Metal-Binding Abilities of Radioprotective Organic Thiosulfates

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Abstract □ Ionization constants and stability constants for the complexation of Cu(II), Al(III), and Fe(III) ions by several aminoalkane and cyanoalkane thiosulfates were determined. Metal-binding avidities of the aminoalkane thiosulfates were found to be significantly less than those for the amino (or guanidino) thiols or for dithiocarbamates having radioprotective properties, but the possibility exists that organic thiosulfates may stabilize cuprous ion-containing macromolecules against radiation damage.

Keyphrases □ Metal-binding abilities, radioprotective organic thiosulfates—with Cu(II), Al(III), Fe(III), ionization, stability constants calculated □ Thiosulfates, radioprotective organic—metal-binding abilities, ionization, stability constants calculated □ Potentiometric titrimetry—determinations

The aminoalkane thiosulfates, or Bunte salts, have been recognized as a particularly effective class of radiation-protective agents (1, 2). All Bunte salts with appreciable protective properties observed so far have had the amino and thiosulfate functions separated by two or three carbons, a molecular arrangement conducive to metal-ion chelation. The role of metal binding in cellular radiation protection has not been adequately explained, but a variety of observations has shown that metal ions are involved (3, 4). For this reason it was considered desirable to determine metal-binding avidities of aminoalkane thiosulfates as well as of other organic thiosulfates having complexing functions. Although the thiosulfate ion has metal-binding properties, and stability constants have been recorded (5), no stability constants for metal binding of organic thiosulfates were found in the literature.

Possible roles for metal-binding agents in radiation protection lie in the complexation of the metal constituents of metalloenzymes and prevention of radiation-induced conversion to an unfavorable oxidation state. A correlation between extent of complexation of catalase by a series of radiation-protective agents and their relative protective abilities in mice has been found (6). All compounds in this series had the ability to bind metals; the sole exception to the correlation was the Bunte salt of 2-mercaptoethylamine, which stimulated rather than inhibited the enzyme. It was supposed that the Bunte salt must have a much different affinity for metal ions than the other compounds of the series, *i.e.*, amino (or guanidino) thiols, trithiocarbonates, and dithiocarbamates; therefore, the metal-binding abilities of aminoethane and aminopropane thiosulfates, as well as of cyanoethane and cyanomethane thiosulfates, were determined.

METHODS

Materials—Melting points were taken in capillaries using a Mel-Temp apparatus. Elemental analyses were obtained.[1] 2-Aminoethanethiosulfuric acid[2] was recrystallized from methanol; m.p. 207–209° dec. The preparations of sodium 1-cyanomethanethiosulfate monohydrate and sodium 2-cyanoethanethiosulfate monohydrate were previously described (7).

3-Aminopropanethiosulfuric Acid—The method of Kaluszyner *et al.* (8) was used. A solution of 15.3 g. (0.07 mole) of 3-bromopropylamine hydrobromide[3] and 17.4 g. (0.07 mole) of sodium thiosulfate pentahydrate in 50 ml. of water was refluxed for 75 min. The solution was cooled, diluted with 150 ml. of ethanol, and chilled overnight. The product crystallized in long needles, and 6.4 g. (53%) was obtained and recrystallized from methanol; m.p. 189–190° dec.

Anal.—Calcd. for $C_3H_9NO_3S_2$: C, 21.05; H, 5.26; N, 8.19. Found: C, 21.51; H, 5.58; N, 8.12.

Analytical reagent grade $CuCl_2 \cdot 2H_2O$, $AlCl_3 \cdot 6H_2O$, and $Fe(NO_3)_3 \cdot 9H_2O$ were used, and carbonate-free 0.01 *M* potassium hydroxide was prepared according to Armstrong (9). The solutions were stored in polyethylene bottles under nitrogen and diluted quantitatively with boiled distilled water just prior to use. Normalities were checked against potassium biphthalate.

The purity of the organic ligands was ascertained by TLC. Eastman chromatogram sheets were spotted with approximately 0.1% solutions of the compounds in water. The developing solvent contained water and methanol (1:9). When the solvent front was 1.27 cm. (0.5 in.) from the top of the sheet, the sheet was air-dried and placed in a jar saturated with iodine vapor. The presence of one spot confirmed the absence of contaminating compounds.

