Model Studies on the Coordination of Copper in Enzymes. IV. Structure and Stability of Cuprous Complexes with Sulfur-Containing Ligands[1]

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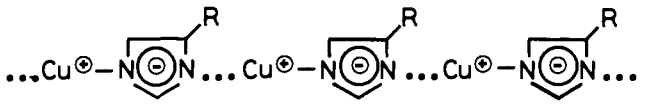
Abstract: Cuprous complexes with biologically relevant protic sulfur ligands have been investigated by anaerobic, acidimetric titration in aqueous acetonitrile solution, by using $Cu^I(CH_3CN)_4ClO_4$ as a source of univalent copper. Monodentate alkylmercaptans, RSH, form linear μ-thiolato complex polymers $[Cu^ISR]_n^0$ predominantly, which can be kept in solution if R contains an auxiliary ionic function devoid of metal affinity, such as $-N(CH_3)_3^+$ or $-SO_3^-$. For an excess of Cu^I, metal hydrolysis is inhibited up to stoichiometric formation of $[Cu^I_2SR]^+$. For an excess of ligand, a second RSH per metal is coordinated, as shown by liberation of up to one-half further proton equivalent of moderate acidity, indicating degradation of the polymer down to maximally $[Cu^I_2(RS)_3]^-$, but not $[Cu^I(RS)_2]^-$. If the auxiliary function of R is sufficiently high in metal affinity, such as $-NH_2$ and $-COO^-$, but not -NHCO- or -OH, five-membered chelates may occur under strictly limited conditions, thus allowing the metal to accept trigonal or tetrahedral configuration in the abscence of large ligand excess. Chelate formation of Cu^I with sulfur ligands is only found in systems where the sulfur is three-coordinated. This is verified in the cases of Cu^I-S—Cu^I-X for mercaptide and RS-Cu^I-X for thioether ligands. Cuprous copper does not form tetrahedral complexes, unless at least one of the metal binding sites allows metal–ligand back-donation. Stability constants β_n' have been determined for various systems, whose quadratic dependence on the acetonitrile concentration supports the postulated chelate structures. Characteristically, overcrowding of the sulfur site by methyl groups, such as in penicillamine, suppresses linear complexation and favors bidentate chelation. Finally, the biologically relevant cuprous complexes have been discussed with respect to a redox active copper coordination sphere.

In an earlier communication[3] we have demonstrated that complex reactions of univalent copper[4] can be studied titrimetrically in homogeneous dilute aqueous solution, using the stable compound $Cu(CH_3CN)_4ClO_4$[5] according to eq 1.

$$Cu(CH_3CN)_2^+ + nLH_m \rightleftarrows [Cu(LH_{m-1})_n(CH_3CN)_{2-n(m-1)}] + n(m-1)H^+ + n(m-1)CH_3CN \quad (1)$$

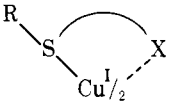
Applying Pearson's concept of "hard and soft acids and bases" (HSAB principle)[6] and the ligand classification of Jorgensen,[7] to all prosthetic groups to which copper might be bound in biological systems, imidazole and cyst(e)ine residues seem to be most suitable constituents of a redox-active copper coordination sphere.[8] After having shown that imidazole forms polynuclear chains (structure I)[3] in preference to bidentate chelation in the case of $R = CH_2CH_2NH_2$, we now report the coordinative properties of biologically relevant sulfur ligands towards Cu^I.



I R= H, $-CH_2CH_2NH_2$

Most recently, much experimental data have been published indicating the direct presence of cyst(e)ine sulfur in the nearest neighborhood of both the "type 1" or "Blue" and "type 3" or "EPR-nondetectable" copper.[9] Another aspect of Cu–S interaction arises from the low molecular weight protein, metallothioneine,[10,11] which exhibits a relatively high content of cysteine and unusually large binding constants towards both uni- and divalent copper,[12] thus leading to heavily disputed postulates in connection with Wilson's disease.[13,14]

Despite some early experiments by Pirie[15] on the isolation of L-cysteinyl peptides as their cuprous 1:1 compounds and several electrometric equilibrium studies on Cu^I complexes with cysteine and penicillamine,[16-19] little is known about the structure and stability of Cu^I complexes with ligands in which sulfur is bound only to sp^3-hybridized carbon atoms, i.e., the class of aliphatic thiols (RSH) and thioethers (RSR'). Similar to the coordination of imidazole and histamine the following questions are of considerable interest for the understanding of catalytic activity and coordination sphere of protein bound copper. (1) What maximum coordination number can be achieved by Cu^I with mercaptans and thioethers in dilute solution? (2) How is the formation of chelates dependent upon



ring size and upon the nature of "X" and R? (3) Is *one* $(d,\pi^*)_\pi$- or $(d,d)_\pi$-acceptor ligand sufficient to change Cu^I coordination from linear to tetrahedral? With how many acceptor ligands is the stabilization of tetrahedral Cu^I maximal?

