonitrile–methanol solution of **1** and $[Cu(CH_3CN)_4]BF_4$[11] yielded crystalline $[Cu(xypz)(BF_4)]_x$,[12] **2**. X-ray analysis of **2** revealed its binuclear composition.[13]

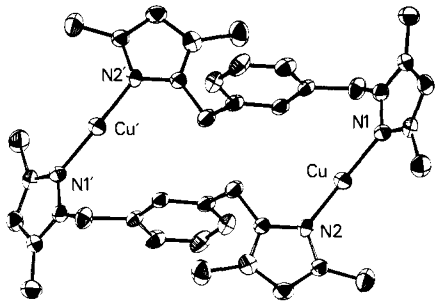

Figure 1. Structure of the $[Cu(xypz)]_2^{2+}$ cation showing the 40% probability thermal ellipsoids: selected distances (Å) Cu–N1 = 1.873 (4), Cu–N2 = 1.875 (4), Cu···Cu = 6.350 (3); angle (deg): N1–Cu–N2 = 159.7 (2).

The structure of the $[Cu(xypz)]_2^{2+}$ cation is shown in Figure 1. The molecule sits on a crystallographic inversion center so that both copper centers are identical. The extremely short Cu–N distances (Figure 1) are the same within experimental error, and they compare well with the values of 1.869 and 1.876 Å found in the only other structure of a two-coordinate Cu(I) N-heterocycle complex.[14] The short bond lengths result from the low coordination number, although backbonding into the π^* orbital of the aromatic pyrazole ring cannot be ruled out. The N1–Cu–N2 angle of 160° is smaller than the expected linear arrangement of donors in two-coordinate Cu(I)[15] and is fully 10° more acute than the previously characterized complex.[14] Although there appear to be no unusual contacts between the pyrazole rings, conformational effects in the ligand may force the smaller N–Cu–N angle. The Cu···Cu distance is large enough to presume that each copper atom acts as it would in a monomeric complex.

The reaction of $[Cu(xypz)]_2^{2+}$ with O_2 varies greatly with solvent. In acetonitrile, the complex is unreactive toward dioxygen; in fact, its synthesis can be performed in a flask open to the atmosphere. In DMF, Me_2SO, or nitromethane, **2** undergoes moderately rapid oxidation to give a green species within several minutes to several hours and a brown complex over several weeks. The nature of these species is under investigation. Cyclic voltammetry of **2** provides more evidence of its stability toward oxidation: an irreversible wave with an anodic peak potential of 1.27 V vs. SCE is observed in CH_3CN at a glassy carbon electrode scanning at 200 mV s^{-1}. We attribute the high potential to the low coordination number, which would stabilize the cuprous state.[16,17]

Exposure of **2** to carbon monoxide in any of the solvents listed above has no effect on the infrared spectrum in the region 2400–1600 cm^{-1}. However, addition of cyclohexylamine or *N*-methylimidazole to a nitromethane solution of **2** and CO results in the formation of carbonyl derivatives with ν_{CO} of 2065 and 2070 cm^{-1}, respectively.[18] These values are in the same range (2055–2083 cm^{-1}) as those for other $CuN_3(CO)$ species prepared previously.[19] Addition of anionic ligands, such as phenoxide, hydroxide, methoxide, or thiolate to $[Cu(xypz)]_2^{2+}$ results in the destruction of the complex and precipitation of $(CuX)_n$ species.

We feel that two ligands, especially those that may participate in backbonding, do not provide the copper with enough electron

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(1) Abbreviations used in this paper: Hc, hemocyanin; xypz, α,α'-bis(dimethylpyrazoyl)-*m*-xylene; DMF, dimethylformamide; Me_2SO, dimethyl sulfoxide.

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(8) Brown, J. M.; Powers, L.; Kincaid, B.; Larrabee, J. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 4210–4216.

(9) Alben, J. O.; Yen, L.; Farrier, N. J. *J. Am. Chem. Soc.* **1970**, *92*, 4475–4476.

(10) EXAFS data do not indicate the presence of two different copper centers in deoxy-Hc. One presumes that if the two coppers were in different environments, the EXAFS spectra could not be fit by a simple model (cf. ref 22).

(11) This was prepared by boiling copper powder with $Cu(BF_4)_2$ in CH_3CN containing a few drops of HBF_4. The complex crystallized upon cooling, and it was stored under an inert atmosphere.

(12) Anal. Calcd for $Cu(C_{18}H_{22}N_4)$ BF_4: C, 48.6; H, 4.99; N, 12.6. Found C, 48.3; H, 4.47; N, 12.4.

