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Biological Analogues. Spectroscopic Characteristics of Mercapto- and Disulfide–Copper(II) Coordination in Relation to Type I Proteins

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Possible methods of stabilizing mercapto- and disulfide–copper(II) coordination are described, and preparation of ligands incorporating some of the required features are given. The electronic absorption spectra of copper(II) complexes with mercapto and disulfide ligands are recorded. The observed sulfur-to-copper(II) charge-transfer transitions of these complexes are apposite in defining the structural and electronic characteristics of blue copper proteins.

The plant and bacterial proteins[1] plastocyanin, azurin, and stellacyanin are intensely blue and contain a single copper atom per molecule. The electronic absorption spectra[2] in the visible and near-infrared regions observed for the copper(II) proteins appear to be entirely due to transitions associated directly (d–d) or indirectly (charge transfer) with the copper atom. No synthetic copper complex has been prepared which plausibly matches the spectacular complexity of these protein spectra.

It is now known that plastocyanin has a copper atom surrounded by a quasi-tetrahedral array of ligands, two imidazole (histidine), one thioether (methionine), and one mercapto (cysteine) ligands.[3] This discovery poses a challenge of considerable complexity if attempts are made at synthesizing a low molecular weight facsimile of the copper site of the protein. Moreover, the electronic spectra of plastocyanin, azurin, and stellacyanin are, superficially at least, remarkably similar, yet unlike the first two, stellacyanin contains no methionine.[4]

This paper concerns two crucial aspects related to the evolution of low molecular weight synthetic facsimilies of the copper binding sites of these proteins. Perhaps the most difficult problem relates to finding conditions for stabilizing the mercapto–copper(II) bond under normal conditions. We describe here a reasonably successful device for achieving this. The other aspect concerns the question of whether the spectroscopic characteristics of a copper(II)–thioether bond resemble those of a copper(II)–disulfide bond. An affirmitive answer may lend support to one of the current conjectures, namely, that although stellacyanin and the other type I proteins have similar structures and ligand environments, the former has a disulfide ligand (cystine) instead of the thioether ligand (methionine) of the others. We report here a method of stabilizing a disulfide ligand coordinated to copper(II) and the associated electronic spectra.

1. Mercapto–Copper(II) Stability. The instability of mercapto–copper(II) complexes arises from the rapid reaction

$$2RS^- + 2Cu(II) \rightarrow 2Cu(I) + RSSR$$

which, under normal circumstances, appears to be essentially irreversible. The mechanism of this reaction is not fully understood, but it seems probable that radical (sulfur) coupling is involved and we assume this to be the case. Thus we postulate that although the mercapto–copper(II) bond is inherently stable, upon dissociation, a one-electron reductive elimination may occur to produce copper(I) and a (neutral) mercapto radical.

$$Cu^{II}\text{–}SR^+ \rightleftharpoons Cu(I) + RS\cdot$$

Such a reaction is expected to be reversible, but the copper(II) is driven to complete reduction because of the formation of the corresponding stable disulfide by radical coupling.

$$2RS\cdot \rightarrow RSSR$$

If the above mechanism is correct, the following conclusions about the stability of mercapto–copper(II) systems may be drawn. First, the one-electron reductive elimination will be suppressed either if the copper(II) complex is made less reducible or if the mercaptide ligand is made less oxidizable by appropriate substitution. Neither adjustment is realistic if models of the blue copper site are sought, since "soft" ligands and a tetrahedral environment are present in the proteins and both of these features lead to positive redox potentials of the resultant complexes.[5] Similarly, only the highly oxidizable aliphatic mercaptans resemble the sulfur of cysteine. Second, copper(II) reduction will be suppressed if ligand–copper(II) dissociation is suppressed. It is known, however, that copper(II) complexes are generally exceedingly labile and considerable elaboration of the ligand structure is required to suppress rapid dissociation. Of course, if the complex were isolated rapidly at low temperatures, a stable *solid* complex would be expected. We reported recently[6] that a nonlabile $[Cu(cyclam)]^{2+}$ complex,[7] which probably has a very negative redox potential, associates with mercapto ligands to give stable mercapto complexes in solution, and a stable mercapto–copper(II) complex incorporating a similar macrocyclic quadridentate ligand has been structurally characterized.[8] The third device that may be employed is to provide steric crowding about the sulfur atom in order to reduce the possibility of coupling of the sulfur radicals. Steric hindrance should also tend to reduce sulfur μ-bridge polymerization which is a common feature of stable mercapto–metal complexes.[9] It seems reasonable to suppose that the stability of the mercapto–copper(II) complex of the proteins themselves arises from the elaborate steric constraints of the peptide structure which is sufficient to prevent rapid dissociation and sulfur-radical coupling.

We chose this steric hindrance approach, together with another device, in order to obtain stable copper(II)–mercapto complexes in solutions under ambient conditions.

2. Ligand Synthesis. The ligand molecule VI chosen is shown in Figure 1 together with an outline of its method of synthesis. It will be noted that all of the saturated carbon

(1) (a) Malkin, R.; Malmstrom, G. B. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1970**, *33*, 177. (b) Malkin, R. *Inorg. Biochem.* **1973**, 689.
(2) Solomon, E. J.; Har, J. W.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1389.
(3) Colman, P. M.; Freeman, H. C.; Guss, J. M.; Murata, M.; Norris, V. A.; Ramshaw, J. A. M.; Venkatappa, M. P. *Nature (London)* **1978**, *272*, 310. Adman, E. T.; Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *J. Mol. Biol.* **1978**, *123*, 35.
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(5) (a) Patterson, G. S.; Holm, R. H. *Bioinorg. Chem.* **1975**, *4*, 257. (b) Yokoi, H.; Addison, A. W. *Inorg. Chem.* **1977**, *16*, 1341.
(6) Amundsen, A. R.; Whelan, J.; Bosnich, B. *J. Am. Chem. Soc.* **1977**, *99*, 6730.
(7) Cyclam is the cyclic quadridentate ligand 1,4,8,11-tetraazatetradecane.
(8) Hughey, J. L.; Fawcett, T. G.; Rudich, S. M.; Lalancette, R. A.; Potenza, J. A.; Schugar, H. J. *J. Am. Chem. Soc.* **1979**, *101*, 2617.
(9) Jicha, D. C.; Busch, D. H. *Inorg. Chem.* **1962**, *1*, 872.

