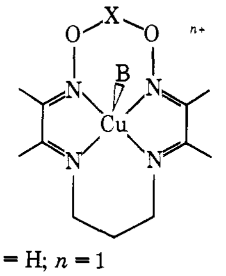
Models for Copper-Containing Proteins: Five-Coordinate Complexes of Copper(I)

Sir:

Hemocyanin, tyrosinase, laccase, cytochrome oxidase, and galactose oxidase are all proteins which contain copper and which bind and/or utilize molecular oxygen. Neither the mode of action nor the nature of active sites of any of these proteins has been clearly defined but all employ Cu(I) in reactions. Moreover, nitrogen ligands, e.g., imidazoles of polypeptidyl histidines, are thought to play a major role in binding copper in these proteins.[1,2] To help elucidate the nature and mechanisms of action of these and related copper-containing proteins we are investigating the structures and reactivities of a number of Cu(I) complexes with nitrogen ligands. Since both Cu(I) and Cu(II) are substitution labile, polydentate ligands, some macrocyclic, have been employed to provide a well-defined ligand environment, even in solution. Herein we report the preparation of unique five-coordinate Cu(I) complexes via the agency of a macrocyclic ligand.

Addition of a hot DMK[3] solution of $Cu(ClO_4)_2 \cdot xH_2O$ to a twofold excess of HDOH,[3,4] in hot DMK yields, upon cooling, dark red-brown, crystalline $Cu(DOH)ClO_4 \cdot H_2O$,[5,6] **1.** Treating **1** with boron trifluoride etherate in boiling dioxane followed by recrystallization from DMK/dioxane yields red crystalline $[Cu(DOBF_2)ClO_4]_2 \cdot C_4H_8O_2$,[6] **2** in which dioxane may be serving as a μ-bidentate ligand.[7] No suitable chemical reducing agents for **1** or **2** have been found; thus far all attempts have led to copper displacement from the macrocycle, often with production of copper metal. However, cyclic voltammograms[8] of **1**[10] and **2**[11] in CH_3CN, DMK, and CH_2Cl_2 indicate nearly reversible electrochemical redox behavior. Constant potential electrolysis[8] (cpe) under N_2 of red CH_3CN solutions of either **1** (at −1.2 V) or **2** (at −1.0 V) yields dark blue solutions of the corresponding Cu(I) complexes. These solutions are very stable at 25 °C, under N_2, giving no indications of disproportionation. When cpe of **2** is effected in DMK (0.1 M in **2**) red, crystalline complex, $Cu(DOBF_2)$, **3,** (blue when ground to a powder) is formed during electrolysis and can be isolated, by filtration.[6] Though the solid Cu(I) complex, **3,** appears stable in air, its blue solutions (DMK, CH_3CN, CH_2Cl_2, C_7H_8) irreversibly turn brown upon exposure to O_2. Neither the O_2 reaction nor the oxidation products are simple but rather are very solvent and temperature dependent; these are the focus of continuing investigation.



1, X = H; *n* = 1
2, X = BF_2; *n* = 1
3, X = BF_2; *n* = 0
4, X = BF_2; B = CO; *n* = 0
5, X = BF_2; B = 1-MeIm; *n* = 0

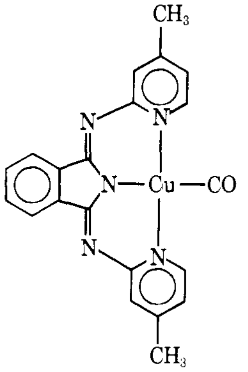
Blue solutions of **3** (DMK, CH_3CN, CH_2Cl_2, C_7H_8) at 25 °C react rapidly with CO to give light yellow solutions. Purging the yellow solutions with N_2 or placing them in vacuo rapidly restores the blue color, a process which can be repeated several times without apparent decomposition. Presumably five-coordinate, orange, crystalline carbonyl complex, $Cu(DOBF_2)(CO)$, **4,** can be isolated from CO saturated DMK solutions of **3** by slow addition of heptane[6] (air stable as solid, ν_{CO} 2068 cm^{-1}).

