acetone–HCl[11] no radioactivity was found in the $PhI(OAc)_2$ peak. Three other hemoproteins, cytochrome P-450 from β-naphthoflavone-induced rat livers,[12] horseradish peroxidase, and catalase, caused no detectable increase in radioactivity in the $PhI(OAc)_2$ peak. Free heme (ferriprotoporphyrin IX) did not catalyze the reaction. Thus, the oxygen transfer appears to be specific for cytochrome P-450 and specificity within the group of cytochromes P-450 exists. Specificity among the cytochromes P-450 has been observed for some substrates but not others.[1,13]

The results of these experiments indicate that oxygen transfer from cytochrome P-450 to a halogen is, in fact, a possible mode of reaction. In aromatic systems epoxidation of the ring is probably favored over oxygenation of bromine or chlorine substituents and there seems to be no reason to postulate halogen oxides as intermediates in aryl hydroxylation. However, halogen oxidation may offer an alternative to oxygen insertion into carbon–hydrogen bonds in aliphatic halocarbons. The iodobenzene–iodosobenzene equilibration described here offers further proof that the actual oxygenating species in cytochrome P-450 is a monoxygenated and not a dioxygenated species. However, an iodine coordinated species, similar to that suggested by Groves et al.[4] for the iron porphyrin system, cannot be ruled out as the intermediate in the oxygen transfer from iodosobenzene to iodobenzene.[14]

(11) Guengerich, F. P.; Ballou, D. P.; Coon, M. J. *J. Biol. Chem.* **1975**, *250*, 7405.
(12) This protein is apparently identical with the major form of P-450 isolated from 3-methylcholanthrene-treated rat liver.[7]
(13) Haugen, D. A.; van der Hoeven, T. A.; Coon, M. J. *J. Biol. Chem.* **1975**, *250*, 3567. Guengerich, F. P. *Ibid.* **1977**, *252*, 3970.
(14) This work was supported by Grants ES00267 and ES01590 to Vanderbilt University from the U.S. Public Health Service.

Leo T. Burka,* Alice Thorsen, F. Peter Guengerich
Center in Environmental Toxicology and Department of Biochemistry
Vanderbilt University, Nashville, Tennessee 37232
Received September 29, 1980

Enhanced Stability of the Imidazolate-Bridged Dicopper(II) Ion in a Binucleating Macrocycle

Sir:

There is great current interest in metal complexes of binucleating ligands[1,2] as models[3] for the coordination environments of metallobiomolecules such as hemocyanin,[4] cytochrome *c* oxidase,[5] and bovine erythrocyte superoxide dismutase (BESOD).[6] Previously we reported[7] the synthesis and structure of $[Cu_2(imH)_2(im)\subset A](ClO_4)_3$ (**1**)[8] which has features analogous to BESOD. Here we show two important new aspects of the chemistry and stability of the related complex $[Cu_2(im)\subset A']^{3+}$ (**2**). The first is the marked hydrolytic integrity of the imidazolate bridge in **2** compared to the corresponding $[(TMDT)_2Cu_2(im)]^{3+}$ complex. The second, and the more unusual, aspect is the spontaneous formation of **2** from the 1:1 complex of A′ with copper(II) in the presence of 1 equiv of imidazole. This insertion of two copper(II) ions into the same binucleating macrocycle to form the imidazolate-bridged dicopper(II) unit is closely parallel to the pH-dependent migration of copper(II) to the vacant zinc-binding site observed for zinc-free BESOD.[9]

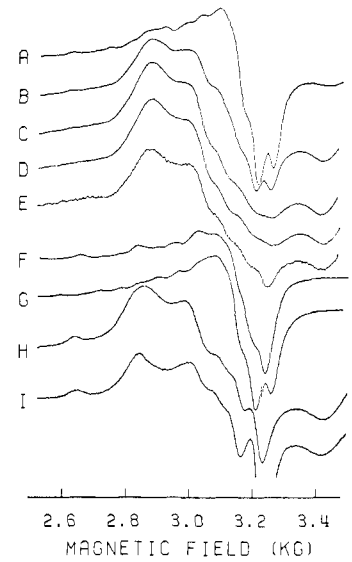
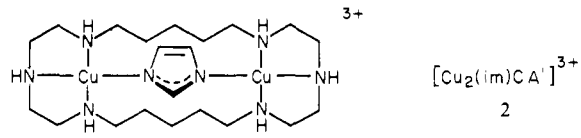


Figure 1. Electron spin resonance spectra of frozen 50% aqueous Me_2SO solutions of $[Cu_2(im)\subset A'](ClO_4)_3 \cdot H_2O$ at 115 K as a function (see ref 20) of pH [(A) 5.6, (B) 6.2, (C) 7.1, (D) 10.4, (E) 11.5] and at 77 K at various Cu–A′–imH–H^+ ratios [(F) 1:1:0:0, (G) 2:1:0:0, (H) 1:1:1:0, (I) 1:1:1:1]. Samples were run in degassed solvents using ~5 mM macrocyle concentrations on a Varian E line X-band spectrometer. Instrument settings were 10 mW of microwave power, 5-G modulation amplitude, and 125 G min^{-1} scan rate.



