Cadmium Binding by Biological Ligands. 2 [1]. Formation of Protonated and Polynuclear Complexes between Cadmium and 2-Mercaptoethylamine

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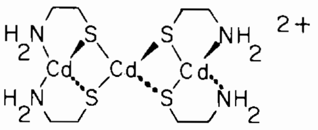
Extensive alkalimetric titrations of the cadmium 2-mercaptoethylamine system in aqueous solutions have revealed the presence of several protonated mononuclear and polynuclear metal complexes. Nearly 600 pH measurements (glass electrode) in 14 separate titrations, were collected with a computer-controlled titrator. The solutions [25 °C, 0.2 M (KNO_3) ionic strength] contained 1–4 mM cadmium nitrate and 3–8 fold excess of mercaptoethylamine. The functional behavior of the data was consistent with the equilibrium model employing the species $Cd(HL)_2^{2+}$, $Cd(HL)_3^{2+}$, $Cd(HL)_4^{2+}$, $Cd(HL)_2L^+$, CdL_2, $Cd_3(HL)_2L_2^{4+}$, *and* $Cd_3L_4^{2+}$, *where* L^- *is the deprotonated form of the ligand. Comparisons are drawn to some of the known properties of multi-cadmium binding proteins, called metallothioneins.*

Introduction

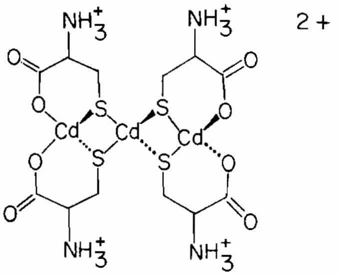
The solution coordination chemistry of cadmium with 2-mercaptoethylamine (mea) is largely unexplored. Aqueous solution studies of the reactions of Cd^{2+} with sulfhydryl ligands are frequently complicated by the tendency of the thiolate group to bridge metal ions into polynuclear frameworks, a subtlety frequently overlooked by early researchers. Such polynuclear aggregations have some biological importance, as exemplified by the multi-cadmium binding proteins, called metallothioneins [3, 4], whose metal structure has been partially elucidated by Otvos and Armitage [5, 6] using ^{113}Cd NMR. The ubiquitous proteins typically contain a single polypeptide chain (~6000 daltons) with 33% cysteine content and can bind up to seven cadmium and/or zinc ions. For each metal ion bound, three thiolate groups participate in coordination, some serving in metal-bridging capacity. The other strong metal-binding groups are lysine residues and their role in binding metal ions in metallothioneins is uncertain.

Felder *et al.* [7] reported the formation of the complexes $[CdL]^+$ and $[Cd(HL)]^{2+}$ in aqueous solution, where L^- represents the fully deprotonated form of mea. Li and Manning [8], using pH-metric techniques, proposed the stepwise formation of $[CdL]^+$ and $[CdL_2]$. Knoblock and Purdy [9] studied the reactions between Cd^{2+} and mea by polarography at pH 7.4 and reported an 'apparent' stability constant for a metal species containing two ligands. Jicha and Busch [10] isolated powdered samples of $[Cd(ML_2)_2][CdCl_4]$, M = Ni^{2+} or Cd^{2+}, and on the basis of absorption spectra, magnetic properties and molar conductance, proposed the trinuclear structure *I*.



I

Such trinuclear species have not been reported in solution for the Cd^{2+}/mea system. Shindo and Brown [11] isolated from low-pH solutions, a similar complex with cysteine and on the basis of infrared evidence proposed the solid-state structure *II*.



II

In the solid state, most cadmium complexes with sulfhydryl ligands are polynuclear and show considerable stereochemical diversity [12]. In solution, the stoichiometric compositions and the factors leading to the formation (or suppression) of polynuclear species have scarcely been studied and are poorly understood [13]. Leussing and Tischer [14] and Perrin and Sayce [15, 16] were the first to study by potentiometric techniques the formation of polynuclear complexes in solution between Zn^{2+} and sulfhydryl ligands. More recently, formations of cadmium polynuclear complexes have also been reported [1, 13, 17].

