intramolecular vibrations. The outer-sphere reorganization energy, ΔG^*_{out}, is

$$\Delta G^*_{out} = \frac{1}{4}\left(\frac{e^2}{2r}\right)\left(\frac{1}{D_o} - \frac{1}{D_s}\right) \quad (6)$$

where r is the radius of the reactant ion, D_o is the optical dielectric constant (the square of the refractive index), and D_s is the static dielectric constant. The work term may be evaluated according to the Debye–Hückel theory:

$$w_r = z_1 z_2 e^2 / 2rD_s(1 + 2\beta r\mu^{1/2}) \quad (7)$$

where z_i are the charges on the reactants, e is the charge on the electron, μ is the ionic strength, and $\beta = (8\pi Ne^2/1000D_s kT)^{1/2}$.

For the porphyrin complexes ΔG^*_{in} is small (<1 kcal) because the structure of the complex changes little on going from Fe(II) to Fe(III).[55] ΔG^*_{out} is calculated according to eq 6 where $r = (r_1 r_2 r_3)^{1/3}$ and the r_i are the radii along the perpendicular axes. For $K[Fe(TPP)(CN)_2]$, $r = 6.2$ Å.[56] In Me_2SO, $1/D_o - 1/D_s = 0.437$ and therefore $\Delta G^*_{out} = 2.9$ kcal/mol. In methanol, $\Delta G^*_{out} = 3.6$ kcal/mol. The Coulombic interaction energy, expressed by eq 7, is 0.4 kcal mol^{-1} in Me_2SO ($\beta = 0.418$ Å^{-1} M$^{-1/2}$ at 37 °C) and 0.5 kcal mol^{-1} in MeOH ($\beta = 0.499$ Å^{-1} M$^{-1/2}$ at 37 °C). Thus, in these systems the activation energy for electron transfer is due mainly to outer-sphere reorganization. The differences in $(\Delta G^*_{out} + w_r)$ between Me_2SO and MeOH is 0.8 kcal. At 37 °C, the reaction is predicted to be approximately 4 times faster in Me_2SO than in MeOH, as was found.

Increased steric bulk on the heme will increase r and decrease both ΔG^*_{out} and w_r. This should give a higher electron self-exchange rate constant.[3,57] Instead, the rate constant is slightly lower. This may be explained as a decrease in orbital overlap between the complexes, resulting in a reaction with a lower probability factor κ. Similar steric effects have been found in the electron self-exchange rate constants of iron phenanthroline complexes[27] and in cross reactions of $Ru(NH_3)_5(py)^{3+/2+}$ complexes with $Co(1,10\text{-phen})_3^{3+/2+}$.[58] Other studies have found either no steric effects or somewhat complicated patterns.[59] In our study steric effects are small, and even a change in macrocyle from tetraphenylporphyrin to the natural protoporphyrin skeleton produces at most a factor of 5 change in the electron self-exchange rate constant. This indicates that electron transfer in low-spin hemes that are not highly charged is relatively insensitive to the exact nature of the macrocycle.

Electron self-exchange rate constants in $FeP(CN)_2^{2-/-}$ (this work) and $Fe(TPP)(RIm)_2^{0/+}$ complexes[15,16] (10^7–10^8 M^{-1} s^{-1}) are only slightly faster than those in the small cytochromes (10^6–10^7 M^{-1} s^{-1}).[15,16] This observation is somewhat surprising; it might have been expected that the proteins would transfer electrons more slowly because most of the heme is covered by the amino acid chain.[60] The difference could be explained in terms of the Marcus theory if w_r and ΔG^*_{out} for proteins were very small. Wherland and Gray have calculated w_r for a number of cytochromes; the values are $0.1 < w_r < 1.0$ kcal.[49] It is difficult to estimate ΔG^*_{out} in proteins because amino acid residues nearby the heme are not free to reorient; it is possible that ΔG^*_{out} is very small. Part of the similarity between the models and proteins may then be explained on the basis of decreases in heme exposure, w_r, and ΔG^*_{out} in the proteins.

However, the large rate constants for electron transfer in small cytochromes may also be a function of factors not considered explicitly in eq 3–7. Possibilities include specific interactions of residues between two proteins, orientation of the proteins in one another's electric field during approach, and formation of complexes. These also may help to explain the wide range of electron self-exchange rate constants, 10^2–10^7 M^{-1} s^{-1}, that have been measured in the heme proteins themselves.

Acknowledgment. We thank the National Institutes of Health (Grants AM 30479 and BRSG S07 RR07054) for support of this work.

Registry No. $Fe^{III}TPP(CN)_2$, 40988-77-0; Fe^{III}(3-MeTPP)$(CN)_2$, 63871-86-3; Fe^{III}(4-MeTPP)$(CN)_2$, 63871-85-2; Fe^{III}(4-MeOTPP)$(CN)_2$, 94929-68-7; Fe^{III}(4-*i*-PrTPP)$(CN)_2$, 94943-94-9; Fe^{III}DPDME$(CN)_2$, 59006-49-4; Fe^{III}PPDME$(CN)_2$, 64060-98-6; Fe^{II}(4-OMeTPP)(CN)(Me_2SO), 94929-69-8; Fe^{II}(4-OMeTPP)$(Me_2SO)_2$, 94929-70-1; $Fe^{II}TPP(CN)_2$, 64060-99-7; Fe^{II}(3-MeTPP)$(CN)_2$, 94929-71-2; Fe^{II}(4-MeTPP)$(CN)_2$, 94929-72-3; Fe^{II}(4-OMeTPP)$(CN)_2$, 94929-73-4; Fe^{II}(4-*i*-PrTPP)$(CN)_2$, 94929-74-5.

(55) (a) Scheidt, W. R.; Gouterman, M. In "Iron Porphyrins"; Lever, A. P. B., Gray, H. B., Eds.; Addison-Wesley: Reading, MA, 1983; Part I, pp 89–139. (b) Spiro, T. G. *Ibid.* Part II, pp 89–159.
(56) Scheidt, W. R.; Haller, K. J.; Hatano, K. *J. Am. Chem. Soc.* **1980**, *102*, 3017–3021.
(57) Brown, G. M.; Sutin, N. *J. Am. Chem. Soc.* **1979**, *101*, 883–892.
(58) Koval, C. A.; Pravata, R. L. A.; Reidsema, C. M. *Inorg. Chem.* **1984**, *23*, 545–553.
(59) The work in this area has been summarized recently by Koval et al.[58]
(60) Sutin, N. *Adv. Chem. Ser.* **1977**, *No. 162*, 156–172.