Experimental Section

Materials. $Cu(CH_3CN)_4ClO_4$ was synthesized by the method of Hemmerich and Sigwart[5] and recrystallized twice from acetonitrile. All chemicals, if not described separately, were commercially available products in reagent grade from Fluka AG, Buchs (Switzerland), and used without further purification. Glutathione and its *S*-methyl derivative were obtained from Sigma Chemical Co., München (Germany). Thioglycolic acid amide,[20] (2-mercaptoethyl)trimethylammonium chloride,[21] 2-mercaptoethanesulfonic acid,[22] 2-mercapto-2-methylpropylamine,[23] 1-(aminomethyl)cyclohexyl-1-mercaptan;[23] 2-tritylmercaptoethylamine (*S*-tritylcysteamine),[24] 2-methylmercaptoethylamine (*S*-methylcysteamine),[25] and 2-benzylmercapto-2-methylpropylamine[23] were synthesized by methods described in the literature.

Acetonitrile (BASF, Ludwigshafen, Germany) was purified according to the procedure by O'Donnell et al.[26] Stock solutions of Cu^I, 0.10 M in acetonitrile, and ligand, 0.010 M in doubly distilled water, were freshly prepared shortly before use. The exact concentration of Cu^I was determined potentiometrically as described elsewhere.[3,5] The absence of Cu^{II} due to autoxidation was checked by electron paramagnetic resonance (EPR). All solvents were flushed carefully for at least 30 min with a stream of argon 99.999%, Messer Griesheim (Germany). Argon also was used to provide an inert atmosphere during titration experiments. In general, oxygen-free solutions were

Table I. Properties of Cuprous Mercaptide Complexes

No.	R(SH)(XH) compounds	pK_{SH}^{H} [a]	pK_{XH}^{H} [b]	Ref	H_{tot}^{+}/Cu [c]	$[Cu^{I}\text{-}SR]_{n\downarrow}$ [d]	Crit Cu/RSH [e]	$\Delta p\text{-}K^{Cu_2O}$	$\Delta p\text{-}K_{CuX}^{H}$ [f]	Log K_{CuL}^{Cu} [g]
1	$HSCH_2CH_2NHCOCH_3$	9.8	—		1.25	3	1	3	—	—
2	$HSCH_2CH_2OH$	9.5	—		1.10	3	1	3	—	—
3	$HSCH_2CONH_2$	8.1	—		1.00	3	1	3	—	—
4	$HSCH_2CH_2SO_3^-$	9.5	—		1.50	—	2	4	—	8.2
5	$HSCH_2CH_2N(CH_3)_3^+$	7.9	—		1.50	—	2	3	—	7.4
6	$HSCH_2COOH$	10.0	3.5	33	1.10	3	2	1	—	—
7	$HSCH_2CH_2COOH$	10.0	4.8	35	1.10	3	1.5	3	—	—
8	$HSCH_2CH_2CH(COO^-)NH_3^+$	8.8	10.4	34	1.30	3	1	3	0.6	—
9	$HSCH_2CHNHCOCH_2CH_2CHNH_3^+$ (CONHCH2COO⁻, COO⁻)	9.2	9.5	34, 36	1.25	3	1	1.5	0.6	—
10	$HSCH_2CH_2NH_3^+$	8.3	10.8	34	1.50	8	1.5	4	2.3	11.6
11	$HSCH_2CH_2N(CH_3)_2H^+$	7.9	10.4		1.50	7	1.5	3	4.7	13.5
12	$HSCH_2CH(COOCH_3)NH_3^+$	7.4	8.4	34	1.55	7	1.5	4	1.7	13.6
13	$HSC(CH_3)_2CH_2NH_3^+$	8.2	10.4		1.40	5	2	2	4.6	16.2
14	$HS(CH_2NH_3^+)C(CH_2)_4CH_2$	8.3	10.4		1.75	5	1.5	3.5	7.0	18.6
15	$HSCH_2CH(COO^-)NH_3^+$	8.5	10.4		1.55	7	1.5	4	3.0	14.0
16	$HSC(CH_3)_2CH(COO^-)NH_3^+$	7.9	10.3		1.25	5	2	3	3.9	14.3

[a] Dissociation constant predominantly attributed to deprotonation of SH;[34] determined potentiometrically in 1.0 M CH_3CN, 0.1 M $NaClO_4$ at 20 °C. [b] Dissociation constant predominantly attributed to deprotonation of XH.[34] [c] Maximum number of SH protons liberated (cf. eq 6). [d] pH at which inhomogeneity occurs in solvent system b. [e] Critical metal to ligand ratio, up to which Cu^{I} hydrolysis (cf. Figure 1) can be suppressed in solvent system b; ΔpK^{Cu_2O} describes the shift of Cu^{I} hydrolysis to higher pH under these conditions (cf. Figure 1, curves d and e). [f] Metal induced decrease of pK_{XH}^{H} in solvent system b (Cu/RSH ≥ 1, cf. Figure 2). [g] Determined in 1.0 M CH_3CN and 0.1 M $NaClO_4$ at 20 °C.

transferred by a gas tight syringe (Hamilton Bonaduz AG, Switzerland) using rubber serum caps on all vessels.