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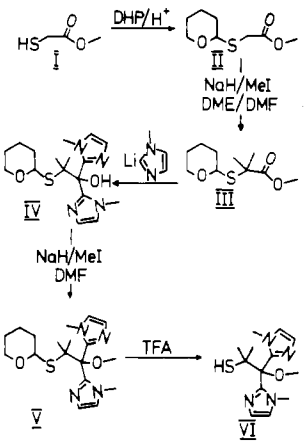


Figure 1. Outline of the method of synthesis of ligand VI.

atoms of VI are fully substituted so as to provide maximum steric hindrance to sulfur-radical coupling and also to prevent polymerization via mercapto bridges.

A notable feature of the synthesis is the use of the tetrahydropyranyl sulfur-protecting group. This protecting group survives strongly basic conditions and is stable under mildly acidic conditions although it is removed with strong acid. We have developed two mild methods of removing the THP group: one employs mercuric acetate in 2:1 methylene chloride–acetic acid at 25 °C for 3 h, another method involves treatment of the protected sulfur compound with neat trifluoroacetic acid at 25 °C. Both of these deprotecting methods are very efficient and clean. The other steps in the synthesis proceeded in high yield and, under appropriate conditions, without incident. The ligand VI is an air-stable crystalline solid.

3. Mercapto–Copper(II) Spectra. An ethanol solution of the ligand VI, 1 equiv of sodium ethoxide, and copper(II) perchlorate gives an intense green color. At low concentrations ($\sim 10^{-4}$ M) the solution slowly fades in color over 1 h at 25 °C to give copper(I) and the disulfide of VI which has been isolated and characterized. Varying the ligand anion to copper(II) ratio establishes that the maximum intensity of the visible absorption spectrum is reached when the ratio is 2:1 *or greater*, and, moreover, the molar extinction coefficient is constant if the concentration is varied from 10^{-3} to 10^{-5} M for the 2:1 ratio solutions.

We have succeeded in obtaining stable spectra which persist for 2 days at 25 °C by means of the following device. If an ethanol solution containing 20 equiv of both the ligand and sodium ethoxide and 1 equiv of copper(II) perchlorate (10^{-4} M) is left exposed to air, the spectrum shown in Figure 2 is observed. This spectrum is the same as that observed for a 2:1 ligand anion to copper(II) solution, but the former's greater (spectral) stability is probably due to the mechanism outlined in Figure 3.

We assume that a standing concentration of the complex $[Cu(VI)_2]^0$ is maintained because although relatively slow reduction of the copper(II) occurs with the formation of the disulfide of VI, copper(I) is rapidly oxidized by oxygen and then complexed by the excess ligand anion. Presumably peroxide is formed during the reaction, but its presence does not interfere with the system. Indeed, addition of small amounts of hydrogen peroxide has no effect on the spectrum. Further, although the disulfide of VI is capable of complexing to both copper(II) and copper(I), this also does not detectably interfere with the concentration of $[Cu(VI)_2]^0$ or with the reoxidation to copper(II). Such a mechanism for obtaining stable concentrations of mercapto–copper(II) complexes depends critically on having the correct mix of thermodynamic and kinetic parameters. We have attempted to use the same device with the ligand 2-aminoethanethiol without success. It

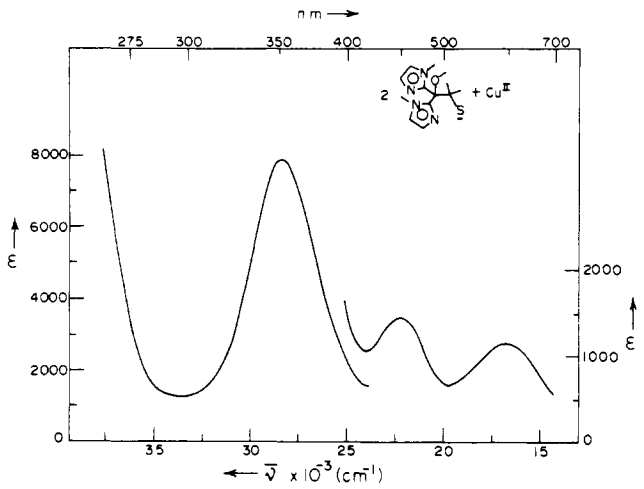


Figure 2. Electronic absorption spectrum of $[Cu(VI)_2]^0$ in ethanol solution at 25 °C.

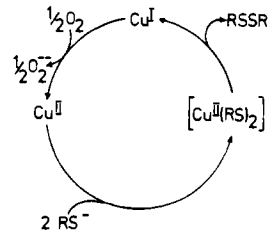


Figure 3. Suggested cycle for obtaining a steady-state concentration of the mercapto complex $[Cu(VI)_2]^0$. Ligand VI is written as RS in the diagram.

is probable that the enhanced stability of the $[Cu(VI)_2]^0$ complex is one of the key features in obtaining stable standing concentrations of the mercapto–copper(II) species.

The stability of the 2:1 ligand anion to copper(II) solutions depends on the concentration of the complex; the higher the concentration, the faster the reduction. We have succeeded in isolating the $[Cu(VI)_2]^0$ complex from ethanol solutions at −50 °C as an intensely green, slightly impure solid. The solid itself is indefinitely stable, but upon dissolution in a variety of solvents, copper(II) is reduced quickly at 25 °C.

