Biochemistry VOLUME 3, NUMBER 6

June 11, 1964

Metal Chelates of Some Sulfur-containing Amino Acids*

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Received October 21, 1963

Acid-dissociation constants of DL-methionine, *S*-methyl-L-cysteine, DL-ethionine, L-cysteine, and DL-penicillamine, and the chelate-stability constants with Ag(I), Ca(II), Cd(II), Co(II), Cu(II), Hg(II), Mg(II), Mn(II), Ni(II), Pb(II), Sr(II), and Zn(II) are reported for 25° and 0.10 M ionic strength. L-Cysteine and DL-penicillamine are terdentate with respect to Cd(II), Hg(II), and Pb(II), and bidentate with the other metals, excepting Ag(I). All ligands are monodentate toward Ag(I); penicillamine forms only a protonated Ag(I) complex, and the cysteinate-Ag(I) complex precipitates. DL-Methionine, *S*-methyl-L-cysteine, and DL-ethionine are bidentate toward all other metals. There was no evidence of chelate formation of any of the ligands with the alkaline earths.

Although chelate stability constants have been published for many metal ions with methionine and cysteine, many different methods and varying temperatures and ionic strengths have been employed. Because of the biological importance of these ligands, several of the hitherto-reported metal-stability constants have been redetermined and those of several new metal chelates have now been measured at constant temperature and ionic strength.

There has been one investigation of the chelation constants of DL-penicillamine (β-mercaptovaline) by Kuchinskas and Rosen (1962). Their findings have been examined and the interactions with several additional metal ions have been investigated. Penicillamine has been known as a degradation product of penicillin since 1943 (Abraham *et al.*, 1943). However, its use in recent years as a drug in treating Wilson's disease (hepatolenticular degeneration) (Walsh, 1960; Osborne and Walsh, 1958; Walsh, 1956a,b), as a protective agent against radiation (Langendorff *et al.*, 1958), and as an antidote in Pb, Tl, and Hg poisoning (Aposhian, 1960; Stavinoha *et al.*, 1959; Harris, 1958) warrant the examination of its metal-chelating properties.

Ethionine was found to be a methionine antagonist (Dyer, 1938; Roblin *et al.*, 1945) in 1938 and, relatively recently, to inhibit leukemia in mice (Higgins, 1956). A comparison of the metal-chelate stabilities between ethionine and methionine might tend to partially clarify the reason for the effectiveness of ethionine as an antimetabolite.

S-Methyl-L-cysteine has been isolated from *Phaseolus vulgaris* (Thompson *et al.*, 1956). It has been suggested that this amino acid is the precursor of the corresponding sulfoxide, which has been found in turnip roots (Morris and Thompson, 1956), and that they may be metabolically interconvertible (Thompson *et al.*, 1956).

* This investigation was supported by a research grant (GM-10834) from the National Institutes of Health, U. S. Public Health Service.

Experimental

Materials.—*S*-Methyl-L-cysteine, DL-ethionine, DL-penicillamine, and L-cysteine hydrochloride monohydrate were Mann Analyzed chemicals purchased from Mann Research Laboratories, New York. DL-Methionine was a synthetic sample from U. S. Industrial Chemicals, Inc. Satisfactory standardization was accomplished by potentiometric titration, and the compounds were therefore used without further purification. Metal-ion solutions were prepared from Fisher Analyzed Reagent grade metal nitrates except for Mn(II) and Hg(II) which were obtained as the sulfate and chloride, respectively. The metal salt solutions were standardized by titration with EDTA[1] using standard procedures (Schwarzenbach, 1956; Welcher, 1958).

Potentiometric Measurements.—Measurements of hydrogen-ion concentration were carried out in a 100-ml jacketed titration cell which was fitted with a magnetic stirrer and a tightly fitting rubber stopper through which were inserted nitrogen inlet and outlet tubes, microburet delivery tube, and glass and calomel extension electrodes. A Beckman Model GS *p*H meter was used to determine hydrogen-ion concentration. The electrodes were calibrated by comparing the meter reading during the titration of acetic acid with the theoretical hydrogen-ion concentration calculated from the data tabulated by Harned and Owen (1950). In the *p*H regions below 3.5 and above 10.5 the *p*H meter was calibrated by adding known amounts of hydrochloric acid and sodium hydroxide, respectively. All potentiometric measurements were made at 25.05 ± 0.05° and at an ionic strength of 0.10 M in potassium nitrate. The concentration of ligand in the cell was 0.003 M.

