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Electron Transfer. 113. Reduction of Bound Superoxide with Thiols. Catalysis by Copper(II)[1]

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The binuclear superoxo complex of cobalt(III) $[Co_2(O_2)(CN)_{10}]^{5-}$ is reduced exceedingly slowly by the mercapto derivatives 2-aminoethanethiol and cysteine at pH 4.5–7.2. Reaction is catalyzed dramatically by dissolved copper but by none of the other usual transition metal centers. Each superoxide anion oxidizes one RSH, yielding the disulfide R_2S_2 and the binuclear peroxo complex $[Co_2(O_2)(CN)_{10}]^{6-}$, which rapidly decomposes to $[Co(CN)_5(H_2O)]^{2-}$ and H_2O_2. All reactions are first order in copper with the unipositive state the active reductant. Transformations are markedly inhibited by high concentrations of thiol, pointing to partial conversion of the Cu(I) center to a much less reactive thiol adduct. With 2-aminoethanethiol, conversion of added Cu(II) to Cu(I) appears to be complete within a few seconds of mixing. Reductions with cysteine in deficiency give linear decay profiles with slopes independent of both [RSH] and [oxidant]. Under these conditions, rates are fixed by the unimolecular generation of Cu^I from $Cu^{II}(SR)$. As [RSH] is raised, progressive conversion of the more active to the less active Cu^I reductant is reflected in a change in the rate-determining step, and reactions become first order in [oxidant]. Integration of differential equations based upon the proposed sequence (eqs 7–13 in text) reproduces the observed profiles, not only in the linear and exponential regions but also in an intermediate region where decay curves are of mixed order. Dissimilarities between the reductive actions of the two thiols are related to the differing stabilities of the $Cu^{II}(SR)$ intermediates. The catalytic effectiveness of Cu^I in thiol systems, in comparison to Fe^{II}, is attributed both to the more negative potential of $Cu^{I,II}$ and to the lesser substitution lability of thiol-bound Fe^{II}.

The last two decades have witnessed a dramatic revival of interest in the reactions of superoxide, reflecting, in part, its intrusion into the area of metabolic processes.[2] Striking progress in superoxide research has been spurred by development of diverse preparative methods[3] and improvements in analytical procedures.[4]

Most important, superoxide has been implicated as an intermediate in some autoxidations[5] and as a byproduct in the functioning of aerobic organisms.[6] Its survival in biosystems is customarily cut short by the action of bimetalloproteins (superoxide dismutases) that catalyze its disproportionation into H_2O_2 and O_2.[7] The role of such proteins is thought to be the protection of the organism from superoxide toxicity, but the detailed nature of such toxic effects remains controversial.[7,8]

Although much of the work on superoxide reactions has dealt with disproportionations in systems featuring substitution-labile transition metal centers (transformations which are presumably related to the action of superoxide dismutases in biosystems),[9] it is recognized that superoxide is a strong nucleophile which can form persistent metal complexes when facile decomposition paths are not at hand. In many instances, superoxo derivatives of substitution-inert metal centers have been characterized,[10] and a newer, comparably interesting, body of chemistry is associated with superoxo adducts of labile d^0 states, which must be examined in solution.[11]

The chemistry of mononuclear superoxo complexes is often complicated by the ease with which they abstract hydrogen atoms from organic substrates[12] and, in some cases, by their loss of molecular oxygen at low partial pressures of O_2. Binuclear (μ-coordinated) superoxo derivatives generally pose fewer problems in preparation and in handling and can frequently be stored as crystalline salts.

Investigations to date on the redox chemistry of $(Co^{III})_2$–superoxide complexes have been centered about reactions at low pH with the metal center reductants Fe^{2+}, Cr^{2+}, V^{2+}, and Eu^{2+} [13] and with the dimeric species $Mo_2O_4^{2+}$.[14] In each case, reaction appears to be initiated by $1e^-$ reduction to the dimeric μ-peroxo cation, which, under the conditions used, decomposes to $2Co^{2+}$ and O_2. A less detailed study[15] of reactions with SO_2 and SO_3^{2-} indicates that reduction of the superoxide function is accompanied by incorporation of the oxidation product, SO_4^{2-}, into the coordination sphere of Co(III).

We find virtually no reports treating the reactions of bound superoxide with such nonmetallic reductants as hydrazine, hydroxylamine, ascorbate, and azide. Moreover, reactions with organic thiols are of particular interest in view of the role of RSH/R_2S_2 couples in modulating the redox potential at biological

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(1) Sponsorship of this work by the donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.

(2) For example: (a) Fridovich, I. *Acc. Chem. Res.* **1972**, *5*, 321. (b) Fridovich, I. *Adv. Inorg. Biochem.* **1979**, *67*, 1. (c) Fee, J. A.; Valentine, J. S. In *Superoxide and Superoxide Dismutase*; Michelson, A. M., McCord, J. M., Fridovich, I., Eds.; Academic Press: New York, 1979; p 19.