The cuprous complexes of *N*-acetylcysteamine and thioglycolic acid were isolated by mixing equimolar amounts of both ligand and $Cu(CH_3CN)_4ClO_4$ (0.005 M in aqueous acetonitrile 50% by volume) under strictly anaerobic conditions in a titration vessel. After adjusting the pH to 5.0 with 0.10 M NaOH, the colorless microcrystalline solid was collected by suction in a stream of argon, then washed carefully with aqueous acetonitrile and finally dried for 24 h over P_2O_5 at 25 °C and 0.1 mmHg.

Anal. Calcd for C_4H_8NOSCu: C, 26.29; H, 4.96; S, 17.55; Cu, 34.78. Found: C, 25.0; H, 4.17; S, 16.65; Cu, 34.0; see text.

Anal. Calcd for $C_2H_3O_2SCu$: C, 15.48; H, 1.95; S, 20.60; Cu, 40.83. Found: C, 15.04; H, 1.83; S, 20.74; Cu, 40.5.

Methods. Potentiometric titration curves were recorded on a E 336 A Combi titrator with the E 436 E multiple stand, the EA 125 glass electrode and a thermostated vessel from Metrohm, Herisau (Switzerland).

Calculation of Stability Constants. At a given concentration of acetonitrile, stability constant K_n can be determined from pH-titration data by applying Bjerrum's method.[27] From eq 1 this constant K_n defines the following relationship:

$$K_n = \frac{[Cu(L_m)_n(CH_3CN)_{2-nm}][CH_3CN]^{nm}}{[Cu(CH_3CN)_2][L_m]^n} \quad (2)$$

(L = *m*-dentate ligand)

Furthermore variation of the CH_3CN concentration shows whether L_m acts upon Cu^{I} as a mono- or bidentate ligand (m = 1 or 2) using expression 3.

$$-\Delta \log K_{CuL}^{Cu}/\Delta \log [CH_3CN] = nm \quad (3)$$

For the mono- and bidentate thiols accurate constants are rather difficult to obtain due to polymerization reactions followed by inhomogeneity of the solution even at low pH (Table I). Despite these problems we estimated the stability constant K_{CuL}^{Cu} for the cuprous mercaptides from their 1:1 titration curves by using only data points where no precipitation had occurred as compiled in Table I. Taking into account the value of 4.34 for β_2 of the $Cu(CH_3CN)_2^+$ cation, which represents the complexation of the hypothetical cuprous aquo ion with CH_3CN,[5] the results obtained for the Cu^{I} cysteine complex (Table I) are in reasonable agreement with the constants reported by Stricks and Kolthoff.[16]

Results and Discussion

Throughout the present investigation we apply mainly two types of solvent systems.

(a) A 1:1 v/v Mixture of Water and Acetonitrile. In this medium hydrolysis of cuprous copper is shifted to pH >10.5, thus facilitating evaluation of the complex equilibria under acidimetric investigation. At the same time, it precludes any but mercaptide interaction with cuprous copper, due to the extraordinarily high Cu^{I} affinity of anionic sulfur.

(b) Aqueous Solutions Containing Acetonitrile in Concentrations between 0.1 and 1.0 M. Under these conditions the concentration of the auxiliary soft ligand CH_3CN is just sufficient to suppress metal disproportionation entirely and metal hydrolysis sufficiently, i.e., up to pH 6-7 (cf. Figure 1).

Furthermore, we are subdividing sulfur ligands into four classes (Tables I and II): (1) monodentate RSH with a metal-inert and uncharged R; (2) monodentate RSH, where R contains a solubilizing charged function, but which is devoid of metal affinity; (3) potentially bidentate RSH, where R contains a metal binding function capable of chelate formation; (4) bidentate RSR′, where R contains a chelating function.

(1) Monodentate RSH with Inert R (Table I, Compounds 1-3). In the case of a metal to ligand ratio 1:1 all RSH protons are liberated below pH 4. Under these conditions a colorless microcrystalline precipitate is formed, which is highly insoluble in all common solvents. This polymer may form the chain structure II, similar to the Cu^{I}-imidazole polymer (structure I).[3]

In two cases the precipitate has been isolated under anaerobic conditions. From its elementary analysis an average ratio of $Cu^{I}:RS^{-}$ = 1:1 is found in agreement with the data of Pirie.[15] Deviations from this composition (±10%) mostly depend upon the titration velocity, the solvent, and the ligand concentration. Comparison of the ir spectra of free and of the complexed ligand clearly demonstrates the absence of the characteristic SH vibration around 2500 cm^{-1} as reported by Misra et al.[28]

The electronic spectra of these complexes as well as of those described later in the text, do not show any significant absorption maxima both in the uv and visible region in agreement with the literature.[29] Furthermore, no appreciable amount of cupric copper due to autoxidation or disproportionation could be detected by EPR. This served as a simultaneous test for the anaerobicity maintained in the systems investigated.