Calculations.—All calculations were made by the IBM 1620 computer at the computation center of Illinois Institute of Technology, using programs written by the author (G.R.L.). To insure accuracy, indi-

[1] Abbreviation used in this work: EDTA, ethylenediaminetetraacetic acid.

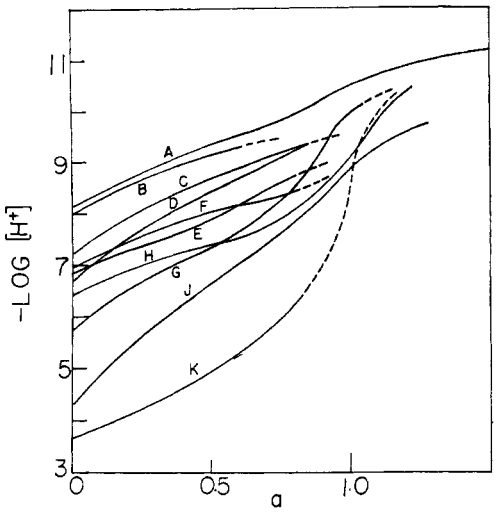


FIG. 1.—Potentiometric equilibrium curves for 1:1 and 2:1 molar ratios of methionine to metal ion. A, Free ligand; B, 2:1 Mn(II); C, 2:1 Cd(II); D, 2:1 Co(II); E, 2:1 Pb(II); F, 2:1 Zn(II); G, 2:1 Ni(II); H, 1:1 Ag(I); J, 2:1 Hg(II); K, 2:1 Cu(II). t = 25°, μ = 0.10 (KNO_3); a = moles of base added per mole of ligand present.

vidual spot calculations were made for each new computer program.

ACID-DISSOCIATION CONSTANTS.—Each of the acidic groups of the ligands, the protonated amino and the mercapto groups, gives rise to a separate buffer region, thus facilitating the computations. If K_1^H is defined as 1,

$$K_1^H = \frac{[H^+][L^-]}{[HL]} \quad (1)$$

If T_L represents the total concentration of the ligand in solution, and a represents the number of equivalents of base added in the particular buffer region under consideration, then it follows that

$$T_L = [HL] + [L^-] \quad (2)$$

$$aT_L + [H^+] - [OH^-] = [L^-] \quad (3)$$

Solving equations (2) and (3) for $[L^-]$ and [HL], and substituting into equation (1), one obtains

$$K_1^H = \frac{[H^+](aT_L + [H^+] - [OH^-])}{T_L - (aT_L + [H^+] - [OH^-])} \quad (4)$$

which may be evaluated at each point in the buffer region.

EQUIMOLAR METAL-LIGAND CURVES; MONOBASIC LIGANDS.—If K_1 is defined as

$$K_1 = \frac{[ML^{+n-1}]}{[M^{+n}][L^-]} \quad (5)$$

with T_M defined as total metal in solution, then

$$T_L = [ML^{+n-1}] + [HL] + [L^-] \quad (6)$$

$$T_M = [ML^{+n-1}] + [M^{+n}] \quad (7)$$

$$aT_L + [H^+] - [OH^-] = [L^-] + [ML^{+n-1}] \quad (8)$$

Solving equations (1) and (8) for $[L^-]$ gives:

$$[L^-] = \frac{([1 - a]T_L - [H^+] + [OH^-])K_1^H}{[H^+]} \quad (9)$$

Solving equations (1), (6), and (7) for $[M^{+n}]$ and $[ML^{+n-1}]$ and substituting into equation (5) gives

$$K_1 = \frac{T_L - [L^-]X}{[L^-]^2X} \quad (10)$$

where $X = 1 + [H^+]/K_1^H$.