(3) (a) Sawyer, D. T.; Roberts, J. L., Jr. *J. Electroanal. Chem.* **1966**, *12*, 90. (b) Valentine, J. S.; Curtis, A. B. *J. Am. Chem. Soc.* **1975**, *97*, 224. (c) McDowell, M. S.; Bakac, A.; Espenson, J. H. *Inorg. Chem.* **1983**, *22*, 847. (d) Jeon, S.; Sawyer, D. T. *Inorg. Chem.* **1990**, *29*, 4612.

(4) (a) Knowles, P. F.; Gibson, J. F.; Pick, F. M.; Brick, F.; Bray, R. C. *Biochem. J.* **1969**, *111*, 53. (b) Gampp, H.; Lippard, S. J. *Inorg. Chem.* **1983**, *22*, 357.

(5) (a) Taube, H. *Prog. Inorg. Chem.* **1986**, *34*, 607. (b) Bakac, A.; Espenson, J. H.; Creaser, I. I.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 7624.

(6) Bannister, W. H.; Bannister, J. V. *Biological and Clinical Aspects of Superoxide and Superoxide Dismutase*; Elsevier: Amsterdam, 1980.

(7) For example: Valentine, J. S.; de Freitas, D. M. *J. Chem. Educ.* **1985**, *62*, 990.

(8) Fee, J. A. In *Oxygen and Oxy-Radicals in Chemistry and Biology*; Rodgers, M. A. J., Powers, E. L., Eds.; Academic Press: New York, 1981; pp 205–239.

(9) For example: Bull, C.; McClune, G. J.; Fee, J. A. *J. Am. Chem. Soc.* **1983**, *105*, 3920.

(10) For a review: Hill, H. A. O.; Tew, D. G. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Ed.; Pergamon: Oxford, U.K., 1987; Vol. 2, Chapter 15.2.

(11) (a) Samuni, A.; Czaspski, G. *J. Phys. Chem.* **1970**, *74*, 4592. (b) Bourke, G. C. M.; Thompson, R. C. *Inorg. Chem.* **1987**, *26*, 903.

(12) Nishinaga, A.; Shimizu, T.; Toyoda, T.; Matsuura, T.; Ishiguro, M. *J. Org. Chem.* **1982**, *47*, 2278.

(13) (a) Hoffman, A. B.; Taube, H. *Inorg. Chem.* **1968**, *7*, 1971. (b) Davies, R.; Sykes, A. G. *J. Chem. Soc. A* **1968**, 2831.

(14) Sasaki, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1939.

(15) Edwards, J. D.; Yang, C.-H.; Sykes, A. G. *J. Chem. Soc., Dalton Trans.* **1974**, 1561.

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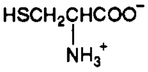
sites.[16] Reactions of this general type are highly favored thermodynamically, but the substitution-inert character of the oxidant and the absence of acceptor sites in the reductant appear to rule out inner-sphere redox routes in these cases. Past experience[17] with reductions of the peroxo complexes of Cr^{IV} (which exhibit redox behavior broadly similar to that of superoxo derivatives of Co^{III}) suggests that such conversions are slow but may be markedly catalyzed by traces of Fe(II), Fe(III), and Cu(II), even at levels as low as 10^{-7} M.

The present study deals with the reductions, using aminoethanethiol (I) and L-cysteine (II), of the dimeric superoxo complex (μ-superoxo)bis[pentacyanocobaltate(III)] (III). As anticipated,

$H_2NCH_2CH_2SH$

I



$HSCH_2CH(NH_3^+)COO^-$

II

$[(NC)_5Co{-}O_2{-}Co(CN)_5]^{5-}$

III

these reductions are slow and are strikingly accelerated by dissolved copper. Although the superoxide complex plays an essential role in these reactions, marked differences between the kinetic pictures associated with these two catalytic systems appear principally to reflect variations in the reducing capabilities of the participating copper(I)–thiol complexes as well as differences in the ease with which they are generated from their copper(II) analogs.

Experimental Section

Materials. Potassium (μ-superoxo)bis[pentacyanocobaltate(III)] hydrate, $K_5[Co_2(O_2)(CN)_{10}]\cdot H_2O$, was prepared by the method of Davies and co-workers[18] from (μ-superoxo)bis[pentaamminecobalt(III)] chloride, $[(NH_3)_5Co(O_2)Co(NH_3)_5]^{5+}(Cl^-)_5$, by treatment with aqueous KCN. After acidification with HNO_3 (hood!), the zinc salt was precipitated by addition of $ZnSO_4$, washed thoroughly with water, and then redissolved in a minimum volume of 10% aqueous KCN. The resulting solution was added, with stirring, to 10 times its volume of 1:1 C_2H_5OH/CH_3OH. The red microcrystalline precipitate so obtained was redissolved in a minimum volume of water, reprecipitated with EtOH/MeOH as above, washed with absolute EtOH, and air-dried. Further reprecipitations in the same manner led to partial decomposition, as did attempted chromatographic separation on Bio-Gel P2. The solid may be stored in the dark at -10 °C for 3 months without significant deterioration. Anal. Calcd for $K_5[(CN)_5Co(O_2)Co(CN)_5]\cdot H_2O$: K, 31.4; C, 19.3; N, 22.5; H, 0.32; Co, 18.8. Found: K, 32.4; C, 18.8; N, 22.8; H, 0.29; Co, 18.7. λ_{max}, nm (ϵ, M^{-1} cm^{-1}): 202 (3.4×10^4), 311 (2.2×10^4), 485 (930).