(2) Monodentate RSH with Solubilizing Charged R (Table

Table II. Properties of Cuprous Thioether Complexes

No.	R(SR')(XH) compounds	pK_{XH}^{H} [a]	ΔpK_{CuX}^{H} [a]	β_2' [b]	Chelate ring size	m [b]
1	$CH_3SCH_2CH_2NH_3^+$	9.45	1.9	8.6	5	2
2	$C_7H_7SCH_2CH_2NH_3^+$	9.33	2.1	10.3	5	2
3	$(C_6H_5)_3CSCH_2CH_2NH_3^+$	8.55	1.0	8.2	5	2
4	$C_7H_7SC(CH_3)_2CH_2NH_3^+$	8.85	2.9	11.6	5	2
5	$CH_3SCH_2CH(COO^-)NH_3^+$	8.75	1.8	9.3	5	2
6	$C_7H_7SCH_2CH(COO^-)NH_3^+$	8.70	2.1	9.7	5	2
7	$CH_3SCH_2CH(COOCH_3)NH_3^+$	6.95	2.0	9.7	5	2
8	$CH_3SCH_2CH_2CH_2NH_3^+$	10.0	0.7	7.2	6	2
9	$CH_3SCH_2CH_2CH(COO^-)NH_3^+$	9.10	0.8	7.4	6	2
10	$CH_3SCH_2CH_2CH(COOCH_3)NH_3^+$	7.15	0.7	7.4	6	2
11	$CH_3SCH_2CH_2CH(CH_2OH)NH_3^+$	9.10	0.7	7.3	6	2
12	$C_7H_7SCH_2COOH$		0.6	Cu^I hydrolysis	5	
13	$C_7H_7SCH_2CH_2COOH$		0.6	Cu^I hydrolysis	6	
14	$S(CH_2CH_2OH)_2$			No complex formation		
15	$S(CH_2COOCH_3)_2$			No complex formation		
16	$CH_3SCH_2CH(NHCOCH_2CH_2CH(COO^-)NH_3^+)CONHCH_2COOH$			No complex formation		

[a] Determined in 1.0 M CH_3CN, 0.1 M $NaClO_4$ at 20 °C. [b] See Experimental Section.

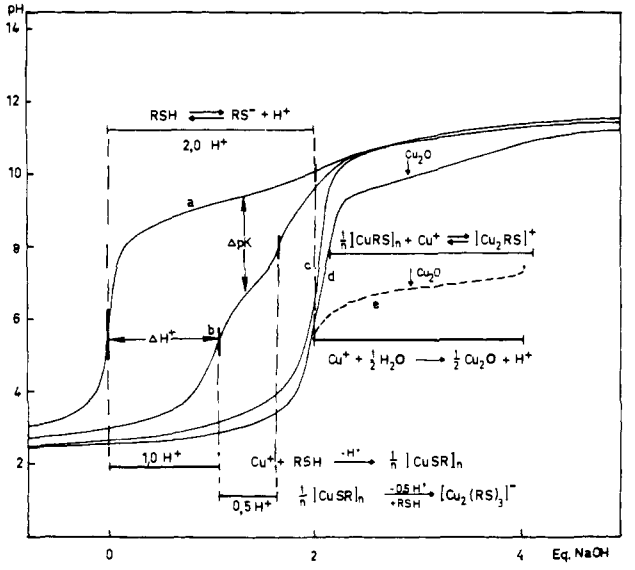


Figure 1. Acidimetric titration curves of β-mercaptoethane sulfonate (LH). [LH] = 3.0×10^{-3} M in solvent system b: (a) in the absence of metal; (b) 1.5×10^{-3} M Cu^+; (c) 3.0×10^{-3} M Cu^+; (d) 6.0×10^{-3} M Cu^+; Cu^+-hydrolysis is suppressed as compared to (e) Cu^+ hydrolysis in the absence of ligand.

I, Compounds 4 and 5). β-Mercaptoethane sulfonate and (β-mercaptoethyl)trimethylammonium (thiocholine) have been applied as prototypes of such ligands. Here the titrations can be kept homogeneous. As shown in Figure 1 the very flat course of the titration curve during the liberation of the first proton at pH 3 is in agreement with concomitant formation of a soluble polymer $[Cu^ISR]_n$.

If an excess of metal is applied, Figure 1 demonstrates inhibition of excess metal hydrolysis up to a ratio of Cu^I:RSH = 2:1. An acetonitrile concentration of 1.0 M is required to maintain the occupation of the residual free metal coordination sites by acetonitrile. The formed positively charged complex $[Cu^I_2SR]^+$ is further stabilized by the negative charge of the residue R in the case of the sulfonate. This result is supported by the polarographic experiments of Stricks and Kolthoff,[16] who also report a binuclear complex $[Cu^I_2SR]^+$ with L-cysteine in ammonia buffer at pH 9.0.

For an excess ligand concentration a second ligand proton per copper atom is liberated in a range of moderate acidity, which depends to some extent upon the charge of R. For the sulfonate this second range of proton liberation is around pH 6, in the case of thiocholine around pH 5.5 (cf. Figure 1). It is important to note that coordination of a second RS^- per metal never reaches the stoichiometric value of 2. In other words, a monomeric, negatively charged species, $[Cu^I(SR)_2]^-$, or its corresponding polymer $[Cu^I(SR)_2]_n^{n-}$ (structure III) is not formed to measurable extent. The limiting value of 1.5 proton equivalents liberated indicates that the smallest unit obtained by degradation of the neutral polymer has the composition $[RSCu^I(SR)Cu^ISR]^-$.