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As part of our continuing interest in the reactions of cadmium with biological ligands, we undertook a detailed potentiometric study of the Cd^{2+}/mea system, over a range of total concentrations. The applications of a new composition-of-species method [18], which is an extension of the so-called FICS ('free ion concentration in solution') analysis developed by Osterberg [19] and others [20–24], led us to propose several novel species, including $[Cd(HL)_3]^{2+}$, $[Cd(HL)_4]^{2+}$ and $[Cd_3(HL)_2L_2]^{2+}$. The possible significance of our interpretations is discussed in terms of metal binding by metallothioneins.

Experimental Section

Chemicals

An acidified cadmium nitrate (Matthey, "puratronic") stock solution was prepared and analyzed by alkalimetric EDTA titrations and Gran plots [25]. 2-Mercaptoethylamine hydrochloride (Aldrich) was used to prepare a ligand stock solution, the concentration of which was determined by alkalimetric titrations. All stock solutions were prepared and stored under moisture-saturated, continuously-purified nitrogen in an inert atmosphere box.

Titration Method

Acidified solutions containing variable amounts of Cd^{2+} and mea were prepared from the stock solutions and titrated with std. KOH, using a computerized titrator [1]. At all times the solutions were kept under nitrogen, due to the sensitivity of the ligand to air-oxidation. The Beckman 39501 combination glass electrode was calibrated by an empirical procedure [26]. The total ionic strength was adjusted to 0.2 *M* with KNO_3. The temperature was maintained at 25 °C. Nearly 600 pH measurements were collected in the pH range 3–11, in 14 separate titrations. The compositions of the titrated solutions are listed in Table I.

Since extended exposure of the pH electrode to the sulfur ligand caused sluggish responses in subsequent uses, the titrator was programmed to conduct each titration in 20 min or less [2]. Also, at the conclusion of each titration, the electrode was immersed in 5% sodium hypochlorite solution for 5 min.

Method of Calculation

The extensive FORTRAN library of routines, STBLTY [27], was used to reduce the data, develop the equilibrium model, and refine the equilibrium constants. Table II contains a glossary of terms used in the following descriptions.

TABLE I. Compositions of Titrated Solutions.

Set No.	Total concentrations (m*M*)				No. Pts.
	$Cd(NO_3)_2$	mea•HCl	HNO_3	KNO_3	
1	0	4.74	17.55	176.9	43
2	0	4.86	17.73	177.3	44
3	0	6.65	20.61	175.6	45
4	0	7.50	17.67	174.2	44
5	0	7.72	17.76	174.2	47
6	1.65	12.93	5.23	177.6	38
7	2.48	12.93	6.11	174.3	40
8	3.31	12.93	6.84	171.4	40
9	3.97	12.93	7.58	168.3	41
10	2.48	8.08	6.00	178.7	38
11	2.48	9.70	6.00	177.5	39
12	2.48	12.93	6.09	174.3	39
13	2.48	16.17	6.07	171.3	40
14	2.48	19.40	5.95	168.2	42

FICS (Free Ion Concentration in Solution) analysis

The concentrations of the free components can be deduced from the FICS relations (see Table II)

$$pM = pM_0 - \int_{pH_0}^{pH} (\partial H/\partial M)_{pH,L}\, dpH|_M \qquad (1)$$

$$pL = pL_0 - \int_{pH_0}^{pH} (\partial H/\partial L)_{pH,M}\, dpH|_L \qquad (2)$$

The integrand functions can be constructed from observed data by a procedure described elsewhere [1, 18, 24]. The above values of pM and pL are determined without any assumptions regarding the identities of the complexes present in solution.