L-Cysteine and 2-aminoethanethiol hydrochloride (Aldrich products) were used as received, as were the Aldrich buffers MES (4-morpholineethanesulfonic acid, pK_A 6.1) and BES (*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, pK_A 7.1). Copper(II) perchlorate solution was prepared by dissolving $CuCO_3$ in $HClO_4$, and sodium perchlorate (for kinetic experiments) was prepared by treatment of $NaHCO_3$ with aqueous $HClO_4$. Because of the sensitivity of the redox reactions to dissolved copper, kinetic runs were carried out in solutions which were made from Millipore water and which had been stirred in contact with zinc amalgam for 7 days, a treatment which was somewhat more effective than passage through Chelex type cation-exchange resin.

Stoichiometric Studies. The stoichiometries of the superoxo–thiol redox reactions were determined spectrophotometrically at 310 nm. Measured deficient quantities of the reductant were added to a known excess of the superoxo complex in the presence of 10^{-6} M Cu(II). After net reaction had ceased, decreases in absorbance were measured. These changes were then compared to those resulting from addition of an excess of the reductant. Results are summarized in Table I.

Kinetic Measurements. Rates were estimated from absorbance changes at 310 nm by using a Beckman Model 5260 or a Cary 14 recording spectrophotometer. Ionic strength was regulated by addition of $NaClO_4$, and pH was generally maintained by using measured quantities of $HOAc{-}OAc^-$ or MES buffer. Conversions were followed to at least 98% completion. All reactions were very nearly first order in copper, and those carried out with the reductant in large excess were first order in oxidant. Dependence on reductant was complex (see Results and Discussion), and in most cases reactions proceeded more slowly at high [Red] than at low. When decay curves were exponential, rate constants were obtained from logarithmic plots of absorbance differences against reaction time. For reactions of this type, specific rates obtained from replicate runs agreed to better than 6%.[19,20]

Table I. Stoichiometry of the Reactions of $[Co_2(O_2)(CN)_{10}]^{5-}$ with Thiols[a]

reductant (Red)	10^4[Red], M	$10^4\Delta$[Ox], M	Δ[Red]/Δ[Ox]
2-aminoethanethiol	2.0	2.38	1.19
	4.0	4.6	1.15
	6.0	5.9	0.99
	7.8	7.1	0.91
cysteine	2.0	2.3	1.15
	4.0	4.2	1.05
	6.0	5.1	0.95

[a] Reactions were carried out in acetate buffers, pH 4.60; μ = 0.50 M ($NaClO_4$); λ = 310 nm; [superoxide] ("Ox") = 8×10^{-4} M and [Cu^{2+}] = 10^{-6} M throughout.

Three different types of profile were obtained from reductions with cysteine. With the reductant in large excess, decay was logarithmic, but with oxidant in excess, profiles were very nearly linear. Although slopes resulting from replicate runs under the latter conditions exhibited some scatter, there was no systematic dependence on the concentration of either redox partner. Curves obtained when the ratio [Red]/[Ox] was between 2 and 5 were neither linear nor exponential and were subjected to a more elaborate analysis (see Results and Discussion).

Reactions carried out in the absence of added Cu^{2+} proceeded about 0.20 times as rapidly as those with 1.1×10^{-6} M Cu^{2+}. Addition of 0.06 M EDTA at pH 6.95 lowered the rate by a further factor of 10.

The reactions of $[Co_2(O_2)(CN)_{10}]^{5-}$ with thiols were not catalyzed significantly by U(IV), Mo(VI), V(IV), Ni(II), Ce(III), Zn(II), Ag(I), or Fe(III) (each at the 10^{-5} M level), either at pH 4.5 (HOAc buffer) or at pH 5.5 (MES buffer).

Results and Discussion

In the media taken for most of our experiments, 2-aminoethanethiol (pK_1 = 8.25; pK_2 = 10.72)[21] exists predominantly as its N-protonated unipositive cation, whereas cysteine (pK_1 = 1.88; pK_2 = 8.16; pK_3 = 10.36)[21] is converted principally in its zwitterionic form (II).