Contribution from the Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Kinetics of Oxidation of Cuprous Complexes of Substituted Phenanthroline and 2,2′-Bipyridyl by Molecular Oxygen and by Hydrogen Peroxide in Aqueous Solution

SARA GOLDSTEIN and GIDON CZAPSKI*

Received September 14, 1984

The kinetics and the reaction mechanism of copper(I) complexes of 5-methyl-1,10-phenanthroline, 5-chloro-1,10-phenanthroline, 5-nitro-1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline, and 2,2′-bipyridyl with oxygen and hydrogen peroxide have been investigated in aqueous solutions with use of the pulse radiolysis technique. The oxidation by O_2 is second order in the copper(I) complex, while the oxidation by H_2O_2 is first order in the copper(I) complex. Both reactions are first order in oxidants. The kinetic results of the oxidation of copper(I) complexes by oxygen are interpreted by a mechanism that proceeds via a superoxide intermediate.

Introduction

Recently, it has been demonstrated that degradation of double-stranded DNA by 1,10-phenanthroline (op) requires the presence of copper salt, a reducing agent, and O_2.[1-5] The degradation is always inhibited by catalase and in some cases by superoxide dismutase (SOD), suggesting the involvement of H_2O_2 and O_2^-, respectively, in the process.[1-3] The degradation is also

(1) Que, B. G.; Doweny, K. M.; So, A. G. *Biochemistry* **1980**, *19*, 5987.
(2) Graham, D. R.; Marshall, L. E.; Reich, K. A.; Sigman, D. S. *J. Am. Chem. Soc.* **1980**, *102*, 5421.
(3) Marshall, L. E.; Graham, D. R.; Reich, K. A.; Sigman, D. S. *Biochemistry* **1981**, *20*, 244.
(4) Gutteridge, J. M.; Halliwell, B. *Biochem. Pharmacol.* **1982**, *31*, 2801.
(5) Sigman, D. S.; Graham, D. R.; D'Aurora, V.; Stern, A. M. *J. Biol. Chem.* **1979**, *254*, 12269.

0020-1669/85/1324-1087$01.50/0

Table I. Kinetic and Spectroscopic Parameters of CuL_2^+

	5-Me-op	op[a]	5-Cl-op	5-NO_2-op	neocup	2,2′-bpy
λ_{max}, nm	430	435	440	450	454	430
ϵ_{max}, M^{-1} cm^{-1}	5960[b]	7400[b]	6000[b]	7320[b]	7500,[b] 7400[c]	4800,[b] 4000[c]
k_7, M^{-1} s^{-1} [d]	2.3×10^9	1.93×10^9	1.55×10^9	1.55×10^9	3×10^8	2.6×10^9
$k_7\epsilon_{max}/(k_7 + k_8)$, M^{-1} cm^{-1} [e]	6057	5870	4340	4545	4200	3420
$k_{-7}k_8/\epsilon_{max}$, M^{-1} s^{-2} cm [f]	2.26×10^9	1.83×10^9	2.13×10^8	6.87×10^7		2.9×10^9
$k_{12}k_{13}/(\epsilon_{max}k_{-12})$, s^{-1} cm [g]	9.83×10^4	3×10^4	1.75×10^4	625		5×10^3
k_{-7}, M^{-1} s^{-1} [h]	6.57×10^4	5×10^4	4.95×10^3	580		5.82×10^4
ϵ_{max}, M^{-1} cm^{-1} [i]	6660	6770	4935	6965		3660
k_8, M^{-1} s^{-1} [i]	2.3×10^8	2.95×10^8	2.12×10^8	8.25×10^8	$(2.35 \pm 0.4) \times 10^8$ [j]	1.82×10^8

[a] All data from ref 9. [b] Determined directly by pulse radiolysis. [c] Determined from spectroscopic measurements. [d] Determined from the slope of Figure 2. [e] Determined from the slope of Figure 3. [f] Determined from the slope of Figure 5. [g] Determined from the intep of Figure 5. [h] Determined from the slope of Figure 6. [i] Determined by the reiteration in eq 11 and 16. [j] Determined from eq 11 and ϵ_{max}.

inhibited by 2,9-dimethyl-1,10-phenanthroline (neocuproine), a cuprous-specific chelating agent.[1,3,5-7]

When op is replaced by 5-NO_2-op, the cleaving of the DNA is more effective than that of op, while 5-Me-op is less effective.[6] When 2,2′-bipyridyl or 2,2′,2″-terpyridine is used, no degradation of the DNA occurs.[1,2,7,8]

The reaction mechanism for this process has not yet been determined. It is believed that $(op)_2Cu^+$ intercalates with the DNA and the subsequent oxidation by H_2O_2 causes the damage due to the formation of OH· radicals.[1-4]

In our previous work we have determined the reaction mechanism and the kinetics for the oxidation of $(op)_2Cu^+$ by O_2 and H_2O_2 in aqueous solution in the absence of DNA.[9] We have decided to continue our investigation on the other copper complexes mentioned above, and maybe through the differences in their reaction mechanisms and their kinetics in aqueous solution, we will be able to understand more about the biological system containing DNA. However, there may be a difference in the behavior of the copper complexes in the absence of DNA as compared to that of the biological system or the ternary system with DNA.

Cu(I) complexes autoxidize at very different rates by molecular oxygen.[10-13] Copper–dioxygen adducts are often proposed as intermediates in Cu(I) autoxidation reactions,[9,12-17] but until recently[18] these adducts have not been isolated and characterized.

In aqueous solutions, many pieces of evidence speak for one-electron reduction of O_2. However, until recently no direct evidence has been given for the formation of O_2^- or HO_2.[2,9]

Experimental Section

Pulse radiolysis experiments were carried out on a Varian 7715 linear accelerator. The pulse duration ranged from 0.1 to 1.5 μs with 200-mA current of 5-MeV electrons. The analytical light sources were either a 200-W Xe–Hg or a 150-W xenon arc. We used 2.0 or 4.0 cm long irradiation optical cells with three light passes. Appropriate light filters were used to eliminate any scattered light. The detection system included a Bausch and Lomb grating monochromator, Model D330/D331 Mk.II, and an IP28 photomultiplier. The signal was transferred through either a Biomation 8100 or an analog to digital converter to a Nova 1200 minicomputer, which operates the whole pulse radiolysis system.

All solutions were prepared in distilled water, which was further purified by a Milli-Q reagent grade water system (Millipore Corp., Bedford, MA). All chemicals employed were of analytical grade and were used without further purification: 1,10-phenanthroline hydrate, 5-nitro-1,10-phenanthroline (Fluka), neocuproine, 5-methyl-1,10-phenanthroline, 2,2′-bipyridyl, 2,2′,2″-terpyridine (Sigma), 5-chloro-1,10-phenanthroline (Ventron), SOD (Diagnostic Data Int.), H_2O_2 (Hopkin & Williams), cupric sulfate, monosodium and disodium phosphate (Mallinckrodt), and sodium formate (Merck).