Blue DMK solutions of **3,** under N_2, also appear to react with added 1-MeIm. Slow addition of heptane to the resulting emerald green solutions yields the crystalline 1-MeIm adduct, $Cu(DOBF_2)(1\text{-MeIm})$,[6] **5.**

We suggest that the carbonyl, **4,** and 1-MeIm, **5,** adducts are five (or more)-coordinate complexes, a coordination number that has apparently not been previously reported for Cu(I).[12] That these complexes are five coordinate, probably square pyramidyl, is suggested by several observations. (1) Elemental analyses are consistent with the empirical formulas. (2) It is highly unlikely that any of the four macrocycle–nitrogen ligands of **3** are dissociating upon formation of the carbonyl or imidazole adducts. In fact, there are no clear examples of readily reversible dissociation–association of one or more liganding atoms in a tetradentate, macrocyclic complex. (3) Solutions of **3** in DMK, CH_2Cl_2, and C_7H_8 are all royal blue but CH_3CN gives, at 25 °C, an aquamarine colored solution. Cooling the latter to −40 °C yields an emerald green solution, similar to that of $Cu(DOBF_2)(1\text{-MeIm})$, **5,** in DMK at 25 °C. Rewarming the green CH_3CN solution to 25 °C restores the aquamarine color. This temperature sensitivity is best explained by formation of a five (or more)-coordinate Cu(I) complex in which at least one CH_3CN is weakly coordinated to Cu. Thus cooling the solution drives the equilibrium to the CH_3CN complex, **6.** That CH_3CN, which competes only poorly as a ligand at 25 °C, should induce even partial macrocyclic–ligand–nitrogen dissociation at −40 °C seems especially unlikely.

$$\underset{\substack{\text{blue}\\ \mathbf{3}}}{Cu(DOBF_2)} + nCH_3CN \rightleftharpoons \underset{\substack{\text{green}\\ \mathbf{6}}}{Cu(DOBF_2)(CH_3CN)_n}$$

If the carbonyl complex, **4,** is indeed five coordinate (a structural investigation is in progress), its infrared stretching frequency (ν_{CO} 2068 cm^{-1}) is notable. Only three other carbonyl adducts of simple Cu(I) complexes with nitrogen ligands are known and all are four coordinate. Tetrahedral hydrotris(1-pyrazolyl)borato(carbonyl)copper(I)[13,14] has ν_{CO} 2083 cm^{-1}, and its dimethyl analogue, hydrotris(3,5-dimethyl-1-pyrazolyl)borato(carbonyl)copper(I),[15] exhibits ν_{CO} 2066 cm^{-1}, while the isoindoline complex, **6**[16] (probably distorted square planar), has ν_{CO} 2068 cm^{-1}. Thus only small variation in ν_{CO} has been observed despite rather different coordination geometries for Cu(I). Thus the CO derivative of hemocyanin (ν_{CO} 2040–60 cm^{-1} [17]), whose coordination geometry has not been determined, could have one or both copper atoms in the active site as five (or more) coordinate. More generally the possibility of five (or more) coordination in any Cu(I) containing protein must not be overlooked.[18]



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References and Notes

(1) (a) P. Lontie and R. Witters in "Inorganic Biochemistry", Vol. 1, G. L. Eichorn, Ed., Elsevier, Amsterdam, 1973, Chapter 12; (b) J. Peisach, P. Aisen, and W. E. Blumberg, Ed., "The Biochemistry of Copper", Academic Press, New York, N.Y., 1966; (c) F. Ghiretti, Ed., "Physiology and Biochemistry of Haemocyanins", Academic Press, New York, N.Y., 1966; (d) B. L. Vallee and W. E. C. Wacker, "The Proteins", 2d ed, Vol. V, H. Neurath, Ed., Academic Press, New York, N.Y., 1970; (e) R. Osterberg, *Coord. Chem. Rev.*, **12,** 309 (1974).