The close approach to 1:1 stoichiometry (Table I), allows us to represent the primary redox reaction as (1). The spectrum of

$$2[Co_2(O_2)(CN)_{10}]^{5-} + 2RSH \rightarrow 2[Co_2(O_2)(CN)_{10}]^{6-} + R_2S_2 + 2H^+ \quad (1)$$

the final product ($\epsilon_{max}^{375} = 2.7 \times 10^2\ M^{-1}\ s^{-1}$) corresponds to that of $[(CN)_5Co(H_2O)]^{2-}$, in keeping with the report[22] that the decacyano-μ-peroxo anion very rapidly decomposes in water to this monomeric complex and H_2O_2.

Kinetic data for reductions of our superoxo complex with 2-aminoethanethiol are assembled in Table II. As seen, rates are independent of pH within the range 4.5–7.1 and are very nearly proportional to [Cu^{2+}] taken. The very slow reaction in the

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(16) For example: Ziegler, D. M. *Annu. Rev. Biochem.* **1985**, *54*, 305.

(17) Ghosh, S. K.; Gould, E. S. *Inorg. Chem.* **1988**, *27*, 4228; **1989**, *28*, 1658, 1948, 3651.

(18) Davies, R.; Mori, M.; Sykes, A. G. *Inorg. Synth.* **1970**, *12*, 202.

(19) When aminoethanethiol was in excess, but at concentrations below 7×10^{-4} M, departures from strictly exponential decay were slight but reproducible, due almost certainly to the slow release of cyanide by the reaction product and the attendant partial ligation of catalytic copper. In such cases, the specific rate of the reaction of interest was estimated by fitting the observed curves, using a described numerical integration procedure,[20] to a sequence involving both the catalyzed reduction of superoxide and the slow loss of available copper. Rates obtained in this way corresponded closely to those calculated from the initial half-life of the decay curve.

(20) Bose, R. N.; Rajasekar, N.; Thompson, D. M.; Gould, E. S. *Inorg. Chem.* **1986**, *25*, 3349.

(21) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1989; Vol. 6, pp 20, 181. These values pertain to 25 °C and μ = 0.10 M.

(22) Haim, A.; Wilmarth, W. K. *J. Am. Chem. Soc.* **1961**, *83*, 509.

Table II. Kinetic Data for the Reaction of $[Co_2(O_2)(CN)_{10}]^{5-}$ with 2-Aminoethanethiol[a]

[RSH], mM	$10^6[Cu^{2+}]$	pH	10^2k,[b] s^{-1}	[RSH], mM	$10^6[Cu^{2+}]$	pH	10^2k,[b] s^{-1}
0.10	1.0	5.66	2.5 (2.6)	1.25	0	5.67	0.69 (0.69)
0.16	1.0	5.66	3.6 (3.4)	1.25	2.5	5.67	1.38 (1.37)
0.26	1.0	5.69	3.9 (4.0)	1.25	5.0	5.66	1.93 (2.0)
0.32	1.0	5.69	4.1 (4.2)	1.25	15.0	5.67	4.5 (4.8)
0.48	1.0	5.69	4.2 (4.2)	1.25	20	5.68	6.6 (6.1)
0.64	1.0	5.68	3.9 (4.1)	1.25	30	5.69	8.6 (8.9)
1.25	1.0	5.67	3.6 (3.4)	1.25	50	5.70	13.9 (14.3)
2.50	1.0	5.66	3.0 (2.7)	1.25	10.0	7.12	3.4 (3.4)
3.5	1.0	5.66	2.5 (2.4)	1.25	10.0	4.56	3.3 (3.4)
5.0	1.0	5.64	2.1 (2.2)	1.25	10.0	5.64[c]	0.09
10.0	1.0	5.62	1.96 (1.91)	5.0	0	7.05[d]	0.12
20.0	1.0	5.62	1.75 (1.75)	5.0	0	6.95[e]	0.07
40	1.0	5.60	1.63 (1.67)				

[a] Reactions were run at 25.0 ± 0.2 °C; $\mu = 0.5$ M ($NaClO_4$); solutions were buffered with MES, BES, and HOAc–OAc^-; $\lambda = 310$ nm; [Ox] = 2×10^{-5} M throughout. [b] Pseudo-first-order specific rates; parenthetical values were calculated using (4) and parameters listed in text. [c] $[CN^-] = 3 \times 10^{-4}$ M. [d] 0.030 M EDTA added. [e] 0.060 M EDTA added.

absence of added copper may be attributed to traces of this element in our systems, particularly since rates are found to be further depressed by addition of the sequestrant EDTA.