Stock solutions were prepared by mixing cupric sulfate with 2.2 equiv of the ligands. The stock solutions contained CuL_2^{2+} at the concentration of 1–5 mM as indicated by their absorption maxima in the visible region.[19,20]

All solutions, except where noted otherwise, contained 0.02 M sodium formate and were buffered with 1 mM $NaH_2PO_4 \cdot H_2O$–$Na_2HPO_4 \cdot 7H_2O$ (pH 7). The solutions were saturated either by air or by oxygen.

The formation and the decay of copper(I) complexes were followed at their absorption maxima in the visible region. The spectra of CuL_2^+ were measured either by pulse radiolysis experiments or from the measurement of the spectra of copper(I) complexes, which were prepared by reducing deoxygenated solutions of copper(II) complexes at pH 7 by an equivalent amount of ascorbate ions. The decay of O_2^- was followed at $\lambda = 254$ nm ($\epsilon = 2000$ M^{-1} cm^{-1}).[21]

The total concentration of O_2^- in the cell was evaluated with the use of $(op)_2Cu^{2+}$ dosimetry. The yield of $(op)_2Cu^+$ in oxygenated formate solution was assumed to be 6.05, and $\epsilon = 6770 \pm 700$ M^{-1} cm^{-1} at $\lambda = 435$ nm.[9] The concentration of the radicals in the cell was 1.0–12 μM for 0.1–1.5-μs pulse duration.

The concentration of H_2O_2 was determined with use of the Fricke ferrous sulfate method (1 mM $Fe(NH_4)_2(SO_4)_2$, 1 mM NaCl, and 0.8 N H_2SO_4), taking $\epsilon_{302}^{Fe^{3+}} = 2197$ M^{-1} cm^{-1}.

Results and Discussion

In the irradiation of aqueous solutions containing formate ions and oxygen, the following reactions occur:

$$H_2O \rightsquigarrow e_{aq}^-, OH\cdot, H\cdot, H_2O_2, OH^-, H_3O^+ \quad (1)$$

$$e_{aq}^- + O_2 \rightarrow O_2^- \qquad k_2 = 2 \times 10^{10}\ M^{-1}\ s^{-1}\ [22] \quad (2)$$

$$H\cdot + O_2 \rightarrow HO_2 \qquad k_3 = 2 \times 10^{10}\ M^{-1}\ s^{-1}\ [23] \quad (3)$$

$$OH\cdot + HCO_2^- \rightarrow H_2O + CO_2^- \qquad k_4 = 3 \times 10^9\ M^{-1}\ s^{-1}\ [24] \quad (4)$$

$$CO_2^- + O_2 \rightarrow CO_2 + O_2^- \qquad k_5 = 2.4 \times 10^9\ M^{-1}\ s^{-1}\ [25] \quad (5)$$

$$HO_2 \rightleftarrows H^+ + O_2^- \qquad K = (2.05 \pm 0.39) \times 10^{-5}\ M\ [26] \quad (6)$$

When CuL_2^{2+} is also present and $[O_2] > [CuL_2^{2+}]$, all the radicals formed by the radiation are converted into O_2^- which will

(6) Reich, K. A.; Marshall, L. E.; Graham, D. R.; Sigman, D. S. *J. Am. Chem. Soc.* **1981**, *103*, 3582.
(7) D'Aurora, V.; Stern, A. M.; Sigman, D. S. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 1025.
(8) (a) Kathleen, M.; Downey, K. M.; Benito, J.; Que, B. G.; So, A. G. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 1025. (b) Doweny, K. M.; Que, B. G.; So, A. G. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 264.
(9) Goldstein, S.; Czapski, G. *J. Am. Chem. Soc.* **1983**, *105*, 7276.
(10) Zuberbuhler, A. D. *Helv. Chim. Acta* **1970**, *53*, 669.
(11) Zuberbuhler, A. D. *Helv. Chim. Acta* **1970**, *53*, 473.
(12) Pecht, I.; Anbar, M. *J. Chem. Soc. A* **1968**, 1902.
(13) Crumbliss, A. L.; Poulos, A. T. *Inorg. Chem.* **1975**, *14*, 1529.
(14) Zuberbuhler, A. D. *Helv. Chim. Acta* **1976**, *59*, 1448.
(15) Guntensperger, M.; Zuberbuhler, A. D. *Helv. Chim. Acta* **1977**, *60*, 2584.
(16) Meisel, D.; Levanon, H.; Czapski, G. *J. Phys. Chem.* **1974**, *78*, 779.
(17) Crumbliss, A. L.; Gestaut, L. J. *J. Coord. Chem.* **1976**, *5*, 109.
(18) Thompson, J. S. *J. Am. Chem. Soc.* **1984**, *106*, 4057.
(19) James, B. R.; Parris, M.; Williams, R. J. *J. Chem. Soc.* **1961**, 44630.
(20) Hogg, R.; Williams, R. G. *J. Chem. Soc.* **1962**, 341.
(21) Bielski, B. H. J.; Allen, A. O. *J. Phys. Chem.* **1977**, *81*, 1048.
(22) Rabani, J.; Thomas, J. K. *J. Am. Chem. Soc.* **1963**, *85*, 1375.
(23) Sweet, J. P.; Thomas, J. K. *J. Phys. Chem.* **1964**, *68*, 1363.
(24) Matheson, M. S.; Mulac, W. A.; Weeks, J. L.; Rabani, J. *J. Phys. Chem.* **1966**, *70*, 2092.
(25) Adams, G. E.; Willson, R. L. *Trans. Faraday Soc.* **1969**, *65*, 2981.
(26) Bielski, B. H. J. *Photochem. Photobiol.* **1978**, *28*, 645.

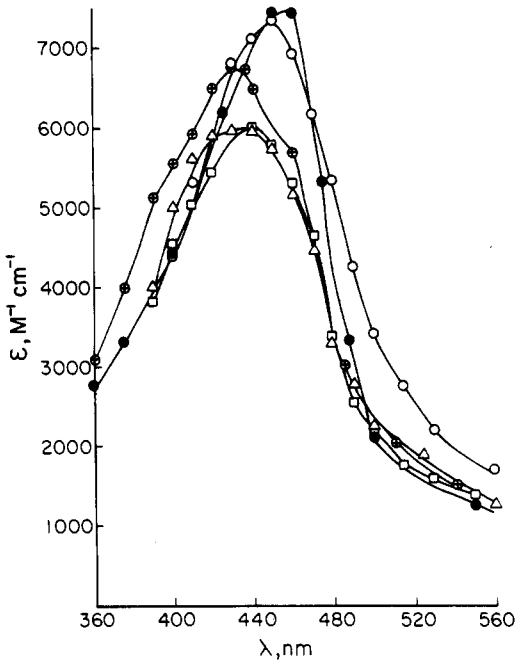

Figure 1. Spectra of CuL_2^+: (▲) $(5\text{-Me-op})_2Cu^+$; (⊕) $(op)_2Cu^+$;[9] (□) $(5\text{-Cl-op})_2Cu^+$; (○) $(5\text{-NO}_2\text{-op})_2Cu^+$; (●) $(neocup)_2Cu^+$. CuL_2^+ was obtained through the reduction of CuL_2^{2+} by O_2^- except for 2×10^{-4} M $(neocup)_2Cu^{2+}$, which was reduced by equivalent amounts of ascorbate ions in deoxygenated solution at pH 7. All solutions where CuL_2^{2+} was reduced by O_2^- were oxygenated and contained 10^{-4} M CuL_2^{2+} and 0.02 M HCO_2Na at pH 7. The pulse duration was 0.5 μs.