(13) Crystal data for **2**: triclinic; $a = 9.729$ (4), $b = 12.479$ (3), $c = 8.584$ (7) Å; $\alpha = 99.09$ (4), $\beta = 104.35$ (5), $\gamma = 73.05$ (2)°; $Z = 2$; space group $P\bar{1}$; Mo Kα radiation; 2894 independent nonzero $[I > 3\sigma(I)]$ reflections with 2θ between 2 and 50°; $R = 6.2$; $R_w = 7.8$. The copper atom position was located by the Patterson function and all nonhydrogen atoms were located on difference Fourier maps. The hydrogens were given calculated positions, and all nonhydrogen atoms were refined anisotropically.

(14) Birker, P. J. M. W. L.; Hendricks, H. M. J.; Reedijk, J. *Inorg. Chim. Acta* **1981**, *55*, L17–L18.

(15) Osterberg, R. *Coord. Chem. Rev.* **1974**, *12*, 309–347.

(16) Another explanation invokes coordination of acetonitrile to the copper. The ligation of nitriles by Cu(I) results in significant stabilization of the reduced state.

(17) Constant-potential coulometry shows the loss of 2.05 electrons/complex during the oxidation process.

(18) Spectra were recorded on a Beckman Model infrared spectrometer using NaCl cells. The carbonyl absorption varied with solvent: in DMF, the values for the cyclohexylamine and the *N*-methylimidazole adducts were 2050 and 2052 cm^{-1}, respectively.

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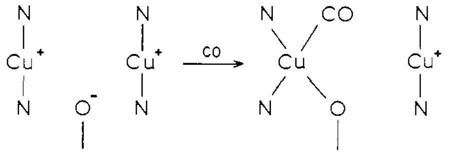
density to engage in binding an excellent π acid like CO. The reactivity of the previously characterized two-coordinate Cu(I) complex[14] with CO is likely a result of coordination from the other nitrogen donors of the ligand framework to the Cu(I) ions.[20]

While we cannot reject the notion that steric effects account for the observed binding of only one CO per binuclear copper unit in deoxy-Hc, we can provide an alternative explanation for the stoichiometry based on electronic effects. If each copper in deoxyhemocyanin were three-coordinate as previously suggested,[8] we would expect that each copper ion would bind CO in a tetracoordinate fashion by analogy with simple copper carbonyls,[19] and one would observe a 1:1 ratio of CO/Cu. Similarly, if each copper is statically two-coordinate, we should expect deoxy-Hc to be inert to carbon monoxide. However, if *one* of the two-coordinate copper ions binds an additional ligand and becomes three-coordinate, then the observed 1:2 stoichiometry obtains. If one accepts the notion that there is ample space for *each* copper to bind a molecule of CO,[22] our results provide indirect evidence that each copper in deoxy-Hc is substantially two-coordinate but that one copper is able to bind an additional donor in the presence of CO.

While the possibility exists that a third endogenous N donor is near one of the copper centers,[23] another notion is that the bridging ligand accepts that role (see eq 1). Binding of the O donor to only one of the copper ions leaves the other copper two-coordinate, hence unreactive toward CO. Unfortunately, we have not been able to generate a species with the $[CuN_2(OR)CO]$ coordination unit, thus we do not know if such a species can even exist or what its properties will be; work toward that goal is in progress.



(1)

Acknowledgment. This work was supported by Grant No. CHE-7817324 from the National Science Foundation. We thank Professor Derek J. Hodgson and his group for their generous assistance with the X-ray crystallographic studies.

Registry No. 1, 81028-65-1; **2**, 81028-23-1; CO, 630-08-0; 3,5-dimethylpyrazole, 67-51-6; α,α'-dibromo-*m*-xylene, 626-15-3.

Supplementary Material Available: Table S1 listing positional and thermal parameters for $[Cu(xypz)]_2[BF_4]_2$ and a figure showing the atom numbering scheme for the $[Cu(xypz)]_2^{2+}$ cation (3 pages). Ordering information is given on any current masthead page.

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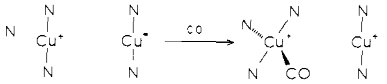
(20) The validity of this statement is suggested by a recent paper[21] that reports the isolation of a bis(carbonylcopper(I)) adduct whose ligand is structurally identical with the one reported by Reedijk.[14] The copper in the former complex is in an $N_3(CO)$ coordination environment.

(21) Gagné, R. R.; Kreh, R. P.; Dodge, J. A.; Marsh, R. E.; McCool, J. *Inorg. Chem.* **1982**, *21*, 254-261.

(22) EXAFS data[7b] suggest that the copper atoms are >5 Å apart; therefore, there should be enough space to form a bis(carbonyl) adduct.

