time and an E_0 near that of the other Os(II) complexes. Consequently, the other Os(II) complexes are to be preferred for exploratory studies.

The $Ir(phen)_3^{3+}$ absorption and blue-green emission are similar to that of $Ir(bipy)_3^{3+}$. Both complexes can be readily pumped at higher concentrations (10^{-3} to 10^{-4} M) in the 350–360 nm region; however, pumping the much more intense $\pi \rightarrow \pi^*$ absorptions at shorter wavelength would minimize transmission corrections. The Os(II) complexes absorb intensely ($\epsilon > 2000$) across the entire visible and are readily pumped in this region as well as the uv. Their emissions are ~700 nm in fluid solutions which makes them difficult to measure on spectrofluorimeters without extended red response photomultipliers. In all cases, the E_0's are not very sensitive to changes in solvent. A 0.1–0.2 kK shift, which is within our experimental error, sometimes occurs on changing from 4:1 (v/v) ethanol–methanol glass to 4:1 (v/v) methanol–water glass.

As is characteristic of charged complexes of this type, the solubility, but not the emission properties, is affected by the counterion. The nitrate salts of the Ir(III) complexes and the chloride salts of the cationic Os(II) complexes used here are soluble in a wide variety of polar organics and water, and we recommend these as the form of choice for most solvents. The perchlorates are all of limited solubilities which would make them more difficult to use as sensitizers.

Especially noteworthy with transition-metal sensitizers is the ability to vary the donor charge and to fine tune E_0 by ligand modifications. The charge factors are especially important with ionic quenchers.

Energies of the thermally equilibrated emitting state, E_0's, were estimated from 77 K emission spectra in ethanol–methanol 4:1 (v/v) glasses using the Fleischauer criterion.[16] τ_0's were estimated using a N_2 laser system which is described elsewhere;[17] semilogarithmic plots were linear over at least 3 half-lives. Photolysis studies used Ar (488 nm at 0.1–1.5 W) and Kr (351, 356 nm at 0.1 W) lasers and water-filtered 500-W tungsten lamps. $Ir(bipy)_3^{3+}$ was synthesized as recently described,[18] and $Ir(phen)_3^{2+}$ can be prepared and purified by an analogous procedure.[15] $Os(bipy)_3^{2+}$ and $Os(phen)_3^{2+}$ can be prepared by literature methods.[19] The other two Os(II) complexes were prepared by reaction of aqueous $Os(phen)_2Cl_2$ with the appropriate ligand. All osmium complexes were purified by column chromatography with Sephadex LH-20 (methanol) and/or Cellex P (aqueous 0.1 F HCl).[20]

Current work is devoted to obtaining other metal complexes with a wider range of E_0's, ionic charges, and longer τ_0's. Attempts to stabilize the Ir(III) complexes by using different solvents or different ligand combinations are underway.[15]

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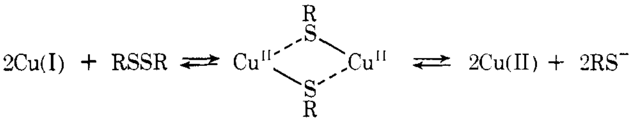
Models for the Electron Paramagnetic Resonance Nondetectable Copper in "Blue Oxidases".[1] A Binuclear Copper(II) Complex with Oxidized Glutathione

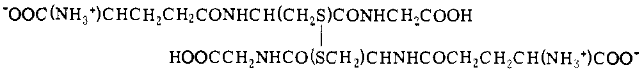
Sir:

In recent years we have studied the properties of binuclear cupric mercaptides,[2] which are formed both from Cu(II) and chelating thiols RSH (R = $-CH_2CH_2NH_2$, $-C(CH_3)_2CH(COO^-)NH_2$, or $-CH_2CH(CONH_2)NH_2$) or Cu(I) and the corresponding disulfides.



$$2Cu(I) + RSSR \rightleftharpoons Cu^{II}(\mu\text{-}SR)_2Cu^{II} \rightleftharpoons 2Cu(II) + 2RS^-$$