reduce CuL_2^{2+} to CuL_2^+ and may oxidize CuL_2^+ to form CuL_2^{2+} and H_2O_2:

$$CuL_2^{2+} + O_2^- \rightarrow CuL_2^+ + O_2 \quad (7)$$

$$CuL_2^+ + O_2^- + 2H^+ \rightarrow CuL_2^{2+} + H_2O_2 \quad (8)$$

Under the condition where $[CuL_2^{2+}] > [CuL_2^+]$, only reaction 7 will take place and, knowing the concentration of O_2^-, one can follow the absorption spectrum of CuL_2^+. The superoxide radicals were able to reduce the various copper(II) complexes except that of 2,2′,2″-terpyridine. In Figure 1, the extinction coefficients of CuL_2^+ are given for $(5\text{-Me-op})_2Cu^+$, $(op)_2Cu^+$, $(5\text{-Cl-op})_2Cu^+$, $(5\text{-NO}_2\text{-op})_2Cu^+$, and $(neocup)_2Cu^+$. The strong electron-accepting properties of the ligands are directly demonstrated by a metal to ligand charge-transfer band at 430–454 nm with the maximum extinction coefficients of $\epsilon_{430} = 5960$ M^{-1} cm^{-1}, $\epsilon_{435} = 6770$ M^{-1} cm^{-1}, $\epsilon_{440} = 6000$ M^{-1} cm^{-1}, $\epsilon_{450} = 7320$ M^{-1} cm^{-1} and $\epsilon_{454} = 7400$ M^{-1} cm^{-1}, respectively.

$(neocup)_2Cu^{2+}$ and $(bpy)_2Cu^{2+}$ were reduced both by ascorbate ions and by O_2^-. We found that for $(bpy)_2Cu^{2+}$ there is a difference of 20% in the reduction yield between the two methods (Table I). We have no explanation for this difference.

Kinetics of the Formation of CuL_2^+. Under the conditions where $[CuL_2^{2+}] > [CuL_2^+]$ rate eq 9 is obtained. The second-order

$$-\frac{d[O_2^-]}{dt} = \frac{d[CuL_2^+]}{dt} = k_7[CuL_2^{2+}][O_2^-] = k_{obsd}[O_2^-] \quad (9)$$

rate constant k_7 was obtained by plotting k_{obsd} as a function of CuL_2^{2+} concentration (Figure 2). The values of k_7 are listed in Table I.

At low CuL_2^{2+} concentration, where $[CuL_2^{2+}] < [O_2^-]$, the yield of CuL_2^+ was less than the initial concentration of CuL_2^{2+}. Therefore, we must assume that reaction 8 also takes place. Under these conditions the catalytic decay of O_2^- predominates and the noncatalytic dismutation can be ignored.[23,26] Assuming steady state for the concentration of CuL_2^+, we obtain

$$[CuL_2^+]_{ss} = \frac{k_7[CuL_2^{2+}]}{k_8} = \frac{k_7}{k_7 + k_8}[CuL_2^{2+}]_0 \quad (10)$$

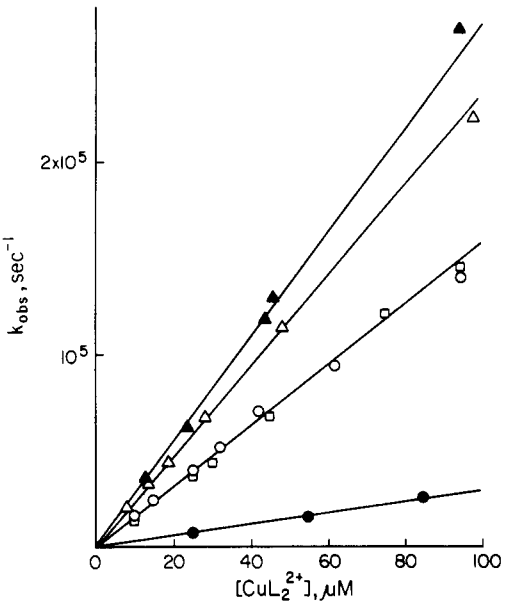

Figure 2. Formation rate constant of CuL_2^+ as a function of $[CuL_2^{2+}]$: (▲) L = 5-Me-op; (□) L = 5-Cl-op; (○) L = 5-NO_2-op; (●) L = neocup; (▲) L = 2,2′-bpy. All solutions contained 0.02 M HCO_2Na at pH 7 and were air saturated.

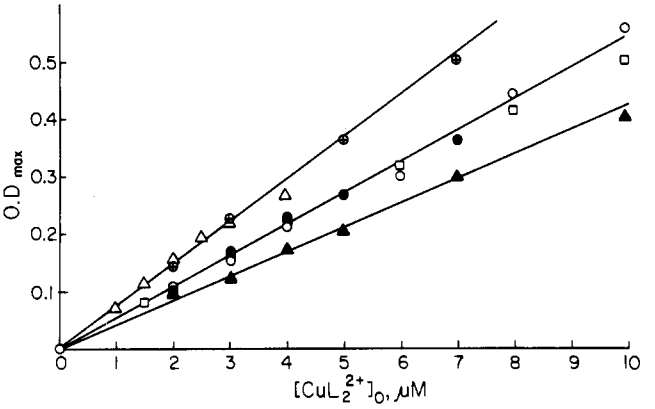

Figure 3. OD_{max} (the OD at the absorption peak) of CuL_2^+ as a function of the initial concentration of CuL_2^{2+}: (▲) L = 5-Me-op; (⊕) L = op;[9] (□) L = 5-Cl-op; (○) L = 5-NO_2-op; (●) L = neocup; (▲) L = 2,2′-bpy. All solutions contained 0.02 M HCO_2Na at pH 7 and were air saturated. The pulse duration was 1.5 μs, and the optical path was 12.1 cm.

