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Antiradiation Compounds XIX: Metal-Binding Abilities of Thioureas

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Abstract □ Metal-binding stability constants for a series of *N*- and *N,N'*-substituted thioureas with Cu(II), Ni(II), Al(III), and Fe(III) ions were determined by potentiometric titration. The sequence of constants for thiourea, *N*-methylthiourea, and *N,N'*-dimethylthiourea indicated steric effects of the methyl groups and that both nitrogen and sulfur were involved in the complexation. The magnitude of the constants was somewhat lower than those of the simple peptides. The mechanism of protection against ionizing radiation by thioureas is probably due to hydrogen-atom transfer rather than binding of metal ions that catalyze cellular oxidations.

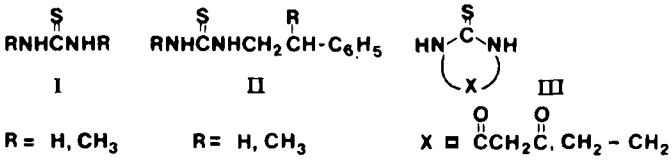
Keyphrases □ Thioureas—metal-ion complexes, stability constants, antiradiation protection □ Antiradiation protection—thioureas, metal-ion complexes, stability constants □ Stability constants—thioureas, metal-ion complexes, antiradiation protection

Metal-ion complexation has been shown or postulated to be involved in a number of biological activities. The effect of complexing agents such as dithiocarbamates (1) and some thioureas (2) on the inhibition of dopamine-β-oxidase, a copper-containing enzyme, is well known. Metal-ion complexation has also been postulated for the antithyroid activity of thiouracil (3), the inhibitory effects of dithiocarbamates against *Mycobacterium tuberculosis* (4), and the fungistatic effects of dithiocarbamates and other complexing agents (5). The nitrate reductase complex of *Neurospora crassa* is also sensitive to inhibition by metal-binding agents, including thiourea (6), and polyphenol oxidase is also inhibited by thiosinamine (allylthiourea) (7).

Several postulations (8–10) regarding the protective effects of a number of metal-binding radiation-protective agents have also been made. Although thiourea and its simple derivatives have some protective ability for whole-body irradiation of animals, these effects have not been appreciable. Thioureas have, however, shown substantial radiation-protective effects in other systems, such as mouse lung (11), human erythrocytes (12), T_2 phage (13), Ehrlich ascites cells (14), and macromolecules (15) (Table I). The radiation-protective effects of thiourea have been attributed to metal binding (11).

With such a significant number of postulations involving the metal-binding aspects of thioureas, it is surprising to find that, other than for thiourea itself (16), metal-binding abilities of substituted thioureas have not been determined. Stability constants for a series of cyclic thioureas, including mercaptoimidazoles and mercaptouracils, were determined with copper, iron, and aluminum ions (17), and the constants were surprisingly high. It was considered important, therefore, to measure the metal-binding stability constants for a series of alkyl, aralkyl, and cyclic thioureas. Knowledge of the magnitude of the constants would indicate whether thiourea complexes were capable of existence in the presence of cellular complexing agents, such as peptides, or whether thiourea might function as a transient metal complexer.

Compounds of the general structures I–III were measured for binding abilities to Cu(II), Ni(II), Al(III) and Fe(III) ions using the method of potentiometric titration.



EXPERIMENTAL SECTION

Materials—Analytical reagent-grade[1] aluminum chloride hexahydrate, cupric chloride dihydrate, ferric nitrate nonahydrate, and nickel(II) chloride hexahydrate were used for the metal complexation determination. Carbonate-free 0.01 M KOH was prepared according to the method of Armstrong (18). Solutions of the metal salts were prepared in boiled distilled water and stored in polyethylene bottles under nitrogen; they were diluted quantitatively with carbon dioxide-free water just prior to use. Normalities were checked against potassium biphthalate.

The organic ligands were obtained commercially[2]. *N*-Methyl-*N'*-phenethylthiourea was reported previously (19), and *N*-methyl-*N'*-phenyl-2-propylthiourea was described earlier (20). Purity was ascertained by TLC using chromagram sheets[3]. Solutions (0.1%) of the compounds in ethanol were spotted, and the sheets were developed with benzene–methanol (8:2); spots were detected with iodine vapor.

Ionization Constants—The method of Albert and Serjeant (21) was used, which consisted of titrations of 0.001 M solutions of the compounds in 95% ethanol with 0.01 M KOH in 0.5-mL portions. The pH was recorded with a pH meter[4] with glass and calomel electrodes after each addition. Each titration thus yielded 10 pH values, giving 10 values for the pK_a, which were averaged (Table II). Since most of the pH values fell outside the 5–9 range, corrections were made for hydrogen-ion concentrations.