The absorption spectrum of $[Cu(VI)_2]^0$ has three resolved transitions in the visible and near-ultraviolet regions (Figure 2). Assignment of these transitions seems straightforward; the low-energy band (590 nm) is assigned to the copper d–d bands which are enhanced in intensity by borrowing from higher energy charge-transfer excitations, the 450-nm band is assigned to the $S(\pi) \rightarrow d$ charge-transfer transition, and the intense 350-nm absorption involves the $S(\sigma) \rightarrow d$ charge-transfer transition. These same charge-transfer bands have been assigned[2] to the 600-nm ($S(\sigma) \rightarrow d$) and 740-nm ($S(\pi) \rightarrow d$) absorption of the protein plastocyanin. There is, however, considerable difference in energy between the charge-transfer bands of the $[Cu(VI)_2]^0$ octahedral complex and those of the tetrahedral protein complex, and one has to assume that the copper experiences a very weak tetrahedral field in the protein in order to depress the energy of the highest d level sufficiently to cause the considerable red shift in the "blue" band. On the other hand, it is gratifying to find that the molar extinction coefficient per sulfur for the $S(\sigma) \rightarrow d$ transition in $[Cu(VI)_2]^0$ of about 4000 is the same as that found in the proteins,[2] and the same correspondence holds for the $S(\pi) \rightarrow d$ excitation. Moreover, whereas the thioether–copper(II) complexes show no evidence of the $S(\pi) \rightarrow d$ transition,[6] the present mercapto species and the proteins do. This observation and the extinction coefficients lend strong support to the original protein assignments.[2]

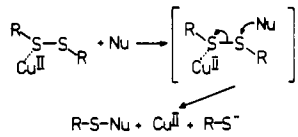
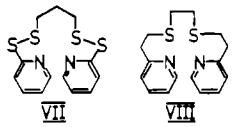


Figure 4. Suggested mechanism for copper(II)-catalyzed scission of a disulfide bond.

4. Disulfide–Copper(II) Stability. It is remarkable that no square-planar disulfide–copper(II) complex has been reported. Initially we had assumed this to be due to the probable weak bonding capacity of disulfides to copper(II) in polar solvents as is observed for thioether–copper(II) species.[6] A much more serious problem became apparent when we attempted to prepare a copper(II) complex with cystamine ($NH_2CH_2CH_2SSCH_2CH_2NH_2$). We had wished to compare the spectrum of this complex with that of the structurally analogous complex formed with the thioether ligand[6] $NH_2(CH_2)_3S(CH_2)_2NH_2$. After cystamine was mixed with copper(II) perchlorate in a variety of solvents, immediate reduction of the copper(II) occurred. In order to check the generality of this reaction, we prepared the ligand VII[10] which is structurally analogous to the thioether ligand VIII.[6] Again rapid reduction of the copper(II) occurred upon mixing solutions of copper(II) and VII.



VII VIII

These observations suggest that a series of reactions depicted in Figure 4 occurs. If water is the nucleophile (Nu), the sulfenic acid (RSOH) will disproportionate to the mercaptan and the sulfinic acid (RSO_2H)

$$2RSOH \rightarrow RSH + RSO_2H$$

The production of RSH in this and the other steps of the reaction leads to the reduction of the copper(II). Even under aprotic and nearly anhydrous conditions, the nitrogeneous ligands we have used, cystamine and VII, cause reduction, which suggests that the basic nitrogen atoms of the ligands themselves act as nucleophiles. The rate of uncatalyzed disulfide cleavage by nucleophiles is slow; for example, the half-life of cleavage of cystamine in 1.0 N NaOH in 80% ethanol solution at 37 °C is 84 h.[11] Whereas it is true that electrophilic assistance greatly speeds up the disulfide scission,[12] the rate increase observed in the case of the reaction between dimethyl disulfide and triethyl phosphite catalyzed by mercuric acetate[13] does not appear to approach the "instantaneous" cleavage we have observed with copper(II).

With the assumption of the S_N2 mechanism in Figure 4, the most direct way of suppressing the copper(II)-catalyzed disulfide scission is to build in steric hindrance about the disulfide linkage. We therefore synthesized the sterically crowded analogue of cystamine, namely, tetramethylcystamine ($NH_2CH_2C(CH_3)_2SSC(CH_3)_2CH_2NH_2$ = Me_4-cystam). Having the disulfide at an inner position of the tridentate ligand with strongly coordinating primary amines at the terminal positions ensures the coordination of the sulfur.

5. Ligand Synthesis. The ligand Me_4-cystam was isolated as its highly crystalline dihydrogen sulfate and monohydrogen perchlorate salts and was prepared by the scheme outlined in Figure 5. We draw attention to molecule IX; this may be described as a *protected sulfhydryl anion equivalent*. We developed it for a number of reasons; in the present case we wished to introduce the sulfur into the acetonitrile molecule to give the protected thiocyanohydrin which, without protection, we expected to be an unstable molecule. The anion $CH_3OCH_2S^-$ is rapidly generated in situ with 1 equiv of sodium exthoxide in ethanol solutions. We have found this anion to be an excellent reagent for displacement reactions which can be carried out below 25 °C; above this temperature, increasing amounts of decomposition appear to occur although it can still be used. The subsequent methylation and reduction reactions proceeded smoothly in high yield, and, as in the case of the tetrahydropyranyl protecting group, the present methoxymethyl ether blocking group could be removed with mercuric acetate. Aerial oxidation of the mercaptan in basic solutions (pH ~8.5) gave the disulfide, which was isolated and purified as its various salts. The free disulfide base is a solid but is hygroscopic.

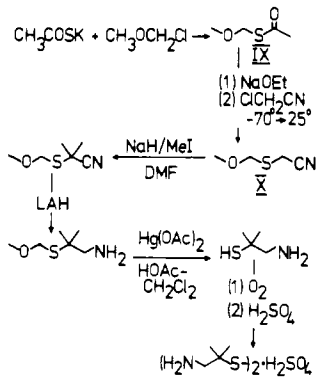


Figure 5. Outline of the synthesis of tetramethylcystamine.