In Figure 3, OD_{max}, the OD at the absorption peak, is plotted vs. $[CuL_2^{2+}]_0$. From the slope of the straight line one obtains the value of $\epsilon_{max}k_7/(k_7 + k_8)$ (Table I), which is very close to the value of ϵ_{max}:

$$\frac{\text{slope}}{l} = \frac{\epsilon_{max}k_7}{k_7 + k_8} \quad (11)$$

Since the measured ϵ_{max} had an error of at least 10%, one cannot determine accurately the value of k_8 from eq 11.

Reactions 7 and 8 lead to the rate equation

$$-\frac{d[O_2^-]}{dt} = \frac{2k_7k_8}{k_7 + k_8}[CuL_2^{2+}]_0[O_2^-] = k_{obsd}'[O_2^-]$$

and we define the "turnover" rate constant, k_{cat}, as

$$k_{cat} = \frac{2k_7k_8}{k_7 + k_8}$$

We can measure k_{cat} directly by following the decay of O_2^- at $\lambda = 254$ nm. Since the absorption of CuL_2^{2+} in the UV region is too high, we were not able to follow the decay and to measure k_{cat} at concentrations above 0.5–1 μM of CuL_2^{2+}. These are very low concentrations, and since we were not able to use EDTA, one

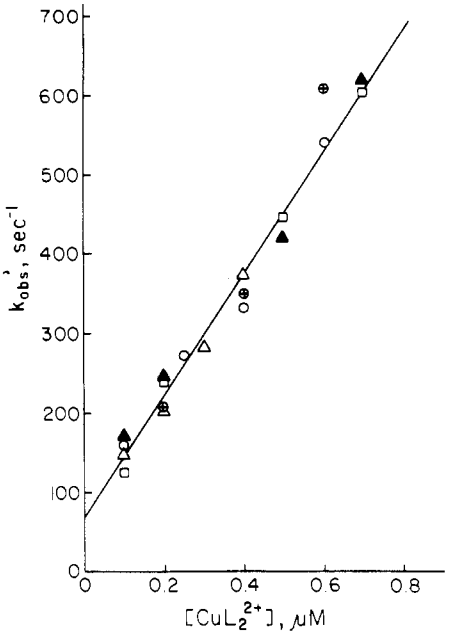

Figure 4. Decay rate constant of O_2^- as a function of $[CuL_2^{2+}]$: (△) L = 5-Me-op; (⊕) L = op; (□) L = 5-Cl-op; (○) L = 5-NO_2-op; (▲) L = 2,2′-bpy. All solutions contained 0.02 M HCO_2Na at pH 7 and were air saturated. The pulse duration was 1.5 μs.

must bear in mind that catalytic impurities exist in the solutions ($k_{obsd}' = 70$–80 s^{-1} at $[CuL_2^{2+}] = 0$). In Figure 4, k_{obsd}' is plotted vs. CuL_2^{2+} concentration. From the slope of the line one can measure k_{cat}. We found that except for $(neocup)_2Cu^{2+}$ and $(terp)_2Cu^{2+}$, k_{cat} for all the complexes is $(7.6 \pm 0.6) \times 10^8$ M^{-1} s^{-1}. We did not observe any catalytic activity for $(neocup)_2Cu^{2+}$ up to 0.5 μM. Nevertheless, the decay of O_2^- was first order with respect to $[O_2^-]$ with $k_{obsd}' = 80$–100 s^{-1}. On the other hand, $(terp)_2Cu^{2+}$ did not catalyze at all the dismutation of O_2^- and the decay of O_2^- turned out to be second-order dependence with respect to O_2^-, as if we added EDTA to the solution without the complex.

Reaction Mechanism for the Oxidation of CuL_2^+ by O_2. The mechanism for the oxidation of the various cuprous complexes by oxygen was the same as was determined earlier for the oxidation of cuprous 1,10-phenanthroline. The exception was cuprous neocuproine, which is not oxidized by molecular oxygen at any measurable rates.

The mechanism is described by the steps

$$CuL_2^+ + O_2 \underset{k_7}{\overset{k_{-7}}{\rightleftharpoons}} CuL_2^{2+} + O_2^- \quad (7)$$

$$CuL_2^+ + O_2^- + 2H^+ \xrightarrow{k_8} CuL_2^{2+} + H_2O_2 \quad (8)$$

$$CuL_2^+ + O_2 \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} CuL_2O_2^+ \quad (12)$$

$$CuL_2O_2^+ + CuL_2^+ + 2H^+ \xrightarrow{k_{13}} 2CuL_2^{2+} + H_2O_2 \quad (13)$$

Assuming steady state for the concentration of O_2^- and $CuL_2O_2^+$, we obtain

$$-\frac{d[CuL_2^+]}{dt} = \frac{2k_{-7}k_8[O_2][CuL_2^+]^2}{k_7[CuL_2^{2+}] + k_8[CuL_2^+]} + \frac{2k_{12}k_{13}[O_2][CuL_2^+]^2}{k_{12} + k_{13}[CuL_2^+]} \quad (14)$$

Under the conditions where $k_{-12} > k_{13}[CuL_2^+]$ and $[CuL_2^{2+}]_0 > [CuL_2^+]$, eq 14 reduces to eq 15 and second-order dependence on CuL_2^+ would be observed. In Figure 5 there is a plot of

$$-\frac{d[CuL_2^+]}{dt} = \left[\frac{2k_{-7}k_8}{k_7[CuL_2^{2+}]_0} + \frac{2k_{12}k_{13}}{k_{-12}}\right][O_2][CuL_2^+]^2 = k_{obsd}''[CuL_2^+]^2 \quad (15)$$

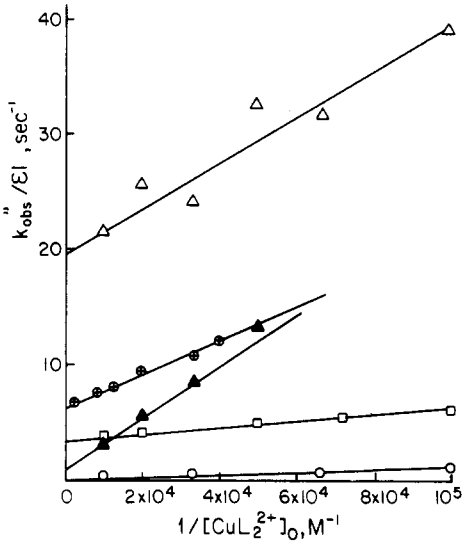

Figure 5. Dependence of the decay rate constant of CuL_2^+ as a function of the reciprocal of the initial concentration of CuL_2^{2+}: (△) L = 5-Me-op; (⊕) L = op;[9] (□) L = 5-Cl-op; (○) L = 5-NO_2-op; (▲) L = 2,2′-bpy. All solutions contained 0.02 M HCO_2Na at pH 7 and were oxygen saturated. The optical path was 12.1 cm.