(2) For example, it has been suggested that the active site of deoxyhemocyanin contains two Cu(I) atoms each bound by the three to five imidazoles. See (a) A. Ghiretti-Magaldi, International Conference on Copper Proteins, Manziana (Rome), Italy, Sept 22–26 (1974); (b) B. Salvato, A. Ghiretti-Magaldi, and F. Ghiretti, *Biochemistry*, **13,** 4778 (1974).

(3) Abbreviations used include: DMK, acetone; HDOH, 3,3′-(trimethylenedinitrilo)-di-2-butanonedioximate; C_7H_8, toluene; 1-MeIm, 1-methylimidazole.

(4) Prepared by condensing 2,3-butanedionemonoxime with 1,3-diaminopropane in ethanol at 25 °C which is much simpler than the previously reported procedure: Von E. Uhlig and M. Friedrich, *Z. Anorg. Allg. Chem.*, **343,** 299 (1966).

(5) This complex has thus far only been isolated in the presence of excess HDOH and even then contains more than the expected amount of Cu. Apparently a second Cu can bind to the two oxime oxygens, similar to the binuclear Cu(II) complex obtained with salicylaldehyde–ethylenediamine: R. M. Countryman, W. T. Robinson, and E. Sinn, *Inorg. Chem.*, **13,** 2013 (1974).

(6) All complexes isolated gave satisfactory elemental analyses which were submitted to the referees.

(7) Adding very small amounts of dioxane to a concentrated acetone solution of **2** causes crystallization of the Cu(II) complex which indicates possible complexation of dioxane.

(8) All electrochemistry was performed with platinum electrodes. The supporting electrolyte used in CH_3CN was tetraethylammonium perchlorate (TEAP, 0.1 M) but in DMK and CH_2Cl_2 tetrabutylammonium perchlorate was employed. The reference electrode was a silver wire immersed in a CH_3CN solution 0.1 M in TEAP and 0.01 M in $AgNO_3$, all separated from the bulk of the solution by a sintered glass disk. Potentials are given vs. this Ag|$AgNO_3$ electrode but note that Ag|0.01 M $AgNO_3$ + 0.1 M TEAP||0.1 M TEAP vs. the standard hydrogen electrode = 0.54 V.[9]

(9) (a) H. H. Willard, L. L. Merritt, and J. A. Dean, "Instrumental Methods of Analysis", 4th ed, Van Nostrand Reinhold Co., New York, N.Y., 1965, p 537; (b) R. C. Larson, R. T. Iwamota, and R. N. Adams, *Anal. Chim. Acta*, **25,** 371 (1961).

(10) $E_{p_c} = -1.07$ V, $E_{p_a} = -0.90$ V in CH_3CN. Excess copper in the sample[5] was plated out as Cu(0) thus providing a clean method for preparing pure solutions of the reduced complex.

(11) $E_{p_c} = -0.78$ V, $E_{p_a} = -0.67$ V, in CH_3CN.

(12) (a) W. E. Hatfield and R. Whyman, *Transition Met. Chem.*, **5,** 47 (1969); (b) F. H. Jardine, *Adv. Inorg. Radiochem.*, **17,** 115 (1975).

(13) M. I. Bruce and A. P. P. Ostazewski, *J. Chem. Soc., Chem. Commun.*, 1124 (1972); M. I. Bruce and A. P. P. Ostazewski, *J. Chem. Soc., Dalton Trans.*, 2433 (1973).

(14) M. R. Churchill, B. G. DeBoer, F. J. Rotella, O. M. Abu Salah, and M. I. Bruce, *Inorg. Chem.*, **14,** 2051 (1975).

(15) C. Mealli, C. S. Arcus, J. L. Wilkinson, T. J. Marks, and J. A. Ibers, *J. Am. Chem. Soc.*, **98,** 711 (1976).

(16) R. R. Gagné, R. S. Gall, and L. S. Speltz, manuscript in preparation.

(17) (a) L. Y. Fager and J. O. Alben, *Biochemistry*, 11, 4786 (1972); (b) J. O. Alben, L. Yen, and N. J. Farrier, *J. Am. Chem. Soc.*, **92,** 4475 (1970).