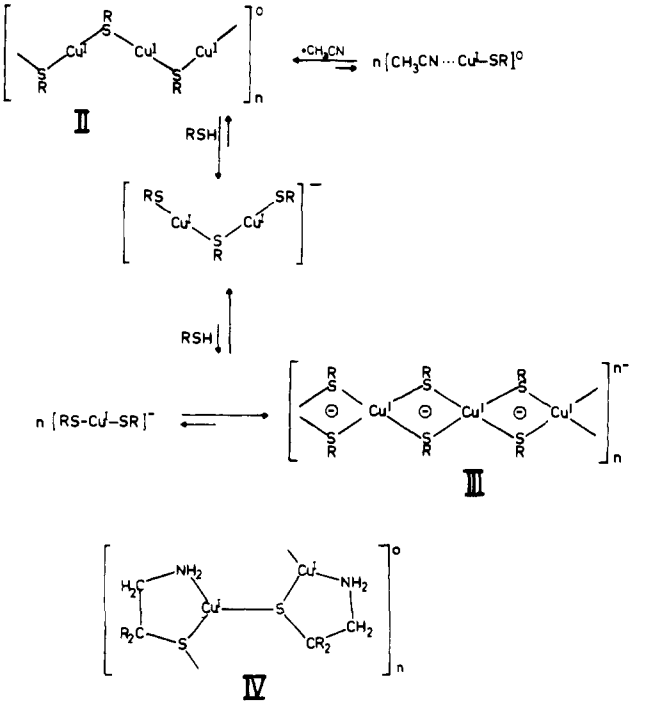
These experimental results can be explained satisfactorily by the following main equilibria (cf. also Scheme I):

$$Cu^+ + RSH \overset{-H^+}{\rightleftharpoons} [Cu^ISR] \rightleftarrows \text{polymer II} \quad (4)$$

$$[Cu^ISR] + Cu^+ \rightleftarrows [Cu^I_2SR]^+ \quad (5)$$

$$2[Cu^ISR] + RSH \overset{-H^+}{\rightleftharpoons} [Cu^I_2SR_3]^- \rightleftarrows \text{polymer III} \quad (6)$$

Scheme I. Cuprous Mercaptide Species in Solution



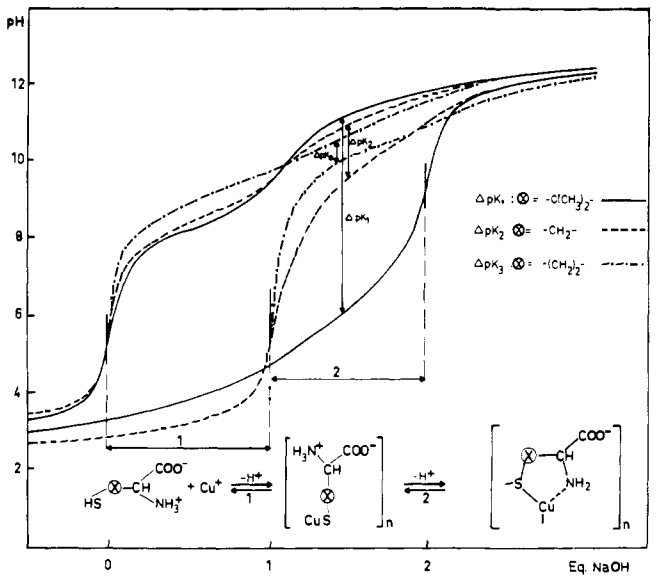


Figure 2. Acidimetric titration curves of L-cysteine (- - -), D-penicillamine (—), and L-homocysteine (·····) in solvent system a. The three upper curves are obtained in the absence of metal and the three lower curves in the presence of metal. [LH] = [Cu] = 2.0×10^{-3} M. Note, that L-homocysteine (⊗ = $-(CH_2)_2-$) yields a first metal induced deprotonation of the same strength as L-cysteine (⊗ = $-CH_2-$), while it yields no metal induction of the second deprotonation (pK_3 = very small); D-penicillamine (⊗ = $-C(CH_3)_2-$) yields a moderately strong first deprotonation, but acts as the strongest chelating agent (large pK_1).

A pertinent question is polymerization of anionic species under ligand excess to yield type III structures. Analogous structures are known in the crystalline state with chloride or cyanide anions as bridging ligands,[30] which are, however, less electron donating than RS^-. Identical structures are verified for mercaptide ligands in the case of the more highly charged Ni^{II} as central atom.[31] A polyanionic structure such as III is less probable in the present case. This is in agreement with conclusions drawn by Martell et al.[32] on related Ag^I complexes with 1,2-dimercaptosuccinic acid as ligand, where again a metal to sulfur ratio of 1:1 is preferred.