The ligand was prepared by reaction of equimolar quantities of the disodium salt of *N,N′,N″*-tris(*p*-tolylsulfonyl)diethylenetriamine[10,11] and 6,9,12-tris(*p*-tolylsulfonyl)-6,9,12-triazaheptadecane-1,17-bis(methanesulfonate)[12] (**4**) in DMF at 90 °C for 2 h. The crude product, isolated upon addition of H_2O, was chromatographed with $CHCl_3$ on silica to give 1,4,7,13,16,19-hexatosylhexaazacyclotetracosane as a white solid in 20% yield. Detosylation[11] was achieved by using 97% H_2SO_4 at 100 °C for

(1) Groh, S. E. *Isr. J. Chem.* **1976–1977** *15*, 277–307.
(2) (a) Gagnē, R. R.; Kreh, R. P.; Dodge, J. A. *J. Am. Chem. Soc.* **1979**, *101*, 6917–6927. (b) Gunter, M. J.; Mander, L. N.; McLaughlin, G. M.; Murray, K. S.; Berry, K. J.; Clark, P. E.; Buckingham, D. A. *Ibid.* **1980**, *102*, 1470–1473. (c) Drew, M. G. B.; McCann, M.; Nelson, S. M. *J. Chem. Soc., Chem. Commun.* **1979**, 481–482. (d) Bulkowski, J. E.; Burk, P. L.; Ludmann, M-F.; Osborn, J. A. *Ibid.* **1977**, 489–499. (e) Agnus, Y.; Louis, R.; Weiss, R. *J. Am. Chem. Soc.* **1979**, *101*, 3381–3384. (f) Gisselbrecht, J. P.; Gross, M.; Alberts, A. H.; Lehn, J. M. *Inorg. Chem.* **1980**, *19*, 1386–1388.
(3) Ibers, J. A.; Holm, R. H. *Science* **1980**, *209*, 223–235.
(4) Himmelwright, R. S.; Eickman, N. C.; Lubien, C. D.; Solomon, E. I. *J. Am. Chem. Soc.* **1980**, *102*, 5378–5388.
(5) Palmer, G.; Babcock, G. T.; Vickery, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73* 2206–2210.
(6) Richardson, J. S.; Thomas, K. A.; Rubin, B. H.; Richardson, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1349–1353.
(7) Coughlin, P. K.; Dewan, J. C.; Lippard, S. J.; Watanabe, E-i.; Lehn, J.-M. *J. Am. Chem. Soc.* **1979**, *101*, 265–266.
(8) Abbreviations: A = 1,4,7,13,16,19-hexaaza-10,22-dioxacyclotetracosane; A′ = 1,4,7,13,16,19-hexaazacyclotetracosane; imH, imadazole; im, imidazolate anion; TMDT, 1,1,7,7-tetramethyldiethylenetriamine; DMF, dimethylformamide; Me_2SO, dimethyl sulfoxide.
(9) Valentine, J. S.; Pantoliano, M. W.; McDonnell, P. J.; Burger, A. R.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4245–4249.
(10) Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268–2270.
(11) Atkins, T. J.; Richman, J. E.; Oettle, W. F. *Org. Synth.* **1978**, *58*, 86–97.
(12) Reaction of 2 equiv of 5-(tetrahydropyran-2-yloxy)pentane-1-methanesulfonate (**5**) with **3** in DMF gave the ditetrahydropyranyloxy derivative of **4**. Hydrolysis of the tetrahydropyranyl groups with *p*-toluenesulfonic acid in 95% EtOH[13] afforded the diol, which was then allowed to react with methanesulfonyl chloride[14] to generate **4**. Compound **5** was obtained by reaction of 2,3-dihydropyran with threefold excess of 1,5-pentanediol.[15] Extraction into benzene followed by vacuum distillation gave the pure monotetrahydropyranyloxy derivative, which was then mesylated to give **5**.
(13) Dauben, W. G.; Bradlow, H. L. *J. Am. Chem. Soc.* **1952**, *74*, 559–560.
(14) Crossland R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195–3196.
(15) Cowie, J. S.; Landor, P. D.; Landor, S. R. *J. Chem. Soc., Perkin Trans. 1.* **1973**, 720–724.