Generally clusters of this type can be regarded as models for the two-electron-accepting EPR-nondetectable copper pair in "Blue Oxidases",[1] which is diamagnetic in the oxidized and the reduced state. From recent results by Byers et al.[3] and Driving and Deinum,[4] it seems now quite clear that cyste(i)ne sulfur does not participate in electron transfer during the catalytic cycle but that there might be antiferromagnetic coupling between the two Cu nuclei via a coordinated but redox inactive disulfide bridge similar to the cupric mercaptide cluster mentioned above, which prompted us to reinvestigate the complexation of Cu(II) by oxidized glutathione (I, GSSG) and related compounds. According to the polarographic measurements of Li et al.,[5] GSSG only forms a 1:1 species with the metal, although theoretically it could bind two copper ions per molecule. In addition to the carboxy protons of glycine, GSSG has six potentially titratable protons, four on peptide and two on amino nitrogen atoms, i.e., LH_8.[6] Upon pH titration of a 2:1 mixture of Cu(II) and the disulfide, precipitation of a pale blue solid occurs around pH 6, which is dissolved at pH greater than 9.5 yielding a clear violet solution with a single absorption maximum at 590 nm (ϵ 90 M^{-1} cm^{-1}). The entire process is accompanied by the liberation of four proton equivalents per Cu present. Furthermore maximum formation of the species absorbing at 590 nm is obtained at a metal:ligand

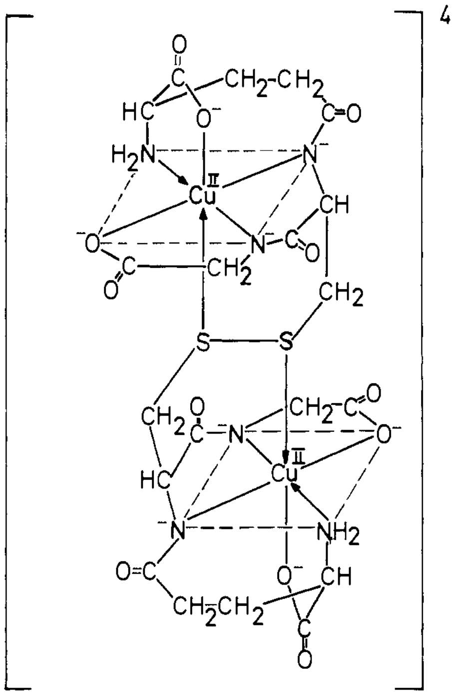


$$\begin{array}{l} ^-OOC(NH_3^+)CHCH_2CH_2CONHCH(CH_2S)CONHCH_2COOH \\ \qquad\qquad\qquad\qquad | \\ \qquad\qquad HOOCCH_2NHCO(SCH_2)CHNHCOCH_2CH_2CH(NH_3^+)COO^- \end{array}$$

I

ratio of 2:1 by the method of continuous variation. The violet compound is extremely stable toward hydrolysis up to pH 13.0 where it decomposes due to alkaline cleavage of the disulfide bond.[7]

Parallel EPR experiments at room temperature and 110°K reveal a broad and featureless spectrum of the violet complex (Figure 1, curve A), which represents 50 ± 2.5% of total Cu by comparison to a Cu(II) standard.[8] Together with the broad main signal around $g = 2$ a rather weak absorption is observed at half field; i.e., $g = 4$. Further addition of GSSG to the 2:1 mixture at pH greater than 9.5 yields a new and well-resolved EPR spectrum (Figure 1, curve C) and full signal strength. Under no experimental conditions is the ligand superhyperfine structure observed. Even at concentrations as high as 3.0 M neither NaCl, urea, nor guanidinium salts have any remarkable effect on the Cu(II)-GSSG EPR spectra with respect to shape and signal strength both in solution and the frozen state in contrast to what has been found in similar Cu(II)-peptide complexes.[9] Surprisingly all three mixtures, A, B, and C (Figure 1; see legend), exhibit identical absorption maxima and molar extinction coefficients independent of the metal:ligand ratio. Upon replacement of GSSG by S-methylglutathione (GSMe) a stable violet 1:1 complex is formed at basic pH with λ_{max} 585 nm (ϵ 85 M^{-1} cm^{-1}) under equivalent conditions but having a type C and fully developed EPR signal. As expected, GSMe does not bind two copper nuclei in contrast to GSSG which is indicated by the hydrolysis of excess metal at pH greater than 6.0.