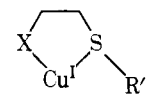
(3) Potentially Bidentate Mercaptans RSH (Table I, Compounds 6–16). Prototypes of potential binding sites in the residue R are $-COO^-$ and $-NR_2$. In the first of these cases, i.e., thioglycolic and β-mercaptopropionic acid (Table I, compounds 6 and 7), the neutral type II polymers $[Cu^IS(CH_2)_{1-2}COOH]_n^0$ are thermodynamically and kinetically sufficiently stable as to impede neutralization of the carboxyl groups in the precipitate once formed (see Experimental Section). However, upon mixing equimolar solutions of metal and ligand in ammonia buffer, pH 9, no inhomogeneity is observed, while precipitation will occur around pH 3.

Easier to handle are the cysteine type ligands (Table I, compounds 10–16). In the acidic range, cysteine behaves like thiocholine. When the pH titration is continued, at a metal to ligand ratio of 1:1 (Figure 2), a proton from the ammonium group is liberated, independently of the acetonitrile concentration, at pH ≈7, as compared to the pK of 10.3 for the free ligand.[34] This large increase in acidity, induced by the neighboring cuprous mercaptide, strongly points towards chelate formation. It must be emphasized that in the trigonal cuprous chelates (structure IV) all sulfur atoms are tricoordinated, too. The chelation is further substantiated by the fact that homocysteine (Table I, compound 8) behaves as a nonchelating ligand since its amino pK is not essentially lowered by Cu^I in contrast to cysteine (Figure 2). Presumably chelation is confined to those cases, where five-membered rings can be formed. Obviously the chelate effect is rather low, because no solvent ligand is mobilized upon formation of the copper nitrogen chelate bond. In addition, it may be concluded, that for "X" = $-COO^-$, solubilization of the type II polymer at pH 9 is due mainly to the presence of a negative charge, producing $[Cu^IS(CH_2)_{1-2}COO^-]_n^{n-}$ without chelation of the carboxylic group. Otherwise stabilization of both five- and six-membered cuprous mercaptide chelates by $-COO^-$ has to be assumed in contrast to "X" = $-NH_2$, which is most unlikely in view of the ideas outlined in the introductory section.

In contrast to the β-mercaptoamino acids, mercaptans with "X" being a peptide function (-CONH-), such as the already discussed *N*-acetylcysteamine, the thioglycolic acid amide, and the tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) (Table I, compounds 1, 3, and 9) do not form soluble type II polymers under any conditions up to pH 14; i.e., metal induced deprotonation of the peptide nitrogen atom never occurs.

Although one might expect restricted complex formation due to steric hindrance, α-alkylated β-aminothiols, such as D-penicillamine and its derivatives 2-mercapto-2-methylpropylamine and 1-(aminomethyl)cyclohexyl-1-mercaptan (Table I, compounds 13, 14, and 16), differ from their parent compounds L-cysteine and cysteamine (Table I, compounds 10 and 15) by increased chelating capacity towards Cu^I, as shown in Figure 2. In biological systems penicillamine effects the mobilization of toxic copper from the body, thus acting as a chemotherapeutic agent against Wilson's disease.[13] Although its mechanism of action is not well understood until today, one of the possibilities discussed most recently by Peisach and Blumberg[19] is the so-called "reductive chelation" of biological copper; i.e., in the first step the nonspecifically bound toxic Cu^{II} may be reduced by one molecule of RSH yielding Cu^I which subsequently forms a stable chelate with a second penicillamine molecule. Alternatively, a mixed-valence species, as proposed by Tanaka et al.[18] and most recently by Wright and Frieden[37] with the metal coordinated to mercaptide sulfur both in its cuprous and cupric state, might be responsible for the drug activity of penicillamine. Because of the complexity of the Cu^{II}/Cu^I-penicillamine/penicillamine disulfide redox couple and the large biomedical interest related to it,[38] the outstanding coordinative properties of C_α-alkylated β-aminothiols towards both Cu^I and Cu^{II} are not discussed in greater detail at this point, but will be published separately within this series.

(4) Potentially Bidentate Thioether Ligands RSR′ (Table II). As mentioned earlier[3,8] thioether ligands (RSR′), such as *S*-benzylcysteine or D,L-methione, can stabilize cuprous copper in aqueous solution. Theoretically, RSR′ imitates the structural element V, postulated above for the complexes of Cu^I with



V

β-aminothiols (Scheme I) with R′ being a second cuprous ion instead of an organic residue. Figure 3 illustrates the pH-titration of 2-benzylmercapto-2-methylpropylamine, *S*-methyl-L-cysteine, and D,L-methionine (Table II, compounds 4, 5, and 9) in the presence of Cu^I in 1.0 M aqueous acetonitrile. For a metal to RSR′ ratio of 1:2, exactly two proton equivalents per total copper are displaced at a pH which is about one to three units lower than the ligand pK. In addition, precipitation of Cu_2O is not observed at pH <10, while upon titrating a 1:1 mixture or in the presence of excess metal, hydrolysis begins around pH 7.