Stability Constants—Potentiometric titrations were carried out under nitrogen in 95% ethanol at 25°C with the described pH meter. Volumes of 50 mL of the 0.001 M solutions of the organic ligands were titrated with 0.01 M KOH in 0.5-mL portions, first in the absence of metal ions, and then in the

[1] J. T. Baker Analyzed Reagents.
[2] Aldrich Chemical Co., Eastman Organic Chemicals, or Fisher Scientific Co.
[3] Eastman Organic Chemicals.
[4] Beckman Instruments.

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Table I—Radiation Protection by Thioureas

Compound	Test System	Radiation Dose	Protection	Ref.
Thiourea	Mice	875 rad	Increased lifespan	31
Methylthiourea	Mice	650–700 rad	No effect on lifespan	32
Allylthiourea	Mice	700 rad	No effect on lifespan	8
Phenylthiourea	Mice	650–700 rad	No effect on lifespan	32
α-Naphthylthiourea	Mice	200 rad	No effect on lifespan	33
Ethyl isothiourea	Mice	800 rad	Increased lifespan	34
Sulfanilylthiourea	Mice	900 rad	Increased lifespan	35
Guanylthiourea	Mice	900 rad	Increased lifespan	35
Thiourea	Human erythrocytes	84 krad	Protection	12
Thiourea	T_2 phage	1000 rad/s	Protection	13
Thiourea	Ehrlich ascites cells	4000 rad	Protection	14
Allylthiourea	Human erythrocytes	84 krad	Protection	12

Table II—Ionization Constants [a]

Compound	pK_a
Thiourea	2.54[b]
N-Methylthiourea	3.06
N,*N*′-Dimethylthiourea	2.98
N-Methyl-*N*′-phenethylthiourea	3.12
N-Methyl-*N*′-phenyl-2-propylthiourea	3.04
2-Imidazolidinethione	3.08
2-Thiobarbituric acid	4.15
Thioacetamide	3.34

[a] Determined in 95% ethanol at 25°C. [b] Literature value (H_2O) is 2.03: T. J. Lane, J. A. Ryan, and J. L. Walter, *J. Am. Chem. Soc.*, **78**, 5560 (1956).

presence of 0.0005 mol of divalent metal salt or 0.00033 mol of trivalent metal salt.

Volumes of 50 mL of the same quantities of the metal salts were also titrated with 0.01 M KOH. The pH readings were recorded 2 min after each addition of titrant to allow equilibrium to be reached. Solvent concentration at the end of the titrations (if all 10 additions were made) was ~72% ethanol.

Calculations were performed as previously described (17) with a computer. The log K values obtained for the divalent metal complexes are recorded in Table III; values for the trivalent metal complexes are recorded in Table IV. Values for K_1, K_2, and K_3 were obtained from Eqs. 1–3, according to Flood and Loras (22) and Albert (23):

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

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

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

where $\bar{n}$ is the average number of ligand molecules bound by a metal ion at any stage in complex formation and $[L^-]$ is the concentration of the free chelating species.

Formation curves were plotted ($\bar{n}$ *versus* $-\log L^-$) to show whether stepwise complexation may have taken place. No steps were shown in the plots, probably because of the closeness of the log K values.

RESULTS AND DISCUSSION

The K_1 values for the Al(III) and Fe(III) complexes were not uncovered, with the exception of the ferric complex of thioacetamide, possibly because of lack of stepwise complexation (no values for $\bar{n}$ below 1 were obtained). No values for the ferric complex of 2-thiobarbituric acid were obtained because of precipitate formation at the beginning of the titration. Values for K_2 for the Cu(II) and Ni(II) complexes were not obtained because of the formation of precipitates, with the exception of the complexes of 2-thiobarbituric acid. Although log β values could not be obtained in most cases, for the purposes of comparison and for determining whether thiourea complexes are capable of existence in the presence of cellular complexing agents, the constants found should suffice.

The sequence of stability constants of thiourea, *N*-methylthiourea, and *N*,*N*′-dimethylthiourea allow some conclusions regarding structure of the complexes. The complexes of *N*-methylthiourea showed decreased stability constants, compared with those of thiourea, for the aluminum and ferric systems, but increased constants for the cupric and nickel systems. For the latter systems, where only K_1 values were observed, steric effects of the methyl group would be minimal. For the aluminum and ferric systems, the decrease in stability constants may be attributed to steric effects, since 2:1 and 3:1 complexes are involved. These sequences suggest that both the sulfur and a nitrogen atom are involved in bond formation of the complexes.

With the complexes of *N*,*N*′-dimethylthiourea, the additional methyl group caused a decrease in constants for all four metal ions observed. Two methyl groups apparently cause steric hindrance of the 1:1 complexes as well. The longer-chain substituents gave somewhat higher constants than the methyl groups, so the α-methylene groups were less interfering than the methyl groups. For the cyclic thioureas, particularly 2-thiobarbituric acid, where steric hindrance would be less than for the open-chain complexes, the stability constants were the largest of the series. It is also possible that some degree of aromaticity is possible with 2-thiobarbituric acid.