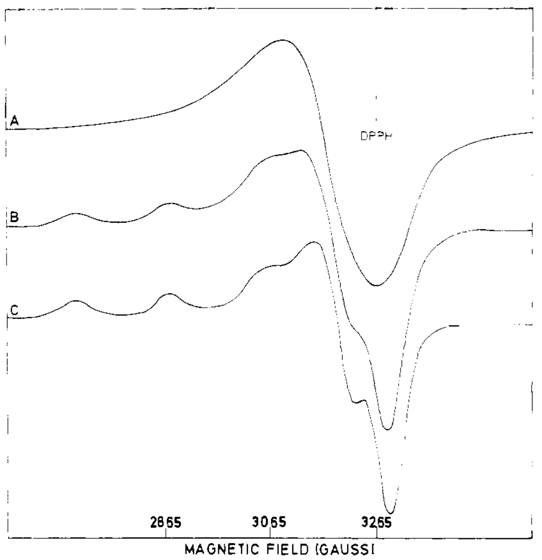
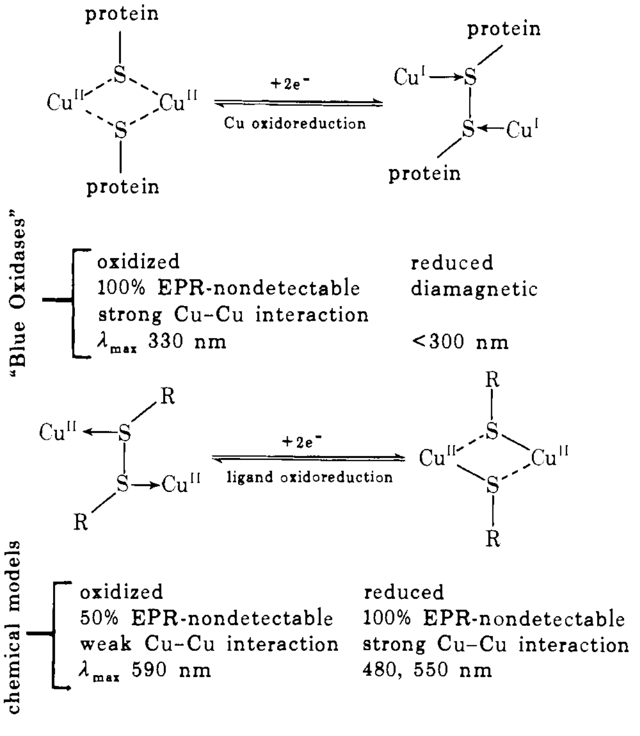


Figure 1. EPR spectra of Cu(II)-GSSG complexes in 0.1 M $NaClO_4$, pH 11.0; Cu(II) = 4.0 × 10^{-3} M (A), 2.0 × 10^{-3} M (B), 1.0 × 10^{-3} M (C), GSSG = 2.0 × 10^{-3} M; EPR-detectable Cu[8] = 50 ± 2.5% (A), 95 ± 4.0% (B), 98 ± 4.0% (C). Experimental conditions: modulation frequency 100 KHz, modulation amplitude 10.0 G, scan time 125 G min^{-1}, scan range 1000 G, temperature 110°K.

From these experimental results and space filling molecular models structure II seems to be most reasonable for the 2:1 complex of Cu(II) with GSSG, indicating metal-sulfur coordination and metal-metal interaction via the disulfide bridge as already proposed by Boas et al.[10] and Zuberbühler and Mason[11] for the binuclear Cu(II)-cystinyl-bisglycine complex with a calculated Cu-Cu distance of about 5 Å. In complete analogy to the Cu(II)-GSSG complex described here this cluster also shows the weak EPR signal at $g = 4$ resulting from the spin-forbidden $\Delta m = 2$-transition in a spin coupled Cu(II) dimer.[10] As in the corresponding Zn(II)-GSSG complex,[6] one copper should be bound to each site of formula I, i.e., to the amino nitrogen and the carboxylic oxygen of the glutamyl moieties of GSSG below pH 6, forming the uncharged pale blue species Cu_2LH_4, which then precipitates. This structural assignment is not only based on simple electrostatic considerations but gains further evidence through comparison with the optical spectra of the well-known Cu(II)-glutamine complexes in this pH range.[12] Once attached to a strong coordinating site, the Cu(II) now provokes deprotonation of adjacent peptide nitrogen atoms at higher pH and their coordination to the metal. This idea is also confirmed by esterification of the carboxy groups to yield the tetraethyl ester of GSSG, which does not form the violet complex under any conditions and cannot prevent copper hydrolysis even in the case of ligand excess.