These acidimetric results are obtained only with RSR′ ligands having the amino group as a second metal binding site (Table II). For "X" being an ester or a hydroxy function, no complexation occurs; i.e., metal hydrolysis takes place inde-

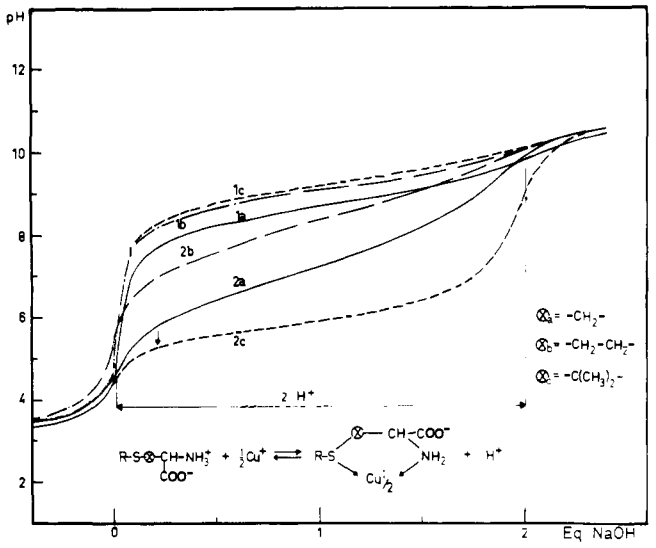


Figure 3. Acidimetric titration curves of *S*-methyl-L-cysteine (a), D,L-methionine (b), and 2-benzylmercapto-2-methylpropylamine (c) in solvent system b. [LH] = 4.0×10^{-3} M, [Cu] = 2.0×10^{-3} M.

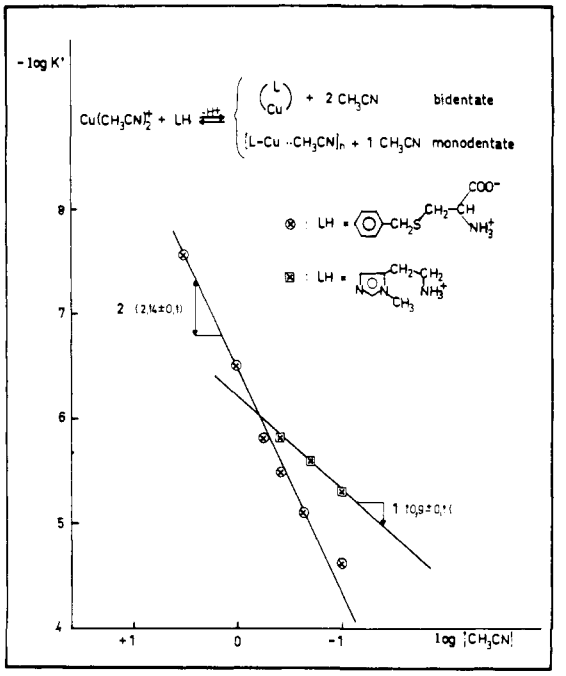


Figure 4. Plot of the first stability constant $-\log K_{CuL}{}^{Cu}$ vs. CH_3CN concentration (cf. eq 3). ⊗ = the bidentate ligand *S*-benzyl-L-cysteine, ⊠ = the monodentate ligand 1-methyl-5-histamine.[3]

pendently of the RSR′ concentration. For "X" being more acidic, as with -COOH, further acidification in the presence of Cu^I cannot be analyzed, but chelate formation can be derived from the inhibition of metal hydrolysis up to pH 8 for a metal to ligand ratio of 1:2 in solvent system b.

In view of the low stability of a cuprous coordination sphere solely constituted by either monodentate RSR′ or RNH_2 ligands, it is reasonable to assume formation of a Cu^I thioether chelate as in the case of bidentate thiols (cf. Scheme I), which is now monomeric because of blocked sulfur bridging by the organic residue R′ (structure V). Furthermore Table II reveals that both five- and six-membered chelate rings are built up at the metal site in contrast to the corresponding aminothiols. Despite the fact that we only investigated one C_α-alkylated RSR′ ligand (Table II, compound 4), its value for β_2', which is the largest one in the series studied, fits nicely the results obtained for the C_α-substituted mercaptans (Table I).

From eq 2 and 3 (see Experimental Section) a quadratic dependence of the first stability constant $K_{CuL}{}^{Cu}$ on the acetonitrile concentration is derived for the stable Cu^I-RSR′ complexes over a range of 0.1-1.0 M CH_3CN, as illustrated in Figure 4 for the bidentate RSR′ ligand *S*-benzyl-L-cysteine.

Conclusions

The following rules apply to Cu^I sulfur complexation.

(1) Two main classes of sulfur ligands must be distinguished: "sp^2 sulfur" as in thiourea, which is biologically irrelevant, and "sp^3 sulfur". This latter class must be subdivided into neutral two-coordinated sulfur as in sulfides (methionine) and disulfides (cystine) and anionic monocoordinated sulfur as in mercaptides (cysteine). Mercaptide sulfur is the most potent biologically relevant ligand for Cu^I, which is uniquely able to displace acetonitrile from $[Cu(CH_3CN)_4]^+$ in 50% aqueous CH_3CN, while all other ligands require dilution of Cu^I-specific CH_3CN down to a few percent v/v by water in order to complex Cu^I efficiently.