$k_{obsd}''/\epsilon_{max}l$ vs. the reciprocal of $[CuL_2^{2+}]_0$ in oxygenated solutions. From the intercept of the line one obtains the value of $k_{12}k_{13}/\epsilon_{max}k_{-12}$ and from the slope of the value of $k_{-7}k_8/\epsilon_{max}$ using the values of l, k_7, and $[O_2]$ (Table I). We obtain

$$\frac{k_{-7}k_8}{\epsilon_{max}} = \frac{(\text{slope})k_7 l}{2[O_2]} \quad (16)$$

When SOD is present, the disproportionation of O_2^- is very rapid and reactions 12 and 13 can be neglected. Under the conditions where the concentration of CuL_2^+ is lower relative to that of $[CuL_2^{2+}]_0$ and SOD, we get

$$-\frac{d[CuL_2^+]}{dt} = \frac{k_{-7}k_E[E]_0[O_2][CuL_2^+]}{k_7[CuL_2^{2+}]_0 + k_E[E]_0} = k_{obsd}'''[CuL_2^+] \quad (17)$$

k_E is the "turnover" rate constant of SOD. We measured it directly with our SOD and found $k_E = (3 \pm 0.3) \times 10^9$ M^{-1} s^{-1}. $[E]_0$ is the initial concentration of the enzyme. In Figure 6 the reciprocal of k_{obsd}''' is plotted vs. the initial concentration of CuL_2^{2+} at constant concentration of 1.96 μM of SOD. From the slope of the line we obtained the value of k_{-7} (Table I).

Knowing the value of k_{-7} and ϵ_{max}, we can calculate k_8 from eq 16. From eq 11 with the value of k_7 and k_8 we can calculate ϵ_{max}. As the value of k_8 is sensitive to the values of ϵ_{max}, we determined both k_8 and ϵ_{max} by reiteration of k_8 and ϵ_{max} in eq 11 and 16. The values thus obtained are given in Table I. The values of ϵ_{max}, except for 5-Cl-op and 2,2′-bpy, which were found by reiteration, are within the experimental error of 10% with those measured directly. For 5-Cl-op and 2,2′-bpy the difference in ϵ_{max} is 20% and 30%, respectively.

From the measured values of k_7 and k_8 we obtained the values of k_{cat}: $(4.2 \pm 0.4) \times 10^8$, $(3.7 \pm 0.35) \times 10^8$, $(1.08 \pm 1) \times 10^9$, $(2.6 \pm 0.3) \times 10^8$, and $(3.4 \pm 0.35) \times 10^8$ M^{-1} s^{-1} for 5-Me-op, 5-Cl-op, 5-NO_2-op, neocup, and 2,2′-bpy, respectively. These values differ from those obtained directly following the decay of O_2^- by 30–50%. We cannot explain this discrepancy.

The kinetics of the oxidation of some substituted phenanthroline complexes of copper(I) by molecular oxygen were investigated with use of stopped-flow spectrophotometry techniques in 2.5 M aqueous acetonitrile.[17] The oxidation was found to be first order with respect to each reactant. The same results were obtained for the 2,2′-bipyridyl–copper(I) complex in aqueous solution.[12] In both studies the authors used a wrong value of the redox potential of O_2/O_2^- (−0.33 V instead of −0.16 V, due to the confusion between standard states of oxygen, 1 M and 1 atm,

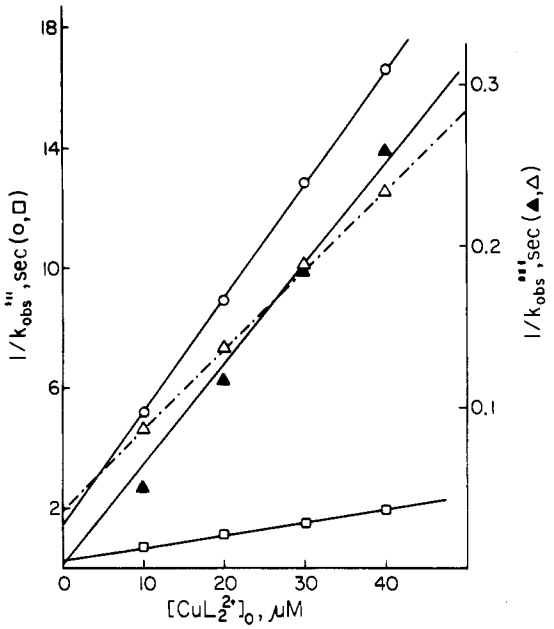

Figure 6. Reciprocal of the decay rate constant of CuL_2^+ as a function of the initial concentration of CuL_2^{2+}: (△) L = 5-Me-op; (□) L = 5-Cl-op; (○) L = 5-NO_2-op; (▲) L = 2,2′-bpy. All solutions contained 1.96 μM SOD and 0.02 M HCO_2Na at pH 7 and were oxygen saturated. The pulse duration was 0.1 μs.

Table II. Redox Potentials of the Copper Complexes

	5-Me-op	op	5-Cl-op	5-NO_2-op	2,2′-bpy
$E^\circ_{CuL_2^{2+}/CuL_2^+}$, mV	108	110	164	219	114

respectively) and therefore on thermodynamic grounds the one-electron reduction of O_2 to give O_2^- seemed to them an unfavorable process. Therefore, they suggested that the autoxidation mechanism involves a simultaneous two-electron reduction of O_2 to give H_2O_2 directly. Our results serve to demonstrate both a stepwise one-electron reduction and a simultaneous two-electron reduction of O_2.

Oxidation–Reduction Potential of the Copper Complexes. From the measured values of k_7 and k_{-7} and with use of the reduction potential $E^\circ_{O_2/O_2^-} = -0.16$ V at 1 M oxygen (−0.33 V at 1 atm of oxygen[27]), we calculate the redox potential $E^\circ_{CuL_2^{2+}/CuL_2^+}$ (Table II).

The result serves to demonstrate a strong influence on the autoxidation rates of copper(I) complexes by electron-donating or -withdrawing groups. In Figure 7, $E^\circ_{CuL_2^{2+}/CuL_2^+}$ is plotted vs. the pK_a[28] of the conjugated acid of the free ligand of the phenanthroline group, yielding a straight line. The pK_a of the conjugate acid of the free ligand can be considered as a measure of σ-electron donor strength. A similar linear correlation is obtained by plotting the logarithm of k_{-7} as a function of the pK_a. The slope is 1.2, which means that 100-fold decrease in the K_a of the conjugate acid of the free ligand results in about 100-fold increase in the second-order rate constant. These results are similar to that obtained in 2.5 M aqueous acetonitrile.[17]

The cuprous neocuproine complex is very stable in the presence of oxygen. This is in contrast to what is expected from plot b in Figure 7, where the oxidation rate constant of this complex is expected to be higher than that of $(op)_2Cu^+$. Neocuproine introduces considerable steric hindrance in the four-coordinate cupric complex, which is usually planar. James and Williams have commented[28] on the importance of steric hindrance in determining the value of the redox potential for CuL_2^{2+}/CuL_2^+. Thus, the