EQUIMOLAR METAL-LIGAND CURVES; DIBASIC LIGANDS.—The formation constants for a dibasic ligand were calculated by programming the equations according to Richardson *et al.* (1959).

2:1 MOLAR RATIO OF LIGAND TO METAL; MONOBASIC LIGANDS.—If K_2 is defined as

$$K_2 = \frac{[ML_2^{+n-2}]}{[L^-][ML^{+n-1}]}$$

then, solving as in the preceding section,

$$K_2 = \frac{T_M - [L^-]X}{[L^-]^2X} - \frac{T_L - [L^-]X}{K_1[L^-]^3X}$$

Fitting the equation by least squares gives K_2 as the intercept and $-1/K_1$ as the slope.

2:1 MOLAR RATIO OF LIGAND TO METAL; DIBASIC LIGANDS.—The formation constants for the dibasic ligands were calculated by programming and fitting to a least squares analysis the equation developed by Gustafson *et al.* (1959).

RESULTS

Acid-Dissociation Constants.—Table I gives the values of $-\log K^H$ obtained for the different ligands and literature values where available. The dissociation constants determined in this work were used throughout the calculations of stability constants, as these values were determined at an ionic strength of μ = 0.10 (KNO_3) and 25°, the same as the conditions used for the ligand-metal systems.

TABLE I
DISSOCIATION CONSTANTS OF LIGANDS[a]

Ligand	K_2^H(-NH_3^+)[b]	K_3^H(-SH)[b]
DL-Methionine	9.04	
	9.10[c]	
S-Methyl-L-cysteine	8.73	
	8.75[d]	
DL-Ethionine	9.02	
	8.60[e]	
L-Cysteine	8.13	10.11
	8.36[f]	10.28[f]
DL-Penicillamine	7.88	10.43
	7.97[g]	10.46[g]

[a] t = 25°; μ = 0.10 M (KNO_3). [b] The constants given are "macroscopic" equilibrium constants, rather than "microscopic" constants for individual groups, since the former may be used directly in the calculation of metal chelate stability constants. [c] Li and Manning (1955); μ = 0.15. [d] Grafius and Nielands (1955); μ = 0.16. [e] Ratner and Clarke (1933); μ = 0.1. [f] Albert (1952); μ = 0.1. [g] Kuchinskas and Rosen (1962); μ = 0.15.

As can be seen from a comparison of the pK_a values of methionine and ethionine, the substitution of an *S*-ethyl for an *S*-methyl group in homocysteine has virtually no effect on the pK, because of its distance from the ionization site. On the other hand, a comparison of cysteine and penicillamine (which may be considered as β,β-dimethylcysteine) shows a considerable decrease in acidity. The decrease in acidity of penicillamine may be due to the two methyl groups which lead to possible steric hindrance of the solvating water molecules.

Chelate-Stability Constants.—The values of the formation constants, log K_1 and K_2, are collected in Table II, together with any literature values available. These constants were calculated from potentiometric curves illustrated in Figures 1 through 5.

The titration of a 2:1 molar ratio of ligand to metal displaces one proton from the monobasic amino acids, giving an inflection in the titration curve at a = 1,

TABLE II
FORMATION CONSTANTS AT 25°[a]