(18) Resonance Raman spectra of Cu(II) "blue" copper proteins are interpretable in terms of five coordination, but there are no available data for the Cu(I) proteins: V. Miskowski, S.-P. W. Wang, T. G. Spiro, E. Shapiro, and T. H. Moss, *Biochemistry*, **14,** 1244 (1975).

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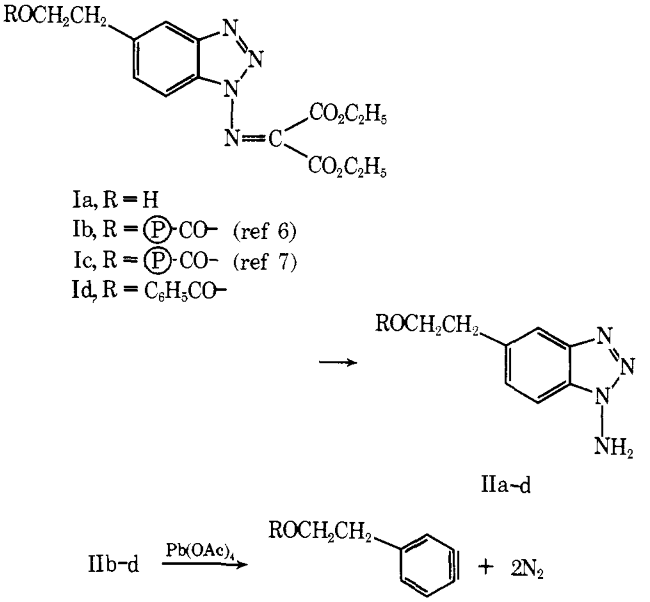
Pseudodilution, the Solid-Phase Immobilization of Benzyne

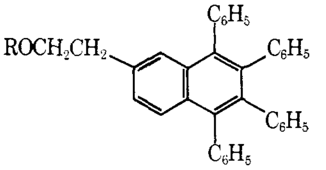
Sir:

Among the various advantages associated with solid-phase synthesis, the possibility of maintaining mutual separation of reactive functional groups bound to the same polymer support is particularly attractive. The results of studies directed at determining the extent of such isolation[1] have not been very encouraging and, in fact, examples of extensive reaction between polymer-bound functional groups have been reported.[2] We have found that the ubiquitous dimerization reaction of benzyne is completely suppressed by attachment of this reactive molecule to a polystyrene resin, thereby extending its lifetime at room temperature to more than a minute.

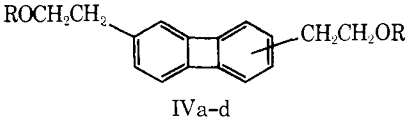
While benzyne has been isolated as a stable entity in rigid matrices at very low temperature,[3] its lifetime in solution and in the gas phase is limited by dimerization to form biphenylene. This process occurs at a rate approaching diffusion control.[4] In order to determine the effect of polymer immobilization the method of Campbell and Rees[5] for the generation of benzyne was adapted.



The ketomalonate imine of 6-β-hydroxyethyl-1-aminobenzotriazole, Ia, was attached to carboxylated polystyrene resin[8] as the ester Ib. For control experiments the benzoyl ester Id was prepared as well. Deprotection of the amino group was effected by selective hydrolysis with aqueous hydroxylamine. Oxidation of the aminotriazole with lead tetraacetate resulted in rapid evolution of N_2 and formation of characteristic aryne addition products.[9] Thus reaction of IIb in dichloroethane with 1 equiv of lead tetraacetate at 23 °C in the presence of excess tetracyclone, followed by saponification of the resin esters, yielded IIIa (99% based on N_2 evolution).



IIIa-d



IVa-d

In the absence of trapping agents, behavior of the resin-bound and solution-phase arynes differed greatly. Oxidation of IId in concentrations as low as 0.006 M provided the two isomeric biphenylenes IVd as the only isolable product (65% yield). By contrast, oxidation of IIb, followed by saponification of the product yielded no detectable amount of the corresponding dimer alcohols, IVa. A yield as low as 0.3% would have been detected. The possibility that resin-bound dimers were formed but resisted saponification was ruled out by the