(2) Cuprous copper does not form tetrahedral complexes, unless at least *one* of the metal binding sites permits metal-ligand back-donation. Except for molecular oxygen, which is a d,π^*-acceptor, three-coordinated sp^3 sulfur is biologically unique in its d acceptor ligand behavior. Hence, chelate formation of cuprous copper with sulfur ligands is only found in complexes Cu^ISRCu^I and Cu^ISRR'.

(3) Cuprous mercaptides, with more than one two-coordinated sulfur atom per metal center, are unstable.

The mechanism of biological electron transfer, as catalyzed by copper enzymes, is still not well understood. In contrast to redox active iron, the great stereochemical difference between Cu^I and Cu^{II} complexes appears to resist an efficient redox shuttle between these two valences. Two independent ways to avoid this dilemma have been proposed.

(a) With respect to the mononuclear "Blue copper" in proteins such as *Stellacyanine* and *Plastocyanine*, Blumberg[39] has proposed a geometry intermediate between square planar and tetrahedral, which is also supported by the work of Gould and Ehrenberg.[40] From the present data only very few cases can be found, where cuprous copper can obtain a coordination number of four in complexes exhibiting none but biologically relevant ligands. Hence, copper proteins do not have a large choice of ligands for a copper dependent mononuclear redox-active site; since mercaptide, as we find, is exclusively σ-donating towards Cu^I, an additional protein site is required for π back-donation, which is a prerequisite for the stabilization of four-coordinated cuprous complexes. This is in good agreement with data reported by Zuberbühler[41] on the autoxidation of cuprous imidazole complexes, where apparently oxygen as single d-acceptor ligand is sufficient to change the coordination number of Cu^I from strictly two to three or four.

(b) For the "type 3" and apparently binuclear copper in redox proteins,[42] a model based on copper-mercaptide and copper-copper interaction has been devised.[43] The present data suggest that the cuprous mercaptide state has to be ruled out as a constituent for this copper type, since the potential is very low[16] and the oxygen affinity very high, and there are three possible states of oxido-reduction in this system, i.e., first cuprous mercaptide,[2] then second, an intermediate state which may vary from cupric mercaptide to cuprous disulfide[43] and third, cupric disulfide.[1] The biological redox shuttle might well be between the two upper levels as already discussed in part III of this series.[1]

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Electronic Spectra of α,β-Unsaturated Carbonyl Compounds. I. An Evaluation of Increments Characteristic of Changes in Configuration (cis/trans) and Conformation (s-cis/s-trans) Based on Direct Observation of the Isomerization of Enamino Aldehydes and Ketones

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Abstract: The trans,s-trans → cis,s-cis and trans,s-cis → cis,s-cis isomerization of enamino aldehydes and ketones has been followed directly by uv measurements. This enabled us to assign unambiguously the absorption maxima of those three configuration-conformation combinations and, consequently, to evaluate the spectral increments characteristic of the cis ⇌ trans and of the s-cis ⇌ s-trans isomerization. Three such calculation paths leading to identical results are described. The increments thus obtained are: 18 nm $\leq \Delta\lambda^{\text{s-cis}}_{\text{s-trans}} \leq$ 27 nm and −7 nm $\leq \Delta\lambda^{\text{cis}}_{\text{trans}} \leq$ 0 nm. A set of spectral increments complementing the basic system of Woodward and the Fiesers has been proposed.

The relationship between the configuration and conformation of α,β-unsaturated carbonyl compounds and the frequency and intensity of their uv absorption has been the subject of numerous investigations during the last three decades. Although many valuable data were obtained, one serious difficulty remained until now: in order to obtain a given conformation, the parent reference compound had to be modified by a ring closure or by introducing bulky substituents. As a consequence, the contributions of (1) the conformational transformation and (2) the electronic properties of the new substituents or ring fragments to the frequency and intensity changes could not be separated. This point has often been ignored or inadequately treated.

For example, Ostercamp,[1] who investigated 78 enamino carbonyl compounds, attempted to circumvent this difficulty by making use of the averaged substituent increments known from other classes of α,β-unsaturated ketones, a procedure obviously suffering from some uncertainty. As a matter of fact, the differences between the calculated and experimental λ_{max} values cited by Ostercamp oscillate within the limits of 23 to −28 nm. Applied to the compounds studied in the present work, Ostercamp's system gives deviations of −15 to 19 nm. Apart from the ambiguities arising from the use of the averaged increments, some inadequacies of Ostercamp's scheme result from neglecting, in the case of conformationally labile compounds, the actual populations of rotamers. Ostercamp relied on earlier papers in which suppositions as to conformational uniformity of such compounds were expressed. However, in some newer papers, a simultaneous occurrence of two rotamers has been experimentally detected.[2] It appears that in a few cases the cis configuration was ascribed by Ostercamp to compounds which in solutions in methanol can be shown (by NMR) to exist predominantly in the trans form.