Ionization Constants—The method employed was that of Albert and Serjeant (10), using a Beckman research pH meter with glass and calomel electrodes. It consisted of titrations of 0.001 *M* aqueous solutions of the compounds with acid and base of such strength that 5 ml. of the titrant contained an equivalent. The titrant was added in 0.5-ml. or 0.25-ml. portions, and the pH was recorded after each addition. Each titration yielded nine pH values, giving nine values for the pKa which were averaged. Where pH values fell outside the range of 5–9, corrections were made for hydrogen- or hydroxyl-ion concentration. The pKa values obtained are listed in Table I.

Stability Constants—Potentiometric titrations were carried out under nitrogen in freshly boiled distilled water at 25° using the described equipment. Fifty-milliliter volumes containing 0.001 mole of the thiosulfates were titrated with 0.01 *N* potassium hydroxide in 0.5-ml. portions, first in the absence of metal ions and then in the presence of 0.0005 mole of divalent metal salt or 0.00033 mole of trivalent metal salt. Titration with 0.01 *N* hydrochloric acid also was carried out with the thiosulfate–$Fe(NO_3)_3$ solutions, since all the necessary values were not uncovered by titration with alkali. Fifty-milliliter volumes of the same quantities of the metal salts were also titrated with 0.01 *N* potassium hydroxide. The pH readings were recorded 2 min. after each addition of titrant to allow equilibrium to be reached. Some values of K_2 or K_3 were not obtained because of precipitation of the metal complexes.

Calculations were done as previously described (11); values for log K_1, log K_2, log K_3, and either log β_2 or log β_3 are recorded in Table II. Values for K_1, K_2, and K_3 were obtained from Eqs. 1–3

Table I—Ionization Constants (25°)

Acid	pKa_1	pKa_2
2-Aminoethanethiosulfuric	9.15	3.12
3-Aminopropanethiosulfuric	9.76	3.16
1-Cyanomethanethiosulfuric	3.53	
2-Cyanoethanethiosulfuric	3.44	

[1] Weiler and Strauss, Oxford, England.

[2] Supplied by Dr. T. R. Sweeney, Walter Reed Army Institute of Research, Washington, D. C.

[3] Aldrich Chemical Co.

Table II—Stability Constants of Metal-Ion Complexes (25°)

Acid	Cu (II) Complexes: Log K_1	Log K_2	Log β_2[a]	Log β_2[b]
2-Aminoethanethiosulfuric	5.58			
3-Aminopropanethiosulfuric	6.34	6.07	12.41	12.21
	Al (III) Complexes			
	Log K_1	Log K_2	Log K_3	Log β_3[a]	Log β_3[c]
2-Aminoethanethiosulfuric	7.50	7.20	7.08	21.78	22.28
3-Aminopropanethiosulfuric	8.10	7.97	7.84	23.91	24.91
1-Cyanomethanethiosulfuric	1.68	1.20			
2-Cyanoethanethiosulfuric	1.88	1.71	1.68	5.23	5.07
	Fe (III) Complexes			
	Log K_1	Log K_2	Log K_3	Log β_3[a]	Log β_3[c]
2-Aminoethanethiosulfuric	9.65	8.91	8.69	27.25	27.49
3-Aminopropanethiosulfuric	10.15	9.66	9.49	29.30	29.25
1-Cyanomethanethiosulfuric	2.56	2.40			
2-Cyanoethanethiosulfuric	3.25	2.90	2.77	8.92	8.69

[a] Determined from the sum of the log K values. [b] Determined from Eq. 4. [c] Determined from Eq. 5.

according to Flood and Loras (11) and Albert (12):

$$K_1 = \frac{n}{(1 - \bar{n})(L^-)} \quad \text{(Eq. 1)}$$

$$K_2 = \frac{(\bar{n} - 1)}{(2 - \bar{n})(L^-)} \quad \text{(Eq. 2)}$$

$$K_3 = \frac{(\bar{n} - 2)}{(3 - \bar{n})(L^-)} \quad \text{(Eq. 3)}$$

Values for log β_2 and log β_3 were obtained from the sum of the log K values. In cases where the difference between values of log K_1 and log K_2, or between log K_2 and log K_3, was less than 0.5, log β_2 and log β_3 were obtained from Eqs. 4 and 5 (13):

$$\log \beta_2 = \log \bar{n} - \log (2 - \bar{n}) - 2 \log (L^-) \quad \text{(Eq. 4)}$$

$$\log \beta_3 = \log \bar{n} - \log (3 - \bar{n}) - 3 \log (L^-) \quad \text{(Eq. 5)}$$

Formation curves were plotted ($\bar{n}$ *versus* $-\log [L^-]$) to show whether stepwise complexation had taken place (13). Figure 1 shows formation curves for 3-aminopropanethiosulfuric acid with Cu (II), Al (III), and Fe (III) ions, which are also typical of the other systems reported here. Since the stability constants for the binding of successive molecules of ligand are not greatly different, no steps are shown in the plots.