Metal Ion	Methionine K_1	Methionine K_2	S-Methyl-L-cysteine K_1	S-Methyl-L-cysteine K_2	Ethionine K_1	Ethionine K_2	L-Cysteine K_1	L-Cysteine K_2	Penicillamine K_1	Penicillamine K_2
Ag(I)	3.17		5.25		5.25		[b]		12.42[c]	
Ca(II)	[d]		[d]		[d]		[d]		[d]	
	−0.66[e]									
Cd(II)	3.67	3.36	3.77	3.32	4.68	4.54	[b]		10.88	
	3.88[f]	3.11							11.4[g]	7.1
Co(II)	4.12	3.44	4.12	3.49	5.13	4.34	[h]		[h]	
	β_2[i] 7.9[j]						9.3[k]	7.6		
Cu(II)	7.87	6.85	7.88	6.84	8.43		[l]		[l]	
	β_2[i] 14.7[j]								16.5[g]	
Hg(II)	6.52	4.93	7.20	5.81	7.25	5.92	14.21		16.15	
							β_2[i] 43.56[m]		17.5[g]	6.0
Mg(II)	[d]		[d]		[d]		[d]		[d]	
							<4[k]			
Mn(II)	2.77	1.80	2.52	1.75	[b]		4.56		[b]	
							4.1[k]			
Ni(II)	5.19	4.65	5.26	4.56	6.15	5.18	9.64	9.40	11.11	10.68
							β_2[i] 19.3[k]		11.4[g]	10.9
Pb(II)	4.38	4.24	4.43	3.54	[b]		11.39		12.37	
	4.40[f]						12.20[f]		13.0[g]	4.3
Sr(II)	[d]		[d]		[d]		[d]		[d]	
Zn(II)	4.37	3.96	4.46	4.06	5.22	5.14	9.04	8.50	9.51	9.00
	4.38	4.09[f]					9.86[f]	8.84	10.0[g]	8.9

[a] μ = 0.10 (KNO_3). [b] Precipitation. [c] For the protonated complex [AgHL]/([Ag^+][HL^-]). [d] No evidence of chelate formation. [e] Schubert (1954). [f] Li and Manning (1955). [g] Kuchinskas and Rosen (1962). [h] Nonreproducible titrations. [i] $\beta_2 = K_1K_2$. [j] Albert (1950). [k] Albert (1952). [l] Redox measurements. [m] Stricks and Kolthoff (1953).

TABLE III
COMPARISON OF METAL-CHELATE STABILITY CONSTANTS OF AMINO ACIDS WITH AND WITHOUT MERCAPTOALKYL GROUPS

	Cu(II)	Hg(II)	Ni(II)	Ag(I)	Pb(II)	Zn(II)	Co(II)	Cd(II)	Mn(II)
Aspartic acid	8.57[a]		7.12[a]			5.84[a]	5.90[a]	4.37[a]	3.74[b]
Alanine	8.13[c]		5.96[d]	3.64[d]	5.00[d]	5.16[c]	4.83[c]	4.2[e]	3.24[c]
Ethionine[f]	8.43	7.25	6.15	5.25		5.22	5.13	4.68	
S-Methyl-cysteine[f]	7.88	7.20	5.26	5.25	4.43	4.46	4.12	3.77	2.52

[a] Chaberek and Martell (1952b). [b] Kroll (1952). [c] Maley and Mellor (1949, 1950). [d] Monk (1951). [e] Perkins (1954). [f] This investigation.

thus indicating the formation of a 2:1 complex. In the case of silver(I), titration of a 1:1 molar ratio gave an inflection at $a = 1$, indicating a 1:1 complex.

In the case of the dibasic ligands, the metals nickel(II) and zinc(II) displace two protons per ligand in systems containing a 2:1 ligand-metal ratio. Thus a simple 2:1 chelate is formed. However when a 2:1 molar ratio of ligand to the metals cadmium(II), mercury(II), and lead(II) is titrated, a steep inflection at $a = 1$ is observed. An examination of the 1:1 curve (Figs. 4 and 5) shows the displacement of the two acidic protons of the ligands, indicating that the ligands are probably terdentate. This had previously been observed in the case of the lead(II)-cysteinate system by Li and Manning (1955).

With silver(I) and DL-penicillamine, the titration of a 1:1 ratio gives an inflection at $a = 1$ (Fig. 5), corresponding to the formation of a 1:1 complex. This first buffer region is followed by a second buffer region extending to $a = 2$. As the first buffer region is the formation of a simple 1:1 complex, the second buffer region might be the dimerization or polymerization of the initially formed complex. This phenomenon has been extensively investigated by Martell *et al.* (1957), Courtney *et al.* (1959). The possibility of dimerization or polymerization is currently being investigated.

The dibasic acids, cysteine and penicillamine, have a free sulfhydryl group each. As a consequence of the well-known reaction of copper with free sulfhydryl groups (Albert, 1952; Klotz *et al.*, 1958; Kolthoff and Stricks, 1951) to form cuprous cysteinate which disproportionates to form cysteine and cuprous ion, the interactions of copper(II) ion with cysteine and penicillamine (β,β-dimethylcysteine) were not studied.