6. Disulfide–Copper(II) Spectra. Solutions for spectra were made by dissolving the ligand perchlorate salt in the requisite solvent (acetonitrile, ethanol, methanol, or dimethylformamide) and successively adding 1 equiv of sodium methoxide (1 M in methanol) and 1 equiv of copper(II) perchlorate hexahydrate in the requisite solvent. A green solution was obtained in all solvents, but the solutions slowly decolorized over 1–3 h depending on the solvent. No significant spectral changes were observed during the time required to record the spectra. Addition of a second equivalent of sodium methoxide to an acetonitrile solution of the disulfide–copper(II) complex caused "instantaneous" decoloration presumably because of rapid cleavage of the disulfide bond. We have not been able to isolate the $[Cu(Me_4\text{-cystam})X](ClO_4)_2$ (X = H_2O, acetonitrile, or *N*-methylimidazole) complexes because of their very high solubilities. When the solutions were concentrated, an insoluble purple material, probably a tetraamine complex, deposited. A concentration study of the 1:1 ligand–copper(II) solutions showed that the spectra were unchanged in the range 3×10^{-3}–1×10^{-5} M in acetonitrile or ethanol. At higher concentrations, the spectra retained their form but there were small changes in the relative intensities of the absorptions.

The equivalent conductivities (Λ_e) for acetonitrile solutions of the 1:1 ligand–copper(II) perchlorate complex and of $[Cu(H_2O)_6](ClO_4)_2$ were measured in the concentration range 0.02–0.002 M. Negligible decomposition was detected spectrophotometrically during the course of the measurements. Plots of Λ_e against $C^{1/2}$ (C = equivalent concentration) were linear, and the zero concentration conductivities (Λ_0) were obtained by extrapolation. Plots of $\Lambda_0 - \Lambda_e$ vs. $C^{1/2}$ gave almost coincident straight lines for the putative $[Cu(Me_4\text{-cystam})CH_3CN](ClO_4)_2$ complex and the known[14] monomeric 2:1 electrolyte $[Cu(H_2O)_6](ClO_4)_2$. Were the copper(II)–Me_4-

(10) This ligand was prepared by Jeanette Brown.
(11) Rachinskii, F. Y.; Bruk, Y. A.; Pavlova, L. V.; Slavachevskaya, N. M. *Zh. Obshch. Khim.* **1970**, *40*, 2109.
(12) Kice, J. L. *Acc. Chem. Res.* **1968**, *1*, 58.
(13) Bach, R. D.; Rajan, S. J. *J. Am. Chem. Soc.* **1979**, *101*, 3112.
(14) Hathaway, B. J.; Holah, D. G.; Postlethwaite, J. D. *J. Chem. Soc.* **1961**, 3215.

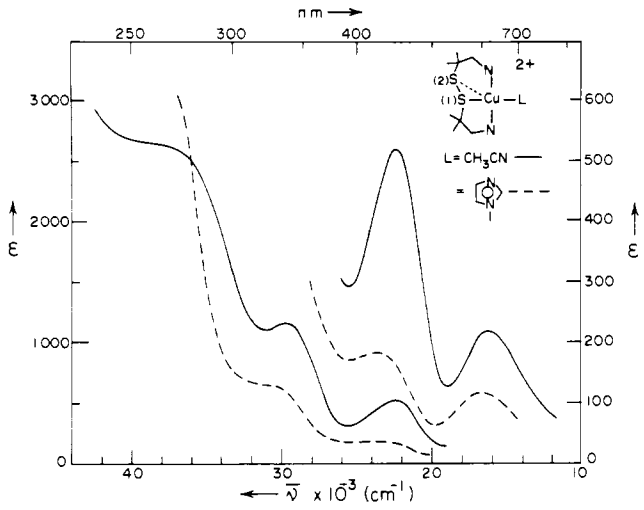


Figure 6. Electronic absorption spectra of $[Cu(Me_4\text{-cystam})X](ClO_4)_2$ in acetonitrile solution at 25 °C.

cystam complex a dimer or higher oligomer, distinctly different electrolyte behavior would be expected.[15] Thus both the concentration-insensitive spectra and the concentration dependence of the conductivity establish that a unique 1:1 copper(II)–ligand complex exists in solution.

Figure 6 shows the spectra of the disulfide–copper(II) complex in acetonitrile solution at a concentration of 1.36×10^{-3} M. Spectra in all of the other solvents were similar, but the peak maxima were in general less clearly resolved. Addition of 1 equiv of *N*-methylimidazole to the acetonitrile solution causes a change in the spectrum, but all of the original absorption peaks are retained. This suggests that *N*-methylimidazole can occupy the fourth coordination site of the complex. The $N(\sigma) \rightarrow d$ (of the coordinated amino groups) transitions occur around 240 nm in these types of complexes,[6] and the free ligand has no absorption to lower energies of 240 nm. Thus transitions occurring to lower energies of ~260 nm are associated with the metal or the metal–disulfide chromophore.

The lowest energy band (613 nm in acetonitrile) is assigned to the d–d transitions of the copper(II) atom. There are three bands that can be assigned to the disulfide–copper(II) chromophore (at 445, 335, and ~270 nm in acetonitrile). The highest energy band in each case appears as a shoulder on the $N(\sigma) \rightarrow d$ transition. We are unable to give a unique assignment of these disulfide–copper(II) charge-transfer bands, but the presence of at least three transitions suggests that both of the sulfur atoms are associated with the spectroscopic characteristics of the system. This probably arises because of the particular geometric constraints of the ligand which can hold both of the sulfur atoms close to the metal. We think that the metal will select one of the sulfur atoms for direct bonding, the other having weak interaction with the copper by being held in proximity by the chelate arm (see inset of Figure 6).