At 1:1 ratios or ligand excess where no precipitation is

Scheme I



oxidized 100% EPR-nondetectable strong Cu-Cu interaction λ_{max} 330 nm	reduced diamagnetic <300 nm
oxidized 50% EPR-nondetectable weak Cu-Cu interaction λ_{max} 590 nm	reduced 100% EPR-nondetectable strong Cu-Cu interaction 480, 550 nm

observed, predominantly 1:1 complexes are formed with GSSG and GSMe in agreement with Li et al.,[5] which have nearly identical ligand fields around the metal as in the 2:1 species II and therefore should show similar optical but different magnetic properties.

Concerning the biological relevance of the results presented here Scheme I is proposed to explain the chemical and physical properties of the EPR-nondetectable Cu pair in "Blue Oxidases" during oxidoreduction as compared to their corresponding chemical models.

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The Acidity of Water in the Transition State for Methyl Tosylate Hydrolysis

Sir:

In neutral and basic aqueous solutions, the hydrolysis of methyl tosylate obeys a two-term rate law (eq 1).[1] The observed values of k_1' and k_2 can be used to calculate the value of pK_a(‡), the acidity constant for ionization of the activated complex in the k_1' path, to give the activated complex in the k_2 path as its conjugate base (eq 2, 3).[2,3]

$$-\mathrm{d}[\mathrm{MeOTs}]/\mathrm{d}t = (k_1'[\mathrm{H_2O}] + k_2[\mathrm{OH^-}])[\mathrm{MeOTs}] \quad (1)$$

$$[\overset{\delta+}{\mathrm{H_2O}} \cdots \underset{(\delta'-\delta)+}{\mathrm{CH_3}} \cdots \overset{\delta'-}{\mathrm{OTs}}]^{\neq} \rightleftharpoons \mathrm{H^+} + [\overset{(1-\delta)-}{\mathrm{HO}} \cdots \underset{(\delta'-\delta)+}{\mathrm{CH_3}} \cdots \overset{\delta'-}{\mathrm{OTs}}]^{\neq} \quad (2)$$

$$\mathrm{p}K_a(\ddagger) = \mathrm{p}K_a(\mathrm{H_2O}) + \log(k_1'/k_2) \quad (3)$$

We wish to report measurements of the temperature dependences of k_1' and k_2 which are sufficiently precise to allow calculation of the values of $\Delta C_p°(\ddagger)$, $\Delta S°(\ddagger)$, and $\Delta H°(\ddagger)$ for the virtual equilibrium in eq 2. Table I lists values of these parameters together with the directly measured values of $\Delta H^\ddagger$, $\Delta S^\ddagger$ and $\Delta C_p^\ddagger$ which were used in their evaluation.

Rates were measured by following the absorbance at 235 nm of solutions in a 50-ml cell; during each run the solution was stirred and its temperature monitored. Temperature control was within ±0.003°, and the first-order rate constants were reproducible to ±0.2%. Values of $\Delta H^\ddagger$, $\Delta S^\ddagger$, and $\Delta C_p^\ddagger$ were calculated from the rate constants as described elsewhere.[4] The agreement (Table I) between our parameters for k_1' at low ionic strengths and Robertson's[5] conductometrically determined values demonstrates the reliability of our spectrophotometric method; this method allows measurements of $\Delta C_p^\ddagger$ for reactions at ionic strengths which are too high for application of the conductometric method (e.g., for k_2 and for k_1' in solutions containing added salts).