DISCUSSION

A comparison of the metal stability constants of the monobasic acids collected in Table II shows a decrease in stability in the order Cu(II) > Hg(II) > Ni(II) > Zn(II) > Pb(II) > Co(II) > Cd(II) > Mn(II). Comparison of the values for aspartic acid and alanine (Table III) indicates that the same order prevails. Since the values for ethionine and S-methyl-L-cysteine are in the same range as those for the simple bidentate amino acid α-alanine, it would appear that there is little or no involvement of substituted mercapto groups in chelation.

This conclusion is further borne out if the differences between K_1 and K_2 (Δ's) are compared. Iminodiacetic and iminodipropionic acids have been shown to be terdentate (Chaberek and Martell, 1952a) and it was shown that it is possible to correlate Δ with whether the ligand was bi- or terdentate (Chaberek and Martell, 1952a,b). The values of Δ are collected in Table IV. It may be seen that the values of Δ

TABLE IV
VALUES OF LOG K_1 − LOG K_2 FOR METAL CHELATES OF BIDENTATE AND TERDENTATE CHELATES

	Iminodi-acetic Acid[a]	Iminodi-propionic Acid[a]	Alanine[b]	Ethionine[c]	*S*-Methyl-L-cysteine[c]	Methionine[c]
Cu(II)	4.90	5.68	1.52		1.04	1.02
Ni(II)	1.86	2.37	1.26	0.97	0.70	0.54
Co(II)	1.61	1.66	0.88	0.79	0.63	0.68
Zn(II)	1.89		0.84	0.07	0.40	0.41
Cd(II)	1.17			0.14	0.45	0.29

[a] Chaberek and Martell (1952a,b). [b] Perkins (1954). [c] Present investigation.

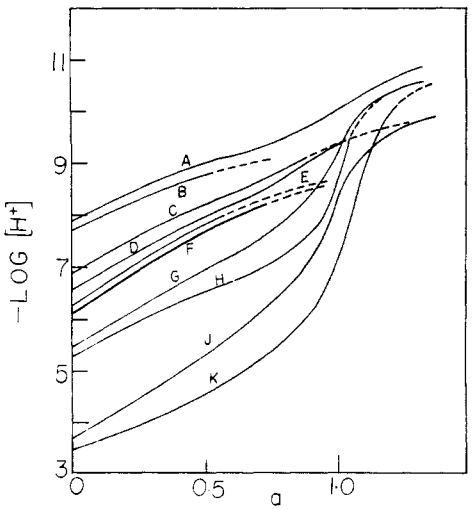


FIG. 2.—Potentiometric equilibrium curves for 1:1 and 2:1 molar ratios of *S*-methyl-L-cysteine to metal ion. A, Free ligand; B, 2:1 Mn(II); C, 2:1 Cd(II); D, 2:1 Co(II); E, 2:1 Pb(II); F, 2:1 Zn(II); G, 2:1 Ni(II); H, 1:1 Ag(I); J, 2:1 Hg(II); K, 2:1 Cu(II). t = 25°; μ = 0.10 (KNO_3); a = moles of base added per mole of ligand present.

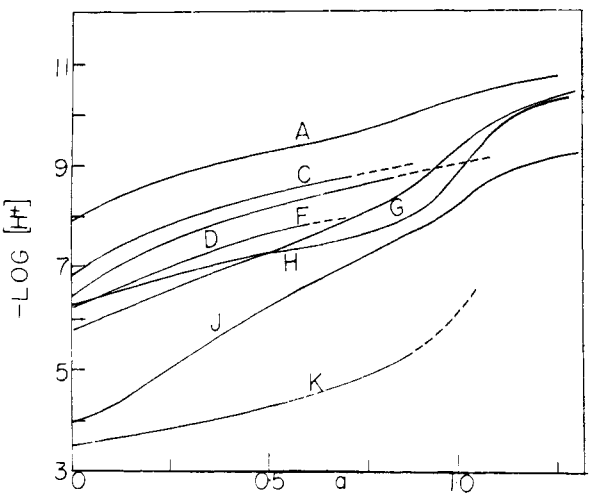
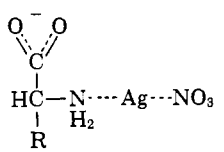