The most notable feature of this system is the kinetic dependence on [reductant]. At very low concentrations, rates increase with [RSH] and are seen to attain a maximum at [RSH] = 0.3–0.5 mM, beyond which they decrease sharply (Figure 1). This trend points to the operation of two equilibria in which RSH participates, along with the catalyst (rather than the oxidant, which is coordinatively saturated). If we represent these equilibria as (2) and (3),[23] this partition leads to expression 4, where k_{CR}

$$\text{Cat} + \text{RSH} \overset{K_2}{\rightleftharpoons} \text{Cat}{\cdot}\text{RSH} \qquad (2)$$

$$\text{Cat}{\cdot}\text{RSH} + \text{RSH} \overset{K_3}{\rightleftharpoons} \text{Cat(RSH)}_2 \qquad (3)$$

$$k_{obsd} = \frac{-d\ln[\text{Ox}]}{dt} = \frac{k_{CR}K_2[\text{RSH}] + k_{CR_2}K_2K_3[\text{RSH}]^2}{1 + K_2[\text{RSH}] + K_2K_3[\text{RSH}]^2}([\text{Cat}] + b) \qquad (4)$$

and k_{CR_2} designate specific rates pertaining to Cat·RSH and Cat$(RSH)_2$. Least-squares refinement of our kinetic data in terms of (4) leads to $k_{CR} = (7.0 \pm 1.1) \times 10^4$ M^{-1} s^{-1}, $k_{CR_2} = (1.26 \pm 0.05) \times 10^4$ M^{-1} s^{-1}, $K_2 = (4.5 \pm 1.1) \times 10^3$ M^{-1}, and $K_3 = (2.1 \pm 0.6) \times 10^3$ M^{-1}. The small quantity b, representing the concentration of Cu^{2+} in our "purified" water, is $(2.5 \pm 0.1) \times 10^{-7}$ M. Rates calculated using (4) are compared with observed values in Table II.

Reductions of the superoxo complex by cysteine, using a greater than 7-fold excess of thiol, also yield exponential profiles and are likewise retarded by increasing [RSH] (Table III). However, this system features no region of positive [thiol] dependence analogous to the rising arm of the curve in Figure 1. Extension of measurements to lower [RSH] yields no hint of such a region but produces distorted decay patterns exhibiting some zero-order, rather than the expected second-order, character. Reactions with the oxidant in excess give linear decay profiles with slopes independent of both [oxidant] and [RSH], thus presenting us with an unusual situation in which the *rate of a redox transformation is zero order in both reaction partners*. Rates are very nearly proportional to catalyst, whether the oxidant or reductant is in excess.

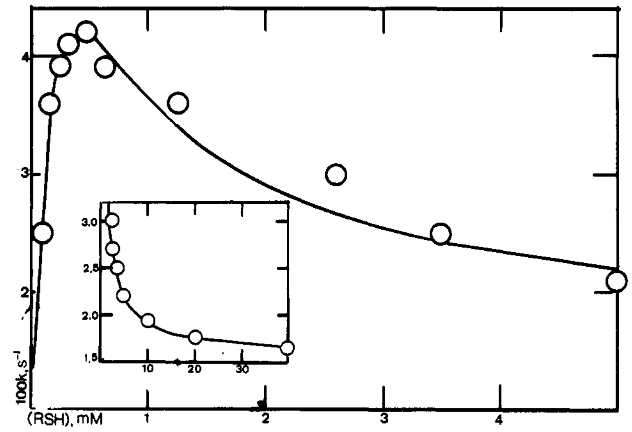

Figure 1. Variation of rates with [thiol] ([RSH]) for the reduction of $[Co_2(O_2)(CN)_{10}]^{5-}$ with 2-aminoethanethiol as catalyzed by Cu^{2+}. Reactions were run at 25 °C, $\mu = 0.50$ M ($NaClO_4$), pH = 5.5–5.6, $[Cu^{2+}] = 1.0 \times 10^{-6}$ M, and [oxidant] = 2.0×10^{-5} M. Circles designate observed pseudo-first-order specific rates; the solid line represents values calculated using eq 4 and parameters listed in the text. The insert shows the continued decrease in rates at higher thiol concentrations.

The observed [cysteine] dependency is in accord with partition of the catalyst into just two forms, Cat and Cat(RSH), both of them active. This distribution should be reflected in rate law 5,

$$k_{obsd} = \frac{-d\ln[\text{Ox}]}{dt} = \frac{k_C + k_{CR}K_{CR}[\text{RSH}]}{1 + K_{CR}[\text{RSH}]}([\text{Cat}] + b) \qquad (5)$$

which is analogous to (4). Here k_{CR} pertains to the bound catalyst, k_C refers to the unbound catalyst, and K_{CR} is the association constant for Cat(RSH). With the reductant in excess, we observe no approach to kinetic saturation at low [RSH], indicating that the product K_{CR}[RSH] ≫ 1 throughout this series of runs and allowing simplification of (5) to (5′). Data may be refined in

$$k_{obsd} = (k_{CR} + k_C/K_{CR}[\text{RSH}])([\text{Cat}] + b) \qquad (5')$$

terms of (5′), keeping b (adventitous Cu^{2+}) = 3×10^{-7} M, to yield $k_{CR} = (2.6 \pm 1.0) \times 10^2$ M^{-1} s^{-1} and the quotient $k_C/K_{CR} = 0.49$ s^{-1}. Although individual values of k_C and K_{CR} cannot be obtained from these exponential profiles, these quantities may be estimated as 3.6×10^4 M^{-1} s^{-1} and 7.3×10^4 M^{-1} from treatment of nonexponential profiles taken when [Red]/[Ox] = 2.5–4 (see below). Rates calculated from (5) using these parameters are compared with observed rates in Table III.