Comparison of the values of pK_a(‡), $\Delta H°(\ddagger)$, etc., to corresponding values for model acids should yield information about the structure of the activated complex. To a first approximation, δ (eq 4, X = pK_a, $\Delta H°$, ...) should be qualitatively related to the displacement of the transition state along the reaction coordinate from reactant (H_2O) to product ($H_2OCH_3^+$).[2]

$$\delta = [X(\ddagger) - X(\mathrm{H_2O})]/[X(\mathrm{H_2OCH_3^+}) - X(\mathrm{H_2O})] \quad (4)$$

The entries in Table I lead to values for δ of 0.21, 0.13, 0.48, and 0.45 based respectively on pK_a, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$. The δ values based on $\Delta S°$ and $\Delta C_p°$ are thus qualitatively larger[9] than those based on pK_a and $\Delta H°$. Since it is well established[11] that the values of $\Delta S°$ and $\Delta C_p°$ for acid dissociation in water are primarily determined by changes in *solvent* structure and polarization which accompany the dissociation, these larger δ values which are derived from $\Delta S°(\ddagger)$ and $\Delta C_p°(\ddagger)$ suggest a hydrolysis mechanism in which solvent reorganization has proceeded to a

Table I. Thermodynamic Parameters for Processes Related to k_1' and k_2

Process	pK_a or −log k	$\Delta H^\ddagger$ or $\Delta H°$ (kcal mol^{-1})	$\Delta S^\ddagger$ or $\Delta S°$ (cal deg^{-1} mol^{-1})	$\Delta C_p^\ddagger$ or $\Delta C_p°$ (cal deg^{-1} mol^{-1})
k_1' activation	6.85[a,c,i]	21.74 ± 0.06[a,c,i]	−17.1 ± 0.2[a,c,i]	−37 ± 1[a,d,i]
	6.84[b,c,i]	21.63 ± 0.03[b,c,i]	−17.5 ± 0.1[b,c,i]	−36 ± 1[b,e,i]
	6.85[a,c,j]	21.45 ± 0.05[a,c,j]	−18.3 ± 0.2[a,c,j]	−30 ± 1[a,f,j]
k_2 activation	3.20[a,c,k]	19.49 ± 0.02[a,c,k]	−7.9 ± 0.1[a,c,k]	−16 ± 1[a,g,k]
pK_a(H_2O) equilibrium	15.74[l,c,i]	13.34[l,c,i]	−27.3[l,c,i]	ca. −26[l,h,i]
pK_a(‡) equilibrium	12.1[m,c,o]	11[m,c,o]	−17[m,c,o]	ca. −12[n,o]
pK_a($H_2OCH_3^+$) equilibrium	−2.0[p]	ca. −4[r]	ca. −5[q]	ca. 5[q]

[a] This work. [b] Reference 5. (Slightly different values are quoted in this reference; the values listed here have been recalculated from the rate constants reported there.) [c] At 25°. [d] At ca. 68° (the midpoint of the temperature range, 45–90°, over which k values were measured). [e] At ca. 48° (temperature range, 15–80°). [f] At ca. 62° (temperature range, 35–90°). [g] At ca. 50° (temperature range, 25–75°). [h] At 55°. [i] Ionic strength $\leqslant 10^{-3}$. [j] In 0.1 F $NaClO_4$. [k] In 0.1 F NaOH. [l] Based on "best" values for water autoprotolysis from ref 6; the values for pK_a and $\Delta S°$ have been corrected to a molar standard state for the dissociating water.[1,2] [m] From parameters related to k_1' and k_2 at I = 0.1 and parameters related to pK_a(H_2O) at I = 0. [n] From $\Delta C_{p}{}^{\ddagger}{}_1$ at I = 0.1 and T = 62°, $\Delta C_{p}{}^{\ddagger}{}_2$ at I = 0.1 and T = 50°, and $\Delta C_p°(H_2O)$ at I = 0 and T = 55°. [o] Inspection of thermodynamic data for electrolytes[7] suggests that errors arising from the combination of data from different ionic strengths (0 and 0.1) should be too small to affect any conclusion discussed here. [p] Reference 8. [q] Estimated from known values for H_3O^+, NH_4^+, and $CH_3NH_3^+$. [r] From the values for pK_a and $\Delta S°$.