Table III—Stability Constants for Cu(II) and Ni(II) Complexes (25°C)

Compound	Cu(II) Complexes[a]			Ni(II) Complexes		
	log K_1	log K_2	log β_2	log K_1	log K_2	log β_2
Thiourea	1.38[b]			1.00		
N-Methylthiourea	1.67			1.39		
N,*N*′-Dimethylthiourea	1.35			1.33		
N-Methyl-*N*′-phenethylthiourea	1.51			1.47		
N-Methyl-*N*′-phenyl-2-propylthiourea	1.44			1.45		
2-Imidazolidinethione	1.66			1.40		
2-Thiobarbituric acid	4.35	3.79	8.14	3.76	3.18	6.94
Thioacetamide	2.30			1.69		

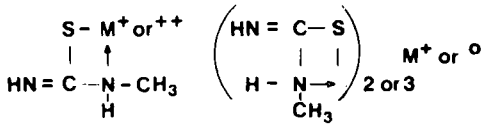
[a] The product of Cu(II) and thiourea is claimed to be a Cu(I) complex: E. I. Onstott and H. A. Laitinen, *J. Am. Chem. Soc.*, **72**, 4724 (1950). [b] The log β_2 for Cu(II) and thiourea was estimated by Bjerrum to be ~2: L. G. Sillén and A. E. Martell, "Stability Constants of Metal-Ion Complexes," The Chemical Society, London, 1964, p. 359.

Table IV—Stability Constants for Al(III) and Fe(III) Complexes (25°C)

Compound	Al(III) Complexes		Fe(III) Complexes			
	$\log K_2$	$\log K_3$	$\log K_1$	$\log K_2$	$\log K_3$	$\log \beta_3$
Thiourea	3.01	1.69		3.55	2.16	
N-Methylthiourea	2.73	1.90		3.00	2.16	
N,N'-Dimethylthiourea	2.61	1.81		2.87	2.28	
N-Methyl-*N'*-phenethylthiourea	3.79	2.21		3.59	2.41	
N-Methyl-*N'*-phenyl-2-propylthiourea	3.59	2.21			2.84	
2-Imidazolidinethione	3.61	2.12		3.92	2.40	
2-Thiobarbituric acid	5.15	3.29				
Thioacetamide	3.06	2.23	4.12	3.40	2.25	9.77

It has been shown previously (17) that stability constants for metal complexes of 2-mercaptoimidazoles and mercaptopyrimidines were relatively high, and that both sulfur and nitrogen were involved in bond formation to give four-membered rings. Four-membered metal chelate rings have also been reported for dithiocarbamates and xanthates (24), and for dithiocarboxylates (25). Ruthenium complexes of thioureas have also been postulated to involve the sulfur and nitrogen in four-membered rings (26).

If complexation to sulfur alone were involved, the presence of *N*-methyl substituents would be expected to increase stability constants by electron release. But the probable effect of steric hindrance by the methyl groups, as indicated for the decreased stability of 2:1 and 3:1 Al(III) and Fe(III) complexes, allow the postulation of four-membered chelate rings involving bonding to both sulfur and nitrogen (IV and V).



IV V

The magnitude of the metal-binding stability constants observed is somewhat lower than that for simple peptides. Log K_1 values reported for the Cu(II) and Ni(II) complexes of glycyl-DL-alanine, for instance, are 5.92 and 4.08, respectively (27). Although metal complexes of thioureas, with the possible exception of cyclic thioureas, should not be expected to exist in the presence of cellular peptides for any appreciable period, they should be capable of binding to the metal constituents of metalloenzymes.

Wheeler and Ribot (28) observed the protective effect of thiourea and methylthioureas for radiation damage to a synthetic polymer. Presence of the methyl groups distinctly lowered the protective ability of thiourea. It would not appear in this system that metal ions are involved in the radiation damage, which must be due largely to the radicals resulting from radiolysis of water. In this case, the ability of the thioureas to act similarly to the thiol radiation-protectors, in transferring hydrogen atoms to the radiation-produced radicals (29), would appear to offer a more probable mechanism of protection. The ability of thioureas to enter a thione–thiol equilibrium would make hydrogen atom transfer a realistic possibility, and in view of the rather low metal-binding stability constants for thioureas, a more likely mechanism than the ability to bind copper or iron ions, which catalyze cellular oxidations. A previous attempt to relate metal-binding ability to radiation protection of a series of aminoalkyl disulfides and thiosulfates did not give a positive correlation (30).

Involvement of thioureas with metalloenzymes, such as dopamine-β-oxidase, nitrate reductase, or polyphenol oxidase, is still a likely possibility by complexation of the metal constituent, however. A correlation between metal-binding ability and the antimicrobial effects of cyclic thioureas has already been observed (17), possibly through the inhibition of metalloenzyme activity.

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