When the oxidant is in excess, the much simpler relationship (6) applies, with $b = (3.1 \pm 1.2) \times 10^{-7}$ M.

$$\text{rate} = \frac{-d[\text{Ox}]}{dt} = (0.21\ \text{s}^{-1})([\text{Cat}] + b) \qquad (6)$$

Any mechanistic description of this system must then accommodate the marked differences in kinetic behavior resulting from variations in conditions. We favor the sequence, represented schematically as (7)–(13), in which the catalytic action of copper entails a recycling between the di- and unipositive states.

Since the direct reaction of thiols with our oxidant has been shown to be negligibly slow (Table II), the active reductant is taken here to be unipositive copper. As a number of workers

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[23] Intervention of complexes of RSH, rather than those of RS^- (which would arise from the equilibrium Cat + RSH ⇌ Cat(RS) + H^+), is proposed in accordance with the lack of pH dependence noted for this system. Because the ratio [RSH]/[Cat] exceeds 10^2 in virtually all runs, it is possible here, as in the sequence (8)–(12), that additional thiol molecules are associated with the catalytic centers. Since the observed [RSH] dependencies give information concerning only *changes* in the number of Cu-bound thiols, additional "kinetically silent" thiols are not shown in the proposed sequences.

Table III. Kinetic Data for the Reaction of $[Co_2(O_2)(CN)_{10}]^{5-}$ with L-Cysteine[a]

A. excess reductant (exponential decay)[b]				B. excess oxidant (linear decay)[c]			
[Cys], mM	$10^6[Cu^{2+}]$	pH	10^3k,[d] s^{-1}	[Cys], mM	$10^6[Cu^{2+}]$	pH	rate × 10^7 [e]
0.30	2.0	5.58	3.9 (4.3)	2.0[b]	1.0	5.59	2.6
0.50	2.0	5.59	2.6 (2.8)	3.0	1.0	5.60	3.2
0.65	2.0	5.59	2.2 (2.3)	4.0	1.0	5.57	3.0
0.80	2.0	5.59	1.83 (1.99)	3.0	1.0	5.57	2.8
1.25	2.0	5.57	1.51 (1.49)	2.0	1.0	5.59	3.3
0.50	1.0	5.58	1.65 (1.61)	4.0	0.50	5.59	1.50
0.50	3.0	5.59	4.0 (4.1)	4.0	2.0	5.58	4.6
0.50	4.0	5.58	5.9 (5.3)	4.0	3.0	5.59	6.8
0.50	6.0	5.56	9.1 (7.8)	4.0	5.0	5.60	10.2
0.50	8.0	5.58	12.8 (10.3)	4.0	1.0	7.15	2.7
0.50	2.0	7.13	2.8 (2.8)				

[a] Reactions were run at 25.0 ± 0.2 °C; $\mu = 0.5$ M; solutions were buffered with 0.010 M (total concentration) MES or BES buffer; $\lambda = 310$ nm. [b] $[Ox] = 4 \times 10^{-5}$ M. [c] $[Ox] = 8.0 \times 10^{-5}$ M unless otherwise indicated. [d] Pseudo-first-order specific rates; parenthetical values were calculated using (5) and parameters listed in text. [e] Pseudo-zero-order rates, $-d[Ox]/dt$ (M s^{-1}).

$$RSH \rightleftharpoons RS^- + H^+ \qquad K_7^A = 7 \times 10^{-9}\ M \qquad (7)^{21}$$

$$RS^- + Cu^{2+} \overset{K_8}{\rightleftharpoons} [Cu^{II}(RS)]^+ \qquad (8)$$

$$[Cu^{II}(RS)]^+ \xrightarrow{k_9} Cu^I + RS^\bullet \qquad (9)$$

$$RSH + Cu^I \overset{K_{10}}{\rightleftharpoons} Cu^I(RSH) \qquad (10)$$

$$Cu^I(RSH) + Ox \xrightarrow{k_{11}} RSH + Cu^{II} + [Co_2(O_2)(CN)_{10}]^{6-} \qquad (11)$$

$$Cu^I + Ox \xrightarrow{k_{12}} Cu^{II} + [Co_2(O_2)(CN)_{10}]^{6-} \qquad (12)$$

$$2RS^\bullet \xrightarrow{k_{13}} R_2S_2 \qquad (13)$$

have demonstrated,[24] the reduction of Cu(II) to Cu(I) with thiols proceeds through Cu(II)–thiol complexes, which, depending upon the structure of the thiol and upon reaction conditions, may be moderately persistent or barely perceptible. The oxidant may react with Cu(I) as such (eq 12) or, more slowly, with the complex Cu^I·RSH (eq 11). The two kinetic contributions correspond to the two terms in the numerator of rate law 5.