FIG. 3.—Potentiometric equilibrium curves for 1:1 and 2:1 molar ratios of ethionine to metal ion. A, Free ligand; C, 2:1 Cd(II); D, 2:1 Co(II); F, 2:1 Zn(II); G, 2:1 Ni(II); H, 1:1 Ag(I); J, 2:1 Hg(II); K, 1:1 Cu(II). t = 25°; μ = 0.10 (KNO_3); a = moles of base added per mole of ligand present.

are comparable to those of the bidentate alanine, rather than to those of iminodiacetic and iminodipropionic acids, showing that the S-substituted sulfur-containing amino acids are bidentate.

Silver(I) ion is the only anomalous metal in the series studied. From basicity and structural observations it would appear that the Ag(I) chelates of methionine and ethionine would have similar formation constants. This is seen not to be the case, however, while the corresponding values of the less similar *S*-methyl-L-cysteine and ethionine chelates are equivalent. Although a satisfying explanation cannot be advanced to account for this, something can be said about the structures of these chelate compounds. Coordination of the Ag(I) ion must occur via monodentate binding by the ligand, through the unshared pair of electrons of the amino nitrogen atom. Further chelation through either the sulfur or a carboxylate oxygen is sterically impossible. Also, since the Ag(I) ion has considerable affinity for nitrate ion in solution (Sidgwick, 1950), the structure of the complex in the electrolyte medium employed may be represented as I:



I

It is known that ethionine is a methionine antagonist (Walsh, 1960; Osborne and Walsh, 1958; Walsh, 1956a,b). A possible explanation might arise from examination of the stability constants which are an average of one log value higher than those of methionine. Since many of the biological reactions are carried out on substrates bound to an enzyme surface through metal coordination (Williams, 1959), the higher stability constant and the similar structure of ethionine will allow it to compete successfully with methionine at an enzyme surface.

The dibasic ligands, cysteine and penicillamine, are either bi- or terdentate depending on the metal ion used in the titration. With nickel(II) and zinc(II), an inflection is observed at a = 2 in the 2:1 ligand-metal ratio. This indicates the formation of a 2:1 complex. Since the ligands are potentially bi- or terdentate, the nickel complex may be either square planar or octahedral. It has been shown that the structure of nickel(II) complexes is indicated by the magnetic moment (Pauling, 1948). The color of the complex may alternatively be used to indicate structure, apparently because of its relationship to magnetic moment (McKenzie *et al.*, 1944; Manch and Fernelius, 1961). Thus octahedral complexes absorb in the yellow-red portion of the spectrum, while square planar complexes absorb in the blue-green portion. The appearance of an orange color and a pink color during the titration of nickel with penicillamine and cysteine, respectively, therefore indicates the formation of square planar complexes involving the mercaptide and amino groups as illustrated by II.

Silver(I) shows an interesting equilibrium in the presence of penicillamine. Potentiometric titration (Fig. 5) shows a one-equivalent buffer region, followed