0002-7863/80/1502-7616$01.00/0

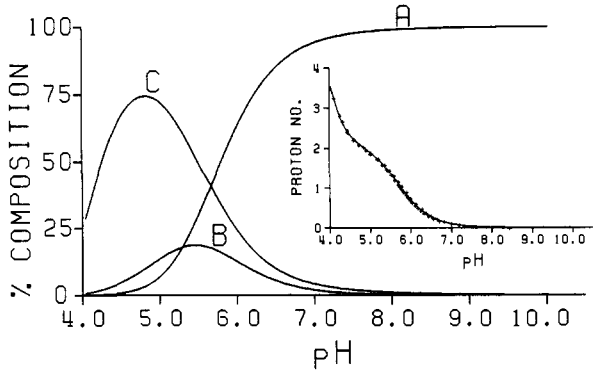


Figure 2. Potentiometric titration (inset) of $[Cu_2(im)\subset A'](ClO_4)_3 \cdot H_2O$ in water showing the average number (every third point is plotted) of protons added per molecule[25] vs. pH. The solid line was calculated by least-squares from eq 1–3 with $\log K_1 = 5.29$ (5), $\log K_2 = 3.32$ (4), $\log K_3 = \log K_4 = 3.97$ (2), $\log K_5 = \log K_6 = 3.90$ (2). The main part of the figure plots the concentrations of $[Cu_2(im)\subset A']^{3+}$ (A), $[Cu_2(imH)(OH_2)\subset A']^{4+}$ (B), and $[Cu_2(OH_2)_2\subset A']^{4+}$ (C) as a function of pH for a 5 mM macrocycle concentration. Species with the macrocycle protonated were omitted from the plot for clarity.

24 h, followed by precipitation of the hydrosulfate salt with ether. Subsequent treatment with excess aqueous 20% NaOH and continuous liquid–liquid extraction into benzene gave 1,4,7,13,16,19-hexaazacyclotetracosane (A′) as a white crystalline solid in 56% yield. The analytical and spectral data[16] are in excellent agreement with the proposed ligand structure.

The compound $[Cu_2(im)\subset A'](ClO_4)_3 \cdot H_2O$ was synthesized by adding 5 mL of a 60 mM solution of cupric perchlorate[17] to 5 mL of a 30 mM solution of A′, both in methanol, followed by 2 mL of a 72 mM methanolic imidazole solution and 0.145 mL of 1 M aqueous NaOH. Concentration of the solution gave a blue precipitate that was recrystallized from water to give the product in 80% yield. Analytical and physical data[18] proved the compound to be a monohydrate of the perchlorate salt of **2**.

The electron spin resonance spectrum of **2** in 50% aqueous Me_2SO (Figure 1 A-E) is similar to that reported previously[20] for $[(TMDT)_2Cu_2(im)]^{3+}$. The spectrum hardly changes in the range $7 \leq pH \leq 11.5$, indicating that the imidazolate brdige remains intact. At low pH (~5.5), the ESR spectrum of **2** shows the presence of a small amount of bridged complex (feature at 3450 G) but essentially resembles that of $[Cu_2\subset A']^{4+}$ (Figure 1G).

Potentiometric titrations of a 2.5 mM aqueous solution of **2** at 25 °C, 0.16 ionic strength, under nitrogen were carried out to obtain a quantitative measure of the species present. Reversible pH-dependent behavior was observed that showed two protons titrating in the range $4.5 \leq pH \leq 7$ (Figure 2, inset). At lower pH values the copper is removed from the ligand. The titration curve was calculated on the basis of eq 1–3 and was refined by

$$[Cu_2(im)\subset A']^{3+} + H_3O^+ \overset{K_1}{\rightleftharpoons} [Cu_2(imH)(OH_2)\subset A']^{4+} \quad (1)$$

$$[Cu_2(imH)(OH_2)\subset A']^{4+} + H_3O^+ \overset{K_2}{\rightleftharpoons} [Cu_2(OH_2)_2\subset A']^{4+} + imH_2^+ \quad (2)$$

$$[Cu_2(OH_2)_2H_n\subset A']^{(4+n)+} + H^+ \overset{K_{3+n}}{\rightleftharpoons} [Cu_2(OH_2)_2H_{n+1}\subset A']^{(5+n)+} \quad (3)$$

$$n = 0\text{–}3$$

least-squares methods to fit all data in the range $4 \leq pH \leq 10$. Figure 2 gives the results of the calculation (caption and solid line, inset) as well as the relative amounts of the various copper-containing species as a function of pH. The imidazolate-bridged complex **2** is the major component in solution from pH 6 to pH 10. This result is in striking contrast to that obtained for $[(TMDT)_2Cu_2(im)]^{3+}$ which only predominates in solution over the narrow range $8.5 < pH < 9.5$.[20] In the methylmercury-(II)–imidazole system, the imidazolate-bridged complex is never more than 40% of the total concentration of imidazole-containing species in solution.[22] The imidazolate bridge in both of these cases breaks above pH 9.5, whereas **2** is stable up to pH 11 (Figures 1 and 2).