With the oxidant in excess, the higher oxidation state of copper is assumed to predominate, and conversion to the cysteinate complex, $[Cu^{II}(SR)]^+$ (eq 8), is very nearly complete. Under these conditions, the rate of the primary reaction is determined by reaction 9, the unimolecular decomposition to Cu(I), showing that the subsequent Cu(I)–superoxide reaction (eq 12) is rapid compared to the generation of Cu(I). In this region of linear decay, rates do not change systematically as reductant is consumed, indicating that partition of Cu(II) between ligation levels involving the thiol is not of consequence.

When the reductant is taken in large excess, the reaction is strongly retarded, and the attendant alteration in the kinetic picture tells us that the consumption of Cu(I) by superoxide has become slow in relation to its formation. The observed rates are then weighted averages of contributions of (11) and (12), with the relative magnitudes of each depending upon partition via equilibrium 10. Since [RSH] suffers only a slight decrease during the course of the reaction, the distribution between slow (11) and rapid (12) paths does not change significantly.

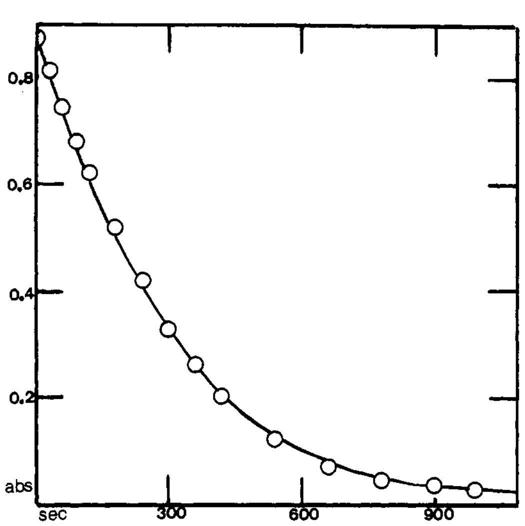

Figure 2. Kinetic profile at 310 nm for the reduction of $[Co_2(O_2)(CN)_{10}]^{5-}$ (4.0×10^{-5} M) with L-cysteine (1.20×10^{-4} M), as catalyzed by Cu^{2+} (1.28×10^{-6} M) at 25 °C. $[H^+] = 2.5 \times 10^{-6}$ M; $\mu = 0.50$ ($NaClO_4$). The solid line is the experimental curve, whereas the circles represent absorbances calculated from numerical integration of differential equations based on the sequence (7)–(13) in the text, taking parameters listed in Table IV. Extinction coefficients used (M^{-1} cm^{-1}): oxidant, 2.2×10^4; product, 2.5×10^2 per cobalt. Optical path length = 1.00 cm.

The "mixed-order" curves obtained when cysteine is in less than 5-fold excess (e.g. Figure 2) reflect the appreciable loss of reductant as the redox reaction proceeds and, consequently, the shift in equilibrium 10 to favor the more effective catalyst.

Expression of the proposed sequence as a series of differential kinetic equations and numerical integration using an adaptation of the program KINSIM[25] yielded the concentration of the various participating species at 15-s intervals during the course of the reaction, and incorporation of the molar absorbances of the superoxo reactant and its reduced peroxo product yielded calculated values for the optical density of the reaction mixture at each point.

Values of rate and equilibrium constants giving agreement between calculated and observed absorbances[26] appear in Table IV. The acidity constant K_7^A is the recorded[21] second ionization constant of cysteine; the high value of K_8 was chosen to ensure virtually complete conversion of Cu(II) to its thiol complex at all acidities examined. The unimolecular specific rate k_9 was determined from experiments in the linear decay region. The

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(24) For example: (a) Cavallini, D.; de Marco, C.; Dupre, S.; Rotilio, G. *Arch. Biochem. Biophys.* **1969**, *130*, 354. (b) Lappin, A. G.; McAuley, A. *J. Chem. Soc., Dalton Trans.* **1978**, 1606. (c) Davis, F. J.; Gilbert, B. C.; Norman, R. O. C.; Symons, M. C. R. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1763.

(25) Barshop, B. A.; Wrenn, R. F.; Frieden, C. *Anal. Biochem.* **1983**, *130*, 134. The original KINSIM program, for which we thank Dr. Carl Frieden, was developed for use on a VAX computer system. This was modified, using FORTRAN-77, to a "fixed-length" format and executed on an IBM 3081D system.

(26) Since the KINSIM procedure does not utilize equilibrium constants as such, these were expressed as ratios of rate constants for forward and back reactions. Typically, for K_7^A ($= 7 \times 10^{-9}$ M), we included k_7 as 70 s^{-1} and k_{-7} as 10^{10} M^{-1} s^{-1}.