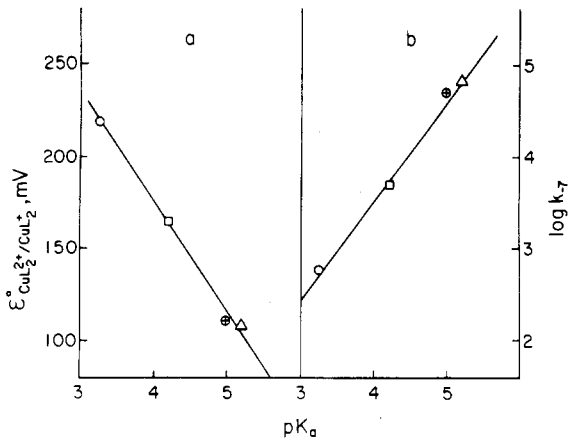

Figure 7. Relations (a) between $E^\circ_{CuL_2^{2+}/CuL_2^+}$ and pK_a and (b) between log k_{-7} and pK_a for a series of 2:1 cuprous complexes with 1,10-phenanthroline and substituted 1,10-phenanthroline: (△) L = 5-Me-op; (⊕) L = op; (□) L = 5-Cl-op; (○) L = 5-NO_2-op.

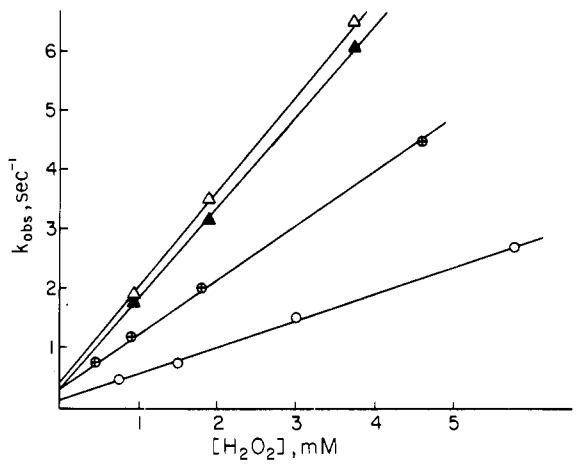

Figure 8. Rate constant of the decay of CuL_2^+ as a function of $[H_2O_2]$: (△) L = 5-Me-op; (⊕) L = op;[9] (○) L = 5-NO_2-op; (▲) L = 2,2′-bpy. All solutions contained 30 μM CuL_2^{2+} and 0.02 M HCO_2Na at pH 7 and were air saturated. The pulse duration was 0.2 μs.

value of the redox potential is much higher than that which is expected from plot a in Figure 7, and therefore k_{-7} should be much lower than that which is expected from plot b in Figure 7. Thus, the stability of $(neocup)_2Cu^+$ is interpreted on the grounds of steric hindrance.

Oxidation of CuL_2^+ by H_2O_2. When H_2O_2 was present in excess relative to CuL_2^+ and oxygen, the rate law for the oxidation of CuL_2^+ was found to be first order with respect to $[CuL_2^+]$ and $[H_2O_2]$:

$$CuL_2^+ + H_2O_2 \rightarrow CuL_2^{2+} + OH^- + OH\cdot \quad (18)$$

$$-\frac{d[CuL_2^+]}{dt} = k_{18}[H_2O_2][CuL_2^+] = k_{obsd}[CuL_2^+] \quad (19)$$

Figure 8 gives the plot of k_{obsd} vs. the concentration of H_2O_2. From the slopes of the lines we measured the values of 1620 ± 40, 440 ± 10, and 1540 ± 40 M^{-1} s^{-1} for 5-Me-op, 5-NO_2-op, and 2,2′-bpy, respectively. The $(neocup)_2Cu^+$ is not oxidized by H_2O_2 at any measurable rate. The oxidation rate constant of $(bpy)_2Cu^+$ was also measured in aqueous solution by using the stopped-flow spectrophotometry method, with $k = 850$ M^{-1} s^{-1}.[12] We have no explanation for the difference by a factor of 2 between our results and those of the flow method.

Correlation between the Kinetic Properties of the Various Copper Complexes and Their Ability To Cleave DNA. 1,10-Phenanthroline and several substituted 1,10-phenanthroline were the only chelating agents found to be effective in inducing degradation of DNA in the presence of Cu(II), a reducing agent, and molecular oxygen.

(27) Ilan, Y. A.; Czapski, G.; Meisel, D. *Biochim. Biophys. Acta* **1976**, *430*, 209.
(28) James, B. R.; Williams, R. J. P. *J. Chem. Soc.* **1961**, 2007.

Our results demonstrate the formation of superoxide in the oxidation of CuL_2^+ by molecular oxygen in aqueous solution. Superoxide potentiates the cleavage reaction because it increases the concentration of CuL_2^+ by reduction of CuL_2^{2+}, which subsequently reacts with hydrogen peroxide to yield the hydroxyl radicals. Our results seem to contradict this hypothesis. For example, the cuprous complex of 2,2'-bipyridyl, which does not cleave DNA, produces diffusible superoxide at rates comparable to those of 1,10-phenanthroline, while the cuprous complex of 5-nitro-1,10-phenantrholien, which damages DNA more effectively than 1,10-phenanthroline, produces the diffusible superoxide at a rate that is 100-fold lower than that of 1,10-phenanthroline. These results are in agreement with those observed earlier by Sigman.[2]

One might also assume that the difference in the DNA cleavage reaction is due to the different rates at which O_2^- reduces the Cu(II) complexes. Our results show that k_7 and k_{cat} have almost the same values for the various Cu(II) complexes. Moreover, according to our results, no correlation exists between the rate at which CuL_2^+ reacts with hydrogen peroxide to form OH· and its ability to damage the DNA.

Therefore, it seems that the main source of damage to DNA in these systems does not originate from OH· radicals being formed in the bulk of the solution.

We believe that the breakdown of the DNA depends on the binding or intercalating of the coordination complex to the DNA during the course of the reaction. One possibility is that the rate constants of the relevant reactions of free and bound CuL_2^+ may be different. Those cuprous complexes that cleave DNA may have higher rate constants when they are bound or intercalated to the DNA than those that do not cleave the DNA.

Another very probable possibility is that the cuprous complexes that cause the damage intercalate into the DNA in a unique orientation. This is in accord with the observation of Pope and Sigman[29] on the difference in the damage of cuprous 1,10-phenanthroline on the A, B, and Z forms of DNA. This seems to be a probable explanation for the inability of cuprous 2,2'-bipyridyl to cleave DNA even though its coordination chemistry is similar to that of 1,10-phenanthroline and the fact that complexes of 2,2'-bipyridyl may bind to DNA.[30]

We expect that a site-specific mechanism may operate in this system and therefore a different orientation in the intercalation of CuL_2^+ into the DNA will yield the hydroxyl radical, according to reaction 18, at different sites and thus will cause the difference in the ability to cleave the DNA for the various cuprous complexes.