Thus the directly bonded sulfur (S_1) atom can, in principle, give rise to $S_1(\sigma) \rightarrow d$ and $S_1(\pi) \rightarrow d$ charge-transfer transitions, but because of the geometry, the other sulfur (S_2) atom may also give rise to $S_2(\sigma) \rightarrow d$ and $S_2(\pi) \rightarrow d$ transitions. Of these four transitions, overlap considerations would suggest the following order of intensities: $S_1(\sigma) \rightarrow d > S_2(\sigma) \rightarrow d \sim S_1(\pi) \rightarrow d > S_2(\pi) \rightarrow d$. The order of intensity for the $S_2(\sigma) \rightarrow d$ and $S_1(\pi) \rightarrow d$ excitations will depend critically on the geometry. We tentatively propose that the transition at ~270 nm which has a molar extinction coefficient of the order of 10^3 is the $S_1(\sigma) \rightarrow d$ transition. The two lower energy, weaker transitions associated with the disulfide–copper(II) chromophore we assign to the $S_2(\sigma) \rightarrow d$ and $S_1(\pi) \rightarrow d$ transitions, but we are not in a position to say which is which.

The structurally analogous copper(II) complex prepared from the thioether ligand $NH_2(CH_2)_3S(CH_2)_2NH_2$ has a symmetrical $S(\sigma) \rightarrow d$ charge-transfer band at 325 nm possessing a molar extinction coefficient of about 2500 and no observable $S(\pi) \rightarrow d$ transition.[6] Thus whereas the disulfide–copper(II) and thioether–copper(II) chromophores have transitions which occur in similar spectral regions and are of similar intensity, the former shows greater complexity for the analogous model systems we have chosen.

7. Sulfhydryl–Copper(II) Spectra. We have found that 2:1 solutions of the ligand VI and anhydrous copper(II) trifluoroacetate in the solvents CH_2Cl_2 and THF give very stable blue-green solutions at ambient conditions. These solutions probably contain a mixture of complexes containing protonated and deprotonated sulfur atoms. In dry THF, addition of up to 4 equiv of trifluoroacetic acid sharpens the spectrum and generates a clean isosbestic point at 378 nm for incremental addition of the acid. When 4 equiv of acid are added in THF, the complex displays a resolved peak at 405 nm (ϵ ~300) which we ascribe to the sulfhydryl (RSH) $S(\pi) \rightarrow d$ transition. This is immediately followed to higher energies by a strong absorption due to the sulfhydryl $S(\sigma) \rightarrow d$ (ϵ ~1750) transition. This latter band is contiguous with other transitions of similar intensity and is not clearly resolved but its maximum is around 310 nm. Thus, as expected, the sulfhydryl–copper(II) charge-transfer bands occur at higher energies than the corresponding transitions of the mercapto–copper(II) chromophore. The d–d transitions occur at around 590 nm (ϵ ~200). Although we are confident that these spectra mainly refer to sulfhydryl–copper(II) coordination, we have been unable to unambiguously establish that a single species exists in the acid solutions.

Discussion

The use of steric hindrance in order to stabilize the mercapto–copper(II) and disulfide–copper(II) systems may provide a basis for the design of low molecular weight models which mimic the physical properties of type I copper proteins. Although steric hindrance is advantageous, we believe that a surer way of stabilizing the mercapto–copper(II) systems is to find ways of making the copper(II)–ligand system *kinetically* inert.

The spectroscopic characteristics of the present mercapto–copper(II) complex are gratifying in that the intensities and general band shapes of the two $S \rightarrow d$ charge-transfer bands match almost exactly these same bands found in the proteins. This observation lends support to both the structural and spectroscopic assignments.

The question of the fourth ligand of stellacyanin is not uniquely resolved by the present results. There are a number of problems associated with this assignment if electronic spectroscopy is used as the final arbiter. Transitions associated with the fourth ligand are expected to occur in the region 400–600 nm. Apart from the strong 600-nm band which is due to the mercapto $S(\sigma) \rightarrow d$ transition, plastocyanin has bands at 423 (ϵ ~100), 464 (ϵ ~300), and 551 nm (ϵ 1163) whereas stellacyanin has two bands in this region, at 443 (ϵ 942) and 560 nm (ϵ 1542).[2] These transitions are probably associated with the imidazole–copper(II) and thioether–copper(II) chromophores of plastocyanin, but, as yet, no persuasive argument has been presented for their precise electronic provenance. Spectra of model systems[6] suggest that the 551-mm transition is the thioether $S(\sigma) \rightarrow d$ charge-transfer band and that the two higher energy transitions could be due to copper(II) → imidazole (π^*) excitations. There is, however, a complication in the structure of plastocyanin which is not

(15) (a) Hayter, R. G. *J. Am. Chem. Soc.* **1962**, *84*, 3046. (b) Feltham, R. D.; Hayter, R. G. *J. Chem. Soc.* **1964**, 4587.

easily resolved by model studies; the protein has an unusually long thioether–copper(II) bond.[16] Such a bond would tend to decrease the intensity and energy position of the $S(\sigma) \rightarrow$ d transition to a degree that is not easily predictable.

With these uncertainties, purely spectroscopic arguments are indecisive; the fourth ligand of stellacyanin could be disulfide or phenolate[6] or mercapto (RS^-) or sulfhydryl (RSH), provided that in the last two the ligand–copper bond is long. If stellacyanin were to have two strongly bonded mercapto ligands, we would expect the 600-nm band to be about twice as intense as this same transition in plastocyanin. It is not.[2]

Experimental Section

Methoxymethyl Thioacetate (IX). Potassium thioacetate (61.1 g, 0.53 mol) was added in portions to dry DMF (150 mL) kept at −5 °C under nitrogen. The mixture was cooled to −30 °C and chloromethyl methyl ether (43.0 g, 0.53 mol) added over 15 min. After an additional 30 min at this temperature, the cooling bath was removed and stirring continued for 2 h at room temperature. The mixture was poured into ice-cold water (700 mL) and thoroughly extracted with Et_2O. The combined organic extracts were washed with water, brine, and then dried over Na_2SO_4. The Et_2O was removed at atmospheric pressure and the residue distilled to give methoxymethyl thioacetate (IX), a colorless oil (52.0 g, 92%, bp 44–46 °C, 15 mm). ^1H NMR: δ 2.40 (3 H, s), 3.32 (3 H, s), 5.05 (2 H, s). Anal. Calcd for $C_4H_8O_2S$: C, 40.0; H, 6.7; S, 26.7. Found: C, 40.1; H, 6.7; S, 26.8.