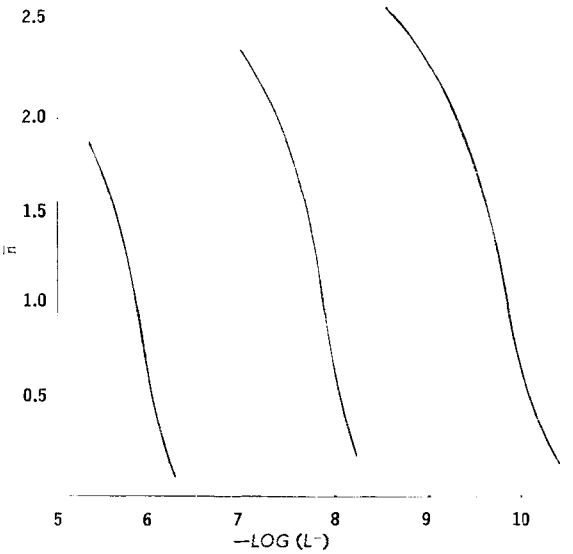


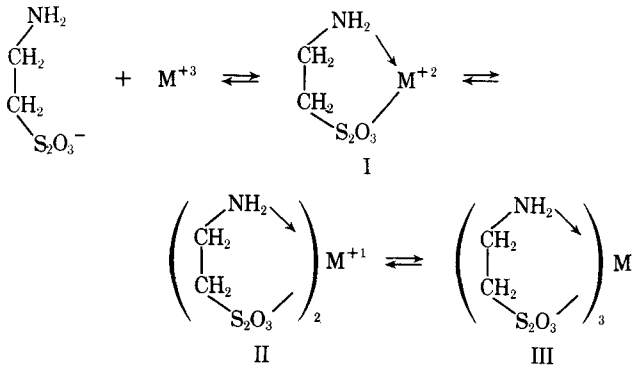
Figure 1—*Formation curves for the 3-aminopropanethiosulfuric acid systems with Cu (II), Al (III), and Fe (III) ions at* $-\log$ (L^-) *values of 6, 8, and 10, respectively.*

RESULTS AND DISCUSSION

The sequence of metal-binding abilities of the organic thiosulfates for cupric, aluminum, and ferric ions in order of decreasing strength was: 3-aminopropanethiosulfuric acid > 2-aminoethanethiosulfuric acid > 2-cyanoethanethiosulfuric acid > cyanomethanethiosulfuric acid. An appreciable difference between the stability constants for the aminoalkane derivatives and those of the cyanoalkane derivatives indicates that the amino groups are involved in the metal binding with the formation of chelates, whereas the cyano groups are probably not involved. Comparison with the metal-binding strength of thiosulfate ion for ferric ion shows a comparable value (log K_1 = 2.65) (14) to that for the binding of ferric ion by the cyanoalkane derivatives (log K_1 = 3.25, 2.56), which indicates little or no contribution from the cyano group.

A similar comparison for the aluminum and cupric ions cannot be made, since no values are recorded for the thiosulfate–Al(III) system, and cupric ion is reduced by thiosulfate ion. Recorded values for the thiosulfate–Cu(I) system, however, in terms of log βn (11.69–14.30) (5), are very similar to those (log β_2 = 12.41) for the 3-aminopropanethiosulfuric–Cu(II) system. This suggests that the amino group is not participating in the binding of cupric ion, at least, and that the Cu(II) is being reduced to Cu(I) in the complex. Stability constants for Cu(I) generally have been higher than those for Cu(II) for a given ligand, where such a comparison has been possible (5), so the close similarity in constants may be taken as a good indication that the organic thiosulfate–copper complexes include Cu(I) ions. Sufficient values for the cyanoalkanethiosulfuric–Cu(II) systems were not obtained to give reliable constants.