Acknowledgment. This work was supported by Grant No. 1409 of the Council for Tobacco Research, by the Gesellschaft für Strahlen Forschung Neuherberg, and by DOE Grant No. EY-(11-1)-3221.

Registry No. $Cu(5\text{-}Me\text{-}op)_2^+$, 17702-23-7; $Cu(5\text{-}Cl\text{-}op)_2^+$, 52152-05-3; $Cu(5\text{-}NO_2\text{-}op)_2^+$, 59751-73-4; $Cu(neocup)_2^+$, 21710-12-3; $Cu(2,2'\text{-}bpy)_2^+$, 36450-97-2; O_2, 7782-44-7; H_2O_2, 7722-84-1.

(29) Pope, L. E.; Sigman, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3.
(30) Howe-Grant, M.; Lippard, S. J. *Biochemistry* **1979**, *18*, 5762.

Contribution from the Chemistry Department, Manchester University, Manchester M13 9PL, England, and Institut für Anorganische Chemie der Universität, D3400 Göttingen, West Germany

Preparation, Crystal Structure, and Spectroscopic Characterization of the Tetranuclear Copper-Thiolate Cluster $[Cu_4(o\text{-}(SCH_2)_2C_6H_4)_3]^{2-}$ as Its $[PPh_4]^+$ Salt

JOHN R. NICHOLSON,[1a] IAN L. ABRAHAMS,[1a] WILLIAM CLEGG,*[1b] and C. DAVID GARNER*[1a]

Received July 23, 1984

$[Cu_4(o\text{-}(SCH_2)_2C_6H_4)_3]^{2-}$ has been prepared by reacting $K_2[o\text{-}(SCH_2)_2C_6H_4]$ with $[Cu(N\text{-methylimidazole})_4][BF_4]$ (1.1:1) in dried acetonitrile and by reacting $Cu(NO_3)_2\cdot 6H_2O$, NEt_3, and $o\text{-}(HSCH_2)_2C_6H_4$ (1:10:5) in ethanol. $[PPh_4]_2[Cu_4(o\text{-}(SCH_2)_2C_6H_4)_3]\cdot MeCN$ crystallizes in the triclinic space group $P\bar{1}$ with $a = 11.900$ (2) Å, $b = 14.000$ (2) Å, $c = 21.550$ (3) Å, $\alpha = 82.09$ (2)°, $\beta = 81.06$ (2)°, $\gamma = 73.21$ (2)°, $V = 3379.1$ Å^3, and $Z = 2$. The structure was solved by direct methods, followed by least-squares refinement using 5374 reflections to a final R value of 0.072 ($R_w = 0.098$). The anion consists of a tetrahedron of copper atoms (Cu---Cu = 2.726 (16) Å), each edge of which is spanned by a μ-thiolato group, and each copper is coordinated by a trigonal-planar array of sulfur atoms (Cu-S = 2.272 (20) Å). The arrangement of the three chelate rings produces an overall symmetry for the anion that is approximately C_3. ^1H and ^{13}C NMR data are consistent with the maintenance of this structure in solution.

Copper-thiolate chemistry is of much current interest, as part of the sustained interest in the thiolate chemistry of the later 3d transition metals,[2-24] together with the cysteinyl ligation of this metal that has been established for plastocyanin[25] and other "blue" copper[26] and metallothionein[27] proteins. The latter are capable

(1) (a) Manchester University. (b) Universität Göttingen. Present address: Department of Inorganic Chemistry, The University, Newcastle upon Tyne, NE1 7RU England.
(2) Holah, D. G.; Coucouvanis, D. *J. Am. Chem. Soc.* **1975**, *97*, 6918.
(3) Christou, G.; Huffman, J. C. *J. Chem. Soc., Chem. Commun.* **1983**, 558.
(4) Costa, T.; Dorfman, J. R.; Hagen, K. S.; Holm, R. H. *Inorg. Chem.* **1983**, *22*, 4091.
(5) Berg, J. M.; Holm, R. H. In "Metal Ions in Biology"; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1982; Vol. 4, Chapter 1 and references therein.
(6) Hagen, K. S.; Holm, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 5496.
(7) Hagen, K. S.; Watson, A. D.; Holm, R. H. *J. Am. Chem. Soc.* **1983**, *105*, 3905.
(8) Hagen, K. S.; Christou, G.; Holm, R. H. *Inorg. Chem.* **1983**, *22*, 309.
(9) Henkel, G.; Tremel, W.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 319.
(10) Hagen, K. S.; Holm, R. H. *Inorg. Chem.* **1984**, *23*, 418.
(11) Swenson, D.; Baenziger, N. C.; Coucouvanis, D. *J. Am. Chem. Soc.* **1978**, *100*, 1932.
(12) Dance, I. G.; Calabrese, J. C. *J. Chem. Soc., Chem. Commun.* **1975**, 762.
(13) Dance, I. G. *J. Am. Chem. Soc.* **1979**, *101*, 6264.
(14) Henkel, G.; Tremel, W.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 318.
(15) Tremel, W., Krebs, B.; Henkel, G. *Inorg. Chim. Acta* **1983**, *80*, L31.
(16) Coucouvanis, D.; Murphy, C. N.; Kanodia, S. K. *Inorg. Chem.* **1980**, *19*, 2993.
(17) Dance, I. G.; Calabrese, J. C. *Inorg. Chim. Acta* **1976**, *19*, L41. Dance, I. G.; Bowmaker, G. A.; Clark, G. R.; Seadon, J. K. *Polyhedron* **1983**, *2*, 1031.
(18) Dance, I. G. *J. Chem. Soc., Chem. Commun.* **1976**, *68*. Bowmaker, G. A.; Clark, G. R.; Seadon, J. K.; Dance, I. G. *Polyhedron* **1984**, *3*, 535.
(19) Dance, I. G. *Aust. J. Chem.* **1978**, *31*, 2195.
(20) Birker, P. J. M. W. L.; Freeman, H. C. *J. Chem. Soc., Chem. Commun.* **1976**, 312.
(21) Dance, I. G. *J. Am. Chem. Soc.* **1980**, *102*, 3445.
(22) Dance, I. G. *J. Chem. Soc., Chem. Commun.* **1980**, 818.
(23) Hencher, J. L.; Khan, M.; Said, F. F.; Tuck, D. G. *Inorg. Nucl. Chem. Lett.* **1981**, *17*, 287.
(24) Dance, I. G. *Inorg. Chem.* **1981**, *20*, 2155.

0020-1669/85/1324-1092$01.50/0