(23) EXAFS measurements are not very sensitive for detecting a low-*Z* atom at a large distance from the metal site, and hence a weakly bound ligand may have been missed.[7b] Since the two coppers appear to be identical in the deoxy form,[10] a third donor near one of the coppers would have to have little influence on the EXAFS measurements yet be near enough to coordinate when CO is present.



Reedijk's complex[14] may be the closest mimic for this type of behavior since the aliphatic nitrogen does not bind in the "deoxy" form, yet it apparently can when CO is added.[20]

Hg^+ ($6s^1$) Ions in KH_2PO_4 and $NH_4H_2PO_4$: New Paramagnetic Probes of Cationic Dynamics Near Phase Transitions in Hydrogen-Bonded Ferroelectrics and Antiferroelectrics

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We present here electron paramagnetic resonance (EPR) evidence to show that Hg^+ ions can be substituted for K^+ in KH_2PO_4 (a hydrogen-bonded ferroelectric) and for NH_4^+ ions in $NH_4H_2PO_4$ (a hydrogen-bonded antiferroelectric[1]). This study was undertaken because, while the (solid–solid) structural phase transitions in these compounds have been considered as models of structural and ferroelectric/antiferroelectric phase transitions, the microscopic details of their own transition mechanisms are not yet understood.[2] Current theoretical models of structural phase transitions need data on the low-frequency ($\sim 10^8$–10^{11} Hz) motion as well as the higher frequency lattice vibrations. The low-frequency motion falls in the domain where EPR spectroscopy is most effective for studying motional effects. Consequently, EPR spectroscopy has been extensively used for studying the dynamics of the anions (PO_4^{3-}) as well as the cations (K^+, NH_4^+, etc.) in the KH_2PO_4-type compounds.[2] In using the EPR technique for studying these (diamagnetic) compounds, of course, it has been necessary to introduce a suitable paramagnetic ion (or free radical) into their lattices. We noticed that the probes used for studying the role of the cations K^+, Cs^+, NH_4^+, etc., were Cr^{3+} ($3d^3$), Fe^{3+} ($3d^5$), Cu^{2+} ($3d^9$), and Tl^{2+} ($6s^1$) ions. It is thus seen that all these probes have one or two units of positive charge higher than that of the alkali cation they are supposed to replace. Because of this extra charge, these ionic probes would exhibit a local electrostatic potential different from that of the substituted cations. Thus the local dynamic and structural properties seen by the probe would be expected to be different from that of the original lattice. We, therefore, searched for a monopositive paramagnetic ion and have found that Hg^+ ($6s^1$) ions might represent such a probe.

The Hg(I) ions were incorporated into KH_2PO_4 or $NH_4H_2PO_4$ by doping these compounds with $Hg(CH_3CO_2)_2 \cdot 2H_2O$ (5% by weight), growing single crystals from aqueous solution, and γ irradiating the doped crystals with a 500-Ci source for a few hours (exact time not critical). The crystals turned yellowish after γ irradiation, an indication that Hg^+ centers had been formed, as reported earlier[3,4] for Hg^+ in $Cd(CH_3CO_2)_2 \cdot 2H_2O$ doped with $Hg(CH_3CO_2)_2 \cdot 2H_2O$.

EPR measurements were made at X-band frequencies by using a Varian E-3 spectrometer. The microwave frequency was measured with a Hewlett-Packard 5345 L digital frequency counter, and the resonance fields were measured relative to the standard free-radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). So that the spin Hamiltonian parameters could be determined, the variation of the EPR signals was investigated in the three mutually orthogonal crystal planes *ab*, *bc*, and *ca* of the tetragonal ($I\bar{4}2d$) phases of KH_2PO_4 and $NH_4H_2PO_4$.[1] The crystallographic axes were easily identified by noting that these crystals grow as "bricks" with the *c* axis as the longest direction. The *a* and *b* axes are parallel to the orthogonal edges of the crystals.

Figure 1 shows typical EPR signals from single crystals of KH_2PO_4 and $NH_4H_2PO_4$ at $T = 300$ K for $\vec{H}//c$, where $\vec{H}$ is the externally applied (Zeeman) magnetic field. The spectra at other

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(1) For a detailed discussion of ferroelectricity and antiferroelectricity, see M. E. Line and A. M. Glass, "Principles and Applications of Ferroelectrics and Related Materials", Clarendon Press, Oxford, England, 1977.

(2) See, for example, R. Blinc in "Magnetic Resonance of Phase Transitions", F. J. Owens, C. P. Poole, Jr., and H. A. Farach, Eds., Academic Press, New York, 1979, Chapter V.

(3) R. S. Eachus and F. G. Herring, *Can. J. Chem.*, *49*, 2868 (1971).

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