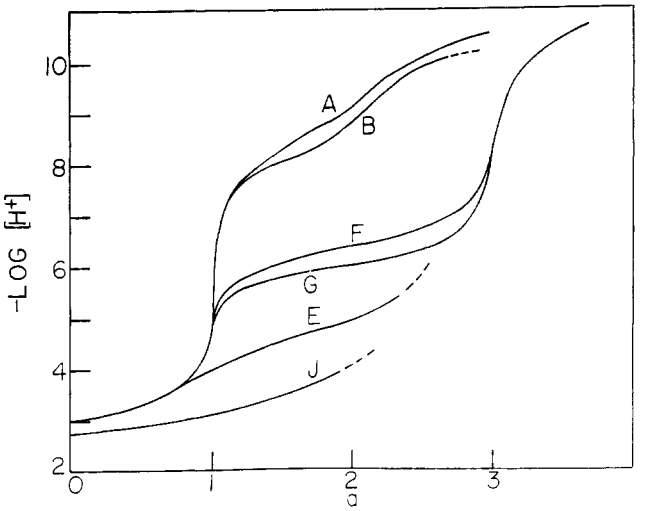


FIG. 4.—Potentiometric equilibrium curves for 1:1 and 2:1 molar ratios of L-cysteine hydrochloride to metal ion. A, Free ligand; B, 2:1 Mn(II); F, 2:1 Zn(II); G, 2:1 Ni(II); E, 1:1 Pb(II); J, 1:1 Hg(II). t = 25°; μ = 0.10 (KNO_3); a = moles of base added per mole of ligand present.

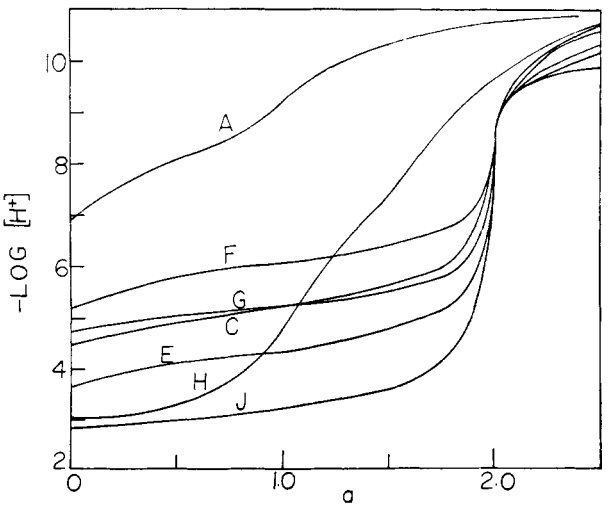
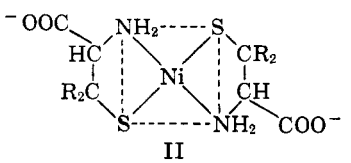


FIG. 5.—Potentiometric equilibrium curves of 1:1 and 2:1 molar ratios of penicillamine to metal ion. A, Free ligand; F, 2:1 Zn(II); G, 2:1 Ni(II); C, 1:1 Cd(II); E, 1:1 Pb(II); H, 1:1 Ag(I); J, 1:1 Hg(II). t = 25°; μ = 0.10 (KNO_3); a = moles of base added per mole of ligand present.



II

(cysteine, R = H; penicillamine, R = CH_3)

by a second, sloping, one-equivalent buffer region. The first buffer region corresponds to the formation of a 1:1 protonated complex with the penicillamine, which functions here as a monodentate ligand coordinating through the mercapto group. This structure is assigned because of the large difference between the formation constants for the S-substituted sulfur acids, where complexing is through the nitrogen, and that of penicillamine ($\Delta \cong$ 7–9 log units). The second buffer region indicated either hydrolysis of the complex (eq. 1) or hydrolysis with dimerization or polymerization, as indicated by the following equilibria:

$$RSAg + OH^- \rightleftharpoons RSAgOH^-$$

$$2\ RSAg + 2\ OH^- \rightleftharpoons (RSAgOH^-)_2$$

Although similar reactions would be expected to form with cysteine, they could not be investigated because of the formation of precipitates.

The titration of cysteine and penicillamine with the metal ions Hg(II), Cd(II), and Pd(II) gives an inflection at one equivalent in solutions containing 2:1 ligand-metal ratios. This indicates that the ligands are probably terdentate in these complexes. This conclusion was further substantiated when a 1:1 molar ratio was titrated and was found to give inflections at two equivalents of base. A similar conclusion concerning the terdentate nature of these ligands was made by Li and Manning (1955) for the lead(II) cysteine chelate.