5-Oxa-3-thiahexanenitrile (X). Methoxymethyl thioacetate (30.3 g, 0.25 mol) was added dropwise under nitrogen to a solution of sodium (5.7 g, 0.25 mol) in EtOH (250 mL) at 10 °C. The solution was then cooled to −78 °C and chloroacetonitrile (15.8 ml, 0.25 mol) added over 10 min. After 1 h of stirring at room temperature, the EtOH was removed under reduced pressure, the residue diluted with water (600 mL), and the resulting solution extracted with Et_2O. The combined organic layers were washed with water and brine and then dried over $MgSO_4$. Et_2O was removed under reduced pressure and the residue distilled through a vacuum-jacketed Vigreaux column to give 5-oxa-3-thiahexanenitrile (X), a colorless liquid (23.5 g, 80%, bp 92–94 °C, 15 mm). ^1H NMR: δ 3.36 (2 H, s), 3.40 (3 H, s), 4.75 (2 H, s). Anal. Calcd for C_4H_7NOS: C, 41.0; H, 6.0; N, 12.0; S, 27.4. Found: C, 41.1; H, 6.2; N, 11.9; S, 27.2.

2,2-Dimethyl-5-oxa-3-thiahexanenitrile. Sodium hydride (35 g of a 50% mineral oil dispersion, 0.73 mol) was washed twice with hexane and then suspended in dry DME (300 mL) and dry DMF (50 mL) under nitrogen. A solution of 5-oxa-3-thiahexanenitrile (X) (40.6 g, 0.35 mol) and methyl iodide (46.0 mL, 0.74 mol) in dry DME (100 mL) was added dropwise over 3 h to the vigorously stirred mixture maintained at 30 °C by external cooling. After 16 h at room temperature, the reaction was quenched by the careful addition of HOAc (10 mL) and then water (200 mL). DME was carefully removed under reduced pressure at room temperature and the residue thoroughly extracted with Et_2O. The combined organic extracts were washed with water, 2% aqueous $Na_2S_2O_3$, water, and brine and then dried over $MgSO_4$. Et_2O was removed under reduced pressure and the residue distilled to give 2,2-dimethyl-5-oxa-3-thiahexanenitrile, a colorless liquid (43.0 g, 86%, bp 76–78 °C, 15 mm). ^1H NMR: δ 1.72 (6 H, s), 3.20 (3 H, s), 4.95 (2 H, s). Anal. Calcd for $C_6H_{11}NOS$: C, 49.6; H, 7.6; N, 9.7; S, 22.1. Found: C, 49.1; H, 7.5; N, 9.5; S, 22.3.

2,2,5,5-Tetramethyl-3,4-dithia-1,6-hexanediamine. A solution of 2,2-dimethyl-5-oxa-3-thiahexanenitrile (10 g, 0.069 mol) in dry THF (30 mL) was added over 10 min to LAH (5.0 g, 0.132 mol) suspended in dry THF (200 mL) under nitrogen. The mixture was stirred for a further 30 min, refluxed for 1.5 h, cooled, and then carefully hydrolyzed by successive additions of water (5 mL), 15% aqueous NaOH (5 mL), and water (15 mL). The mixture was refluxed for 30 min and filtered and the precipitated solid extracted with boiling THF (3 × 100 mL). THF was removed under reduced pressure at room temperature to give 2,2-dimethyl-5-oxa-3-thiahexanamine, a colorless oil (8.0 g, 78%; ^1H NMR δ 1.33 (6 H, s), 1.70 (2 H, s), 2.66 (2 H, s), 3.35 (3 H, s), 4,64 (2 H, s)) which was dissolved in HOAc (50 mL) and CH_2Cl_2 (100 mL). Mercuric acetate (34.0 g, 0.11 mol) was added in portions and the mixture stirred at room temperature for 2 h. Hydrogen sulfide was passed through the vigorously stirred solution, giving an orange precipitate which rapidly turned black. The mixture was stirred for 30 min and filtered through Celite, and the precipitate was washed thoroughly with HOAc–CH_2Cl_2 (1:2). The solvents were removed under reduced pressure and the residue dissolved in water (100 mL). The solution was made basic (pH 8.0) with 1 M NaOH, and air was passed through the solution until an aliquot gave a negative iodine test (17 h). The solution was filtered and 3 M H_2SO_4 (20 mL) added. Crystallization began immediately and was completed by the slow addition of EtOH (400 mL). 2,2,5,5-Tetramethyl-3,4-dithia-1,6-hexanediamine dihydrogen sulfate was filtered, washed thoroughly with ice-cold water, and dried over H_2SO_4 (5.8 g, 70%). A sample was recrystallized from boiling water; mp 285 °C dec. Anal. Calcd for $C_8H_{20}N_2S_2 \cdot H_2SO_4$: C, 31.4; H, 7.2; N, 9.1; S, 31.4. Found: C, 31.6; H, 7.5; N, 9.1; S, 31.6.