(27) A reviewer has suggested that reactions 14 and 15 may compete with,

$$RS^\bullet + RS^- \rightleftharpoons (R_2S_2^\bullet)^- \qquad (14)$$

$$(R_2S_2^\bullet)^- + Ox \rightarrow R_2S_2 + \text{product} \qquad (15)$$

or perhaps overshadow, reaction 13 as processes by which $RS^\bullet$ is consumed (for example: Asmus, K.-D. *Methods Enzymol.* **1990**, *186*, 168). Since these occur after the rate-determining step(s), alteration of the proposed sequence to include (14) and (15) should not affect the overall kinetic profile. If the $(R_2S_2^\bullet)^-$ path is greatly favored over (13), the value of k_9 would be just half that indicated in Table IV.

Table IV. Rate Constants and Equilibrium Quotients Contributing to the Reaction of $[Co_2(O_2)(CN)_{10})^{5-}$ with L-Cysteine[a]

K_7^A	7×10^{-9} M	k_{11}	260 M^{-1} s^{-1}
K_8	10^9 M^{-1}	k_{12}	3.6×10^4 M^{-1} s^{-1}
k_9	0.21 s^{-1}	k_{13}	10^8 M^{-1} s^{-1}
K_{10}	7.3×10^4 M^{-1}		

[a] Parameters pertain to the sequence (7)–(13) in text. Reactions were run at 25 °C; $\mu = 0.50$ M ($NaClO_4$); pH = 5.59; $\lambda = 310$ nm; [Ox] = 4×10^{-5} M; [Red] = $(1.0–1.6) \times 10^{-4}$ M.

component rate constants k_{11} and k_{12} were estimated from refinement of exponential data in terms of (5). The equilibrium quotient K_{10} has also been taken from the latter refinement. The specific rate assigned to the recombination of the RS$^•$ radicals (k_{13}) does not affect the closeness of fit.[23] Absorbances calculated from these parameters (circles) are compared to an experimental curve generated within the "mixed-order" region in Figure 2.

The most pronounced dissimilarities between the reductive actions of the two thiols examined are then evident at low [RSH] and are in accord with the reported[24c] differences in the stabilities of the $Cu^{II}SR$ intermediates. In the pH range studied, N-protonation of 2-aminoethanethiol rules out stabilization of its complexes by chelation. Conversion to Cu(I) (as with the complexes of thiophenol and 2-mercaptoethanol)[24c] is expected to be very nearly complete within a few seconds of mixing with excess thiol. In contrast, the slower unimolecular decomposition of the more stable Cu(II) complex of cysteine becomes kinetically significant when the resulting Cu(I) center is principally in its more reactive form.

The catalytic behavior of dissolved copper in these superoxide–thiol systems brings to mind dramatic $[Cu^{2+}]$-proportional accelerations observed when thiols are oxidized by $Fe(CN)_6{}^{3-}$,[28a–c] O_2,[28d] or peroxo-bound Cr(IV).[17] The striking effectiveness of Cu(I), in comparison to Fe(II), may be attributed in part to the more negative formal potential of $Cu^{I,II}$ and in part to the lesser substitution lability of thiol-bound Fe(II). The data of Hoffman and Taube[13a] suggest that reductions of $(Co^{III})_2$-bound superoxide by Cr(II), V(II), and Eu(II) (each having a strongly negative $E°$ value) proceed by outer-sphere paths, and we suspect that this is the case with our systems as well.

Finally, it may be asked whether the catalytic picture described here is confined to reductions of dimeric Co(III)-bound superoxides or whether it applies also to other superoxo-coordinated systems. Extension of these studies to superoxo complexes of such transition metal centers as Rh(III),[29] Cr(III),[30] Nb(V),[31] and Ti(IV),[32] although entailing synthetic problems more serious than those encountered in the present work, may be instructive.

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(28) (a) Bridgart, G. J.; Fuller, M. W.; Wilson, I. R. *J. Chem. Soc., Dalton Trans.* **1973**, 1274. (b) Bridgart, G. J.; Wilson, I. R. *J. Chem. Soc., Dalton Trans.* **1973**, 1281. (c) Duke, F. R.; Bulgrin, V. C. *J. Phys. Chem.* **1975**, *79*, 2323. (d) Ehrenberg, L.; Harms-Ringdahl, M.; Fedorcsak, I.; Grabath, F. *Acta Chem. Scand.* **1989**, *43*, 177.

(29) Bianchini, C.; Frediani, P.; Laschi, F.; Meli, A.; Vizza, F.; Zanello, P. *Inorg. Chem.* **1990**, *29*, 3402.

(30) Brynildson, M. E.; Bakac, A.; Espenson, J. H. *Inorg. Chem.* **1988**, *27*, 2592.

(31) Richard, P.; Guilard, R. *J. Chem. Soc., Chem. Commun.* **1983**, 1454.

(32) Thompson, R. C. *Inorg. Chem.* **1984**, *23*, 1794.