A previous investigation of the metal chelates of penicillamine with the same metal ions as were used in this investigation has been reported by Kuchinskas and Rosen (1962). Although their experimental results (i.e., titration curves) agree with this investigation they concluded that penicillamine acts as a bidentate ligand towards all metal ions and calculated K_2 values from the second buffer region of their titration curve. These parts of the titration curves are actually composites of the free ligand and the 1:1 curves, and no 2:1 complexes are formed. There was no mention in their report (Kuchinskas and Rosen, 1962) of any 1:1 ligand-metal titrations having been carried out. The values they reported for nickel(II) and zinc(II) are, however, in substantial agreement with the present research.

Kuchinskas and Rosen (1962) also report a value for the formation constant of copper(II) with penicillamine. This system was not investigated by us due to the well-known reaction of copper with mercaptans (Albert, 1952; Klotz *et al.*, 1958; Kolthoff and Stricks, 1951) according to the equation:

$$2\ RSH + Cu(II) \rightleftharpoons {}^1/_2\ RSSR + RS - Cu(I) + 2\ H^+$$

The finding that lead(II) and mercury(II) form 1:1 chelates of exceptional stability, with penicillamine, shows why the ligand is effective as an antidote for lead and mercury poisoning (Aposhian, 1960; Stavinoha *et al.*, 1959; Harris, 1958; Aposhian, 1958). In these chelates the ligand binds three of four coordinating positions to form a stable soluble metal complex which stays in solution over the entire range studied (pH 2–11).

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Metal Complexes of Carnosine*

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Received November 11, 1963

Stability constants of complexes formed by carnosine and twelve metal ions are determined. Evidence is presented for the formation of chelates MHL^{2+} and ML^{+} from divalent metal ions and the ligand HL. The nickel(II) and copper(II) chelates further promote the ionization of a peptide hydrogen. The order of stability constants for the chelates ML^{+} is Cu(II) > Hg(II) > Ni(II) > Mn(II) > Cd(II) ~ Mg(II) ~ Ca(II) ~ Sr(II). With Co^{2+}, Pb^{2+}, and Zn^{2+}, precipitation permitted determination of only the protonated complex constant. Silver(I) gave an insoluble complex, while Ca(II), Cd(II), Mg(II), and Sr(II) formed only the normal chelate compound ML^{+}.

Since the dipeptide carnosine (β-alanyl-L-histidine) was extracted from muscle by Gulewitsch and Amiradzibi (1900a,b), considerable effort has gone into the elucidation of its biochemical function, but none has been found. However, Hanson and Smith (1949) found a protease in swine kidney and in rat liver, pancreas, and kidney, which attacks the peptide bond in carnosine and which was named carnosinase. They discovered that the enzyme was stabilized by the metals Mn(II) and Zn(II). This enzyme was highly purified by Rosenberg (1960a) and he confirmed the observations of Hanson and Smith in that the enzyme was stabilized by Mn(II). Carnosinase was found to be stabilized by the divalent metal ions in the order: Mn > Ca > Mg ~ Cd > Zn (Rosenberg, 1960b). It was also found that carnosinase was activated by the divalent metals in the order Mn > Cd > Zn > Co (Rosenberg, 1960c). To explain these results Rosenberg postulated the existence of two sites on the enzyme, one for stabilization, the other for activation. The nature of the interaction of stabilization is believed to be chelate-ring formation.

In view of the above observation involving activation and stabilization of the peptidase and advancement by Smith and co-workers (Smith, 1949a,b; Smith and Bergman, 1941; Smith and Lumry, 1949) of the theory that metal ions are chelated simultaneously to enzyme protein and substrate, a knowledge of the carnosine metal-formation constants is desirable.

Experimental

Reagents.—A sample of L-carnosine of specific rotation $[\alpha] = +21.3°$ in water, $c = 2$, was purchased from Mann Research Laboratories, New York. To the stock solution of L-carnosine was added exactly one equivalent of hydrochloric acid in order to form the protonated ligand. Metal-ion solutions were prepared from Fisher Reagent Grade metal nitrates, except in the case of manganese and mercury which were the sulfate and chloride, respectively. The metal solutions were standardized by titration with EDTA[1]

* This investigation was supported by a research grant (GM-10834) from the National Institutes of Health, U. S. Public Health Service.

[1] Abbreviations used in this work: EDTA, ethylenediaminetetraacetic acid.