The sulfate salt (2.3 g, 0.0075 mol) was suspended in water (15 mL) and 1 M NaOH (15 mL) added dropwise. The solution was evaporated to dryness and the residue extracted with EtOH (2 × 30 mL). The combined extracts were filtered, and the solvent was removed under vacuum. The residue was dissolved in water (20 mL), 1 M $HClO_4$ (7.5 mL) was added, and the resulting solution was allowed to stand. The white needles of perchlorate salt were filtered off, and a second crop was obtained by concentrating the filtrate to 5 mL and cooling to 0 °C. The compound was recrystallized from boiling water: yield 2.0 g (86%); mp 202–204 °C. Anal. Calcd for $C_8H_{20}N_2S_2 \cdot HClO_4$: C, 31.1; H, 6.9; N, 9.1; S, 20.8. Found: C, 31.1; H, 6.9; N, 9.3; S, 20.6.

Methyl 2-(Tetrahydropyran-2-ylthio)acetate (II). 3,4-Dihydro-2*H*-pyran (67.3 g, 0.80 mol) was added dropwise over 0.75 h to an ice-cold solution of methyl mercaptoacetate (I) (78.1 g, 0.74 mol) and *p*-toluenesulfonic acid (0.1 g) in CH_2Cl_2 (300 mL). The solution was refluxed for 2 h, ice-cooled, washed with 10% aqueous $NaHCO_3$ (50 mL) and water (50 mL), and then dried over Na_2SO_4. CH_2Cl_2 was removed under vacuum and the resiude distilled to give II, a colorless liquid (133.7 g, 95%, bp 76–78 °C, 0.1 mm). ^1H NMR: δ 1.4–2.2 (6 H, m), 3.35 (2 H, AB q, J = 16 Hz, internal chemical shift 18 Hz), 3.2–4.3 (2 H, m), 3.76 (3 H, s), 5.09 (1 H, m). Anal. Calcd for $C_8H_{14}O_3S$: C, 50.5; H, 7.4; S, 16.9. Found: C, 50.4; H, 7.3; S, 16.5.

Methyl 2-(Tetrahydropyran-2-ylthio)-2-methylpropionate (III). Sodium hydride (30.3 g of a 50% mineral oil dispersion, 0.63 mol) was washed twice with hexane and then suspended in dry Et_2O (400 mL) and dry DMF (40 mL) under nitrogen. A solution of II (60.0 g, 0.31 mol) and methyl iodide (39.3 mL, 0.63 mol) in dry Et_2O (300 mL) was added dropwise over 4 h to the vigorously stirred mixture. The vigor of the exothermic reaction was moderated by external cooling. After 16 h at room temperature, the reaction was quenched by the careful addition of HOAc (7 mL) in dry Et_2O (50 mL) and then water (200 mL). The layers were separated and the aqueous layer extracted with Et_2O. The combined organic extracts were washed with water, 2% aqueous $Na_2S_2O_3$, water and brine and then dried over $MgSO_4$. The Et_2O was removed under reduced pressure and the residue distilled to give III, a colorless viscous liquid (59.3 g, 86%, bp 73–76 °C, 0.1 mm). ^1H NMR: δ 1.3–2.0 (6 H, m), 1.55 (6 H, s), 3.2–4.3 (2 H, m), 3.69 (3 H, s), 5.00 (1 H, m). Anal. Calcd for $C_{10}H_{18}O_3S$: C, 55.0; H, 8.3; S, 14.7. Found: C, 55.2; H, 8.4; S, 14.5.

2-(Tetrahydropyran-2-ylthio)-2-methyl-1,1-bis(1-methylimidazol-2-yl)-1-propanol (IV). *N*-Methylimidazole (65.7 g, 0.80 mol) in dry Et_2O (40 mL) was added over 30 min to a solution of *n*-BuLi, made from lithium metal (11.7 g, 1.7 mol) and *n*-butyl bromide (103.5 g, 0.76 mol) in dry Et_2O (400 mL), at 10 °C under nitrogen. Dry THF (500 mL) was added to dissolve the precipitate and the solution cooled to −15 °C, and III (59.0 g, 0.27 mol) in dry THF (40 ml) was added over 30 min. The solution was allowed to come to room temperature and then refluxed for 30 min. The mixture was cooled to 0 °C and quenched by the addition of 17% aqueous NH_4Cl (200 mL). The layers were separated and the aqueous layer thoroughly extracted with Et_2O. The solvents were removed from the combined organic phases, and the residue was treated with water (200 mL). The mixture was then extracted with Et_2O, and the combined organic layers were washed with water and brine and dried over $MgSO_4$. Removal of the Et_2O under reduced pressure left a viscous oil (94.5 g, 100%), shown to be pure by ^1H NMR. Crystals could be obtained from toluene in low yield and recrystallized from cyclohexane (mp 114–115 °C). ^1H NMR: δ 1.4–2.1 (6 H, m), 1.67 (3 H, s), 1.90 (3 H, s), 3.0–4.2 (2 H, m) 3.32 (6 H, s), 4.60 (1 H, m), 5.63 (1 H, s), 6.73

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(2 H, m), 6.92 (2 H, m). Anal. Calcd for $C_{17}H_{26}N_4O_2S$: C, 58.3; H, 7.5; N, 16.0; S, 9.2. Found: C, 58.0; H, 7.6; N, 15.7; S, 9.4.

2-(Tetrahydropyran-2-ylthio)-1-methoxy-2-methyl-1,1-bis(1-methylimidazol-2-yl)propane (V). Sodium hydride (6.5 g of a 50% mineral oil dispersion, 0.14 mol) was washed twice with hexane and then suspended in dry DMF (100 mL) under nitrogen. The vigorously stirred mixture was placed in a cooling bath kept at 15 °C and crude IV (47.0 g, 0.13 mol) in dry DMF (200 mL) added over 15 min. When the gas evolution had ceased (30 min), the mixture was cooled to 5 °C and methyl iodide (19.0 g, 0.13 mol) added at such a rate that the reaction temperature did not exceed 10 °C. The mixture was stirred for 30 min with cooling and then for 1 h at room temperature and then quenched by the addition of water (2.75 L) containing NH_4Cl (15 g). The mixture was extracted with benzene, and the combined organic layers were washed with water and dried over $MgSO_4$. Removal of the benzene under reduced pressure left a residue which solidified on standing. The residue was dissolved in boiling cyclohexane (500 mL) and the product V obtained by the addition of hexane (250 mL). A second crop was obtained by concentrating the filtrate. The total yield of V, a white crystalline solid, was 32.0 g (66%); mp 125–126 °C. ^1H NMR: δ 1.2–1.8 (6 H, m), 1.90 (3 H, s), 2.0 (3 H, s), 3.16 (6 H, s), 3.3–4.5 (3 H, m), 3.54 (3 H, br s). Anal. Calcd for $C_{18}H_{28}N_4O_2S$: C, 59.3; H, 7.7; N, 15.4; S, 8.8. Found: C, 59.6; H, 7.8; N, 15.5; S, 9.0.