An even more interesting effect occurs when imidazole is added to a 1:1 solution of copper(II) and macrocycle A′. As shown by the $g_{\|}$ region of its ESR spectrum (Figure 1F), this $(Cu\subset A')^{2+}$ solution has more than one copper environment, although very little $(Cu_2\subset A')^{4+}$ species is present (compare Figures 1F and 1G). Addition of 1 mol of imidazole/mole of $(Cu\subset A')^{2+}$ results in an ESR spectrum of an imidazolate-bridged complex (Figure 1H), similar to the spectrum of **2** at pH 6.2, at which point 75% of the copper(II) ions are bridged. This bridge remains intact even after the addition of 1 equiv of protons (Figure 1I).

In summary, the binucleating macrocycle A′ conveys unusual stability upon the imidazolate-bridged dicopper(II) ion similar to that observed in forms of the bovine erythrocyte superoxide dismutase protein. This system therefore appears to be well suited to further model studies of the known[23] superoxide dismutase and proposed[24] metal storage and/or transport properties of the protein.

Acknowledgment. This work was supported at Columbia University by NIH Research Grant No. GM-16449 from the National Institute of General Medical Sciences and by Grant No. CHE79-12436 from the National Science Foundation and, at the University of Delaware, by the Center for Catalytic Science and Technology.

(16) Elemental analysis: Anal. Calcd for $C_{18}H_{42}N_6 \cdot H_2O$: C, 59.96; H, 12.30; N, 23.31; mol wt, 343. Found: C, 60.21; H, 12.27; N, 23.23; mol wt (vapor pressure osometry, CH_2Cl_2 solution), 335. Chemical ionization mass spectrometry: m/e 343 $(M + 1)^+$; ^{13}C NMR ($CDCl_3$, Me_4Si internal standard) δ 49.0, 48.8, 48.2, 29.9, and 24.6 (all s); mp 65.5–66.5 °C (uncorrected).

(17) During the addition of the first equivalent of $Cu(ClO_4)_2$ a flocculent light blue precipitate forms which redissolves as the second equivalent is added. Attempts to crystallize the 1:1 Cu–A′ complex have thus far been unsuccessful.

(18) Elemental analysis: Anal. Calcd for $Cu_2C_{21}H_{47}N_8Cl_3O_{13}$: Cu, 14.90; C, 29.57; H, 5.55; N, 13.13; Cl, 12.47; O, 24.38; mol wt, 853.1. Found: Cu, 14.33; C, 29.77 H, 5.78; N, 12.95; Cl, 12.20; O (by difference), 24.97. mol wt (single crystal X-ray diffraction), 856. Magnetic susceptibility: χ vs. T measured by the Faraday method over the range $4.2 \leq T \leq 300$ K showed antiferromagnetic behavior with $J = -29.38$ (1) cm^{-1} (cf. ref 19, 20) and $g = 2.077$ (vs. 2.096 from room temperature ESR measurements on a solid sample). Preliminary X-ray crystallographic work on the 1-methylimidazole adduct of **2**[21] revealed a geometry similar to that found previously for **1**.[7]

(19) (a) Kolks, G.; Lippard, S. J. *J. Am. Chem. Soc.* **1977**, *99*, 5804–5806. (b) Haddad, M. S.; Hendrickson, D. N. *Inorg. Chem.* **1978**, *17*, 2622–2630.

(20) O'Young, C-L.; Dewan, J. C.; Lilienthal, H. R.; Lippard, S. J. *J. Am. Chem. Soc.* **1978**, *100*, 7291–7300.

(21) Coughlin, P. K.; Lippard, S. J. Unpublished results.

(22) Evans, C. A.; Rabenstein, D. L.; Geier, G.; Erni, I. W. *J. Am. Chem. Soc.* **1977**, *99*, 8106–8108.

(23) Fridovich, I. *Science* **1978**, *201*, 875–880.

(24) Fee, J. A. *Int. Sym. Oxidases Relat. Redox Syst. 3rd, 1979*, in press.

(25) Anderegg, G. In "Coordination Chemistry"; Martell, A. E., Ed.; Van Nostrand Reinhold: New York, 1971; Vol. 1, pp 427–490.

Peter K. Coughlin, Stephen J. Lippard*
Department of Chemistry, Columbia University
New York, New York 10027

Andrea E. Martin, John E. Bulkowski
Department of Chemistry, University of Delaware
Newark, Delaware 19711

Received September 15, 1980