Thiosulfate ion is usually unidentate, but it may also be bidentate (15). When unidentate, it is believed to be S-bonded to metal ions, but coordination through both oxygen and sulfur is assumed for bidentate thiosulfate ion (16). Because of the relatively strong binding of thiosulfate ion for Cu(I) and the similarity in binding strengths between thiosulfate ion and the 3-aminopropanethiosulfuric acid, it may be concluded that binding with copper ion by the organic thiosulfates is wholly through the anionic thiosulfate group rather than the central sulfur. With Al(III) and Fe(III) ions, however, the amino function of the organic thiosulfates can be assumed to contribute to the binding; chelate structures (I–III), involving both coordination and ionic bond formation, may be proposed for these complexes (Scheme I).



Scheme I

Comparison of log K_1 values for binding of Cu(II) by a dithiocarbamate, aminoalkanethiol, and aminoalkanethiosulfuric acid shows the latter to be much weaker in binding strength. Diethyldithiocarbamate had a log K_1 value for Cu(II) of 14.9 (17), 2-mercaptoethylamine had a value of 10.05 for Ni(II) [generally comparable to Cu(II) in binding ability] (18), whereas 2-aminoethanethiosulfuric acid had a value of 5.58 for Cu(II). This relatively lower binding ability could account for the failure of 2-aminoethanethiosulfuric acid to inhibit catalase (6), whereas the dithiocarbamate and amino thiol were strong inhibitors.

No indication that organic thiosulfates act as radiation-protective agents through metal-binding ability may be drawn from this study.

However, 2-aminoethanethiosulfuric acid has good protective ability (2), whereas the cyanomethanethiosulfuric acid has none (19). It may be more significant that the thiosulfates can stabilize Cu(I) ion by complex formation; it has been proposed that one mechanism of radiation protection involves stabilization of the valence state of copper in copper-containing enzymes (20) or other macromolecules (21).

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Serum Levels of Chloramphenicol in Children, Rhesus Monkeys, and Cats after Administration of Chloramphenicol Palmitate Suspension

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Abstract □ The absorption of amorphous and polymorph A forms of chloramphenicol palmitate suspensions made with polysorbate 80 was investigated in children, rhesus monkeys, and cats. In children, both forms were absorbed. While polymorph A was absorbed at a slower rate, maintaining the blood level of chloramphenicol for 6 hr., the amorphous form was absorbed maximally at 2 hr. followed by a profound fall in the blood level. Although the absorption of the amorphous form was definitely superior, the polymorph A form in suspension with a surface-active agent was also absorbed to a considerable extent in children, maintaining blood levels of chloramphenicol for a prolonged period. In experiments with cats, polysorbate 80 increased the absorption of both forms of chloramphenicol palmitate, with the amorphous variety having superior absorption. In rhesus monkeys, there was little absorption of the polymorph A form of chloramphenicol palmitate in suspension, possibly indicating the absorption as species specific.

Keyphrases □ Chloramphenicol serum levels—following chloramphenicol palmitate suspension administration, in children, rhesus monkeys, cats □ Polysorbate 80—effects on absorption of chloramphenicol palmitate (amorphous and polymorph A forms), in cats □ Serum levels, chloramphenicol, amorphous *versus* polymorph A forms—comparison in children, rhesus monkeys, cats

Three polymorphic forms of chloramphenicol palmitate have been described; two are crystalline, termed α and β, and one is amorphous. In the solid state, transition occurs from α to β and is irreversible (1). These crystalline forms differ in their physicochemical properties (2). The IR spectrum of the amorphous form is identical to that of the α-type of crystals; but that of the β-type crystals, also termed polymorph A, is different. By using a relative absorbancy ratio of 862–864 $cm.^{-1}$, characteristic bands for α- and β-type crystals and the relative amounts of both types in a mixture can be determined (3).

Contradictory reports appear about the absorption of the different forms of chloramphenicol palmitate or stearate. Altmann *et al.* (4) determined blood levels of chloramphenicol in human adults after feeding chloramphenicol and chloramphenicol palmitate, as large or small crystals, and chloramphenicol palmitate incorporated with surface-active agents. They observed that both the administration of chloramphenicol and chloramphenicol palmitate with surface-active agents yielded the highest blood levels of chloramphenicol, whereas the blood levels following chloramphenicol palmitate as large crystals were definitely lower for the first 4 hr. There was no appreciable difference in the blood levels of chloramphenicol at 6- and 8-hr. intervals following administration of chloramphenicol and the various preparations of chloramphenicol palmitate.