1-Methoxy-2-methyl-1,1-bis(1-methylimidazol-2-yl)-2-propanethiol (VI). A solution of V (20.0 g) in CF_3CO_2H (100 mL) was refluxed for 1 h. Most of the CF_3CO_2H was removed under reduced pressure and the residue, a dark brown oil, was shaken with benzene (400 mL) and water (3 × 700 mL) after which all of the oil dissolved. The combined aqueous layers were extracted with benzene and the extracts discarded. The aqueous phase was neutralized by the addition of solid $NaHCO_3$ and thoroughly extracted with benzene. The combined extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in boiling cyclohexane (500 mL) and the solution allowed to cool to room temperature then rapidly filtered through Celite. The filtrate was concentrated to 150 mL, and upon cooling a white precipitate appeared. Precipitation was completed by the slow addition of hexane (300 mL) to give VI, a white crystalline solid (12 g, 78%, mp 122–124 °C). ^1H NMR: δ 1.75 (6 H, s), 3.34 (9 H, s), 3.60 (1 H, br s), 6.75 (2 H, d, J = 2 Hz), 6.93 (2 H, d, J = 2 Hz). Anal. Calcd for $C_{13}H_{20}N_4OS$: C, 55.7; H, 7.2; N, 20.0; S, 11.4. Found: C, 55.8; H, 7.3; N, 19.7; S, 11.5.

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Registry No. I, 2365-48-2; II, 76024-79-8; III, 76024-80-1; IV, 76024-81-2; V, 76024-82-3; VI, 76036-51-6; IX, 38634-58-1; X, 76024-83-4; $CH_3OCH_2SC(CH_3)_2CN$, 76024-84-5; $(H_2NCH_2C(CH_3)_2S-)_2 \cdot H_2SO_4$, 76024-85-6; $(H_2NCH_2C(CH_3)_2S-)_2 \cdot HClO_4$, 76024-86-7; $CH_3OCH_2SC(CH_3)_2CH_2NH_2$, 76024-87-8; CH_3COSK, 10387-40-3; CH_3OCH_2Cl, 107-30-2; $ClCH_2CN$, 107-14-2; MeI, 74-88-4; DHP, 110-87-2; *N*-methylimidazole, 616-47-7; $[Cu(VI)_2]^0$, 76036-74-3; $[Cu(Me_4\text{-cystam})CH_3CN](ClO_4)_2$, 76036-73-2; $[Cu(Me_4\text{-cystam})(N\text{-methylimidazole})](ClO_4)_2$, 76036-71-0.

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Chlorite Ion Oxidation of the Iron(III) Complex of Deuteroporphyrin IX

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The extent and rate of oxidation of the iron(III) complex of deuteroporphyrin IX by sodium chlorite have been followed via stopped-flow spectrophotometric measurement of the decrease in absorbance in the Soret-band region (384 nm) of the heme spectrum. The reaction results in formation of one or more reaction intermediates (an "intermediate state") containing iron in oxidation state >Fe(III) which play(s) a central role in the (peroxidatic) catalytic activity of the heme. At pH 6.5–7.0 the apparent molar equivalency [heme Fe(III)]:$[ClO_2^-] \simeq 4:1$, but this ratio as well as the rate of intermediate formation decreases with increasing basicity. It is speculated that the extent and rate of intermediate production under given conditions are determined by the relative importance of (1) formation of the intermediate state from heme and oxidant and (2) its subsequent collapse via catalytic or other pathways with accompanying heme regeneration. The apparent stoichiometry at lower pH corresponds to a one-electron oxidation of each of four heme Fe(III) units to Fe(IV) for the transformation of $ClO_2^- \rightarrow Cl^-$, suggesting an intermediate state analogous to peroxidase Compound II species. The possibility of a rate-limiting oxidation of heme Fe(III) to a Compound I analogue, regarded formally as an Fe(V) species, with subsequent comproportionation, Fe(V) + Fe(III) → 2Fe(IV), also is considered. Heme oxidation by hypochlorite ion is measurably faster than oxidation by ClO_2^-, which suggests the possibility of OCl^- as a reactive intermediate in the chlorite reaction. The absorption spectrum of the intermediate state is analogous to that obtained via oxidation of heme with selected peroxobenzoic acids. Comparable kinetic parameters obtained for the oxidation of iodide ion and phenol by intermediate states derived from chlorite and peroxo substrates further indicate that the same oxidized form of heme is obtained via reaction with both types of oxidant.

Protein-free hemes have been widely studied as models of heme-containing enzymes including catalase and various peroxidases which mediate the peroxide oxidation of selected substrates.[1-4] Investigations include those of the kinetics and mechanism of their reaction with peroxo substrates to form intermediate species which play a central role in the catalytic activity of the heme.[5-7] Such intermediates are presumed to represent oxidized forms of heme where the iron center exists in oxidation state >Fe(III) and, indeed, for many years it has been assumed that the principal species so obtained is a product of a two-electron oxidation process and, therefore, an analogue of species derived from catalase and peroxidase enzymes denoted as Compounds I.[8-10]

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