ACSS (Average Composition of Species in Solution) analysis

Using the free concentrations determined by the FICS procedure, it is possible to determine the average stoichiometries of the metal-containing complexes from the relations [18]

$$\bar{e}_m' = (M - m)/(S - lh/K_{a2} - lh^2/K_{a1}K_{a2}) \qquad (3)$$

$$\bar{e}_l' = (L - l - lh/K_{a2} - lh^2/K_{a1}K_{a2})/(S - lh/K_{a2} - lh^2/K_{a1}K_{a2}) \qquad (4)$$

$$\bar{e}_h' = (H - lh/K_{a2} - 2lh^2/K_{a1}K_{a2} - h + K_w'/h)/(S - lh/K_{a2} - lh^2/K_{a1}K_{a2}) \qquad (5)$$

where K_{a1} and K_{a2} are the acid dissociation constants of the ligand. The Sillen 'complexity sum', *S* (see Table II), is calculated by an integration technique [18]. The coefficient $\bar{e}_m'$ refers to the number of metal atoms in a complex at a particular pH. If more

TABLE II. Glossary of Terms.

C_j	concentration of the jth associated species: $C_j = [M_{e_{mj}}L_{e_{lj}}H_{e_{hj}}] = \beta_j m^{e_{mj}} l^{e_{lj}} h^{e_{hj}}$
e_{kj}	stoichiometric coefficient, referring to the number of kth type of atoms in the jth associated species. For example, for the jth species $Cd(pen)_2(OH)$, $e_{mj} = 1$, $e_{lj} = 2$, $e_{hj} = -1$. The value of e_{hj} is negative to signify a hydroxide. Positive values refer to hydrogen atoms.
$\bar{e}_k'$	average stoichiometric coefficient of the kth reactant in *metal-containing* species. In more standard (but less general) nomenclature, $\bar{e}_m' = \bar{q}$, $\bar{e}_l' = \bar{p}$ and $\bar{e}_h' = \bar{r}$; these have been called the 'Mesak' coefficients [34].
H	total hydrogen excess, defined as $A - B + 2L$, where $A = [HNO_3]$, $B = [KOH]$. The factor in front of L refers to the two dissociable protons introduced by the ligand. $H^{calc} = h - K_w'/h + \Sigma_j^N e_{hj}C_j$
h	free hydrogen in concentration, $[H^+]$.
oh	free hydroxide in concentration, $[OH^-]$.
K_w'	$[H^+][OH^-]$, 1.78×10^{-14} at 25 °C and 0.2 M ionic strength.
L	total concentration of the ligand. $L^{calc} = l + \Sigma_j^N e_{lj}C_j$.
l	concentration of the unassociated (and deprotonated) ligand.
M	total concentration of the metal. $M^{calc} = m + \Sigma_j^N e_{mj}C_j$.
m	concentration of the free metal.
$\bar{n}$	$= [L - (H - (h - oh))/\bar{n}_h]/M$. Bjerrum formation function.
$\bar{n}_h$	$= \Sigma_j^N h_j h^j \beta_j^H / \Sigma h^j \beta_j^H$.
N	number of associated species under consideration.
N_h	maximum number of dissociable hydrogens on the ligand.
pH, pL, pM	negative of the logarithm, base 10, of the corresponding free concentrations.
pL_o, pM_0	integration constants.
S	$= \Sigma_j^N C_j$. Sillen's 'complexity sum'.
β_j	cumulative formation constant of the jth associated species, referring to the equilibrium expression. $e_{mj}M + e_{lj}L + e_{hj}H \rightleftharpoons M_{e_{mj}}L_{e_{lj}}H_{e_{hj}}$
β_j^H	protonation constant associated with the reaction $L + jH \rightleftarrows H_jL$.
$\sigma^2(pH)$	$= (0.02)^2 + (0.001\ dpH/dV)^2$, V = vol. (mL) of titrant dispensed.

than one complex is present, then the coefficient refers to the number (not necessarily integral) of metal atoms averaged over all complexes. The $\bar{e}_l'$ and $\bar{e}_h'$ coefficients similarly refer to the number of ligand species and protons in the metal-containing complex(es).

Least Squares Refinement of the Equilibrium Model

During the development of the equilibrium model, constants (logarithmic form) were refined by a Gauss–Newton nonlinear weighted least squares procedure. The function minimized is

$$S = \sum^{N_O} (pH^{obs} - pH^{calc})^2/\sigma^2(pH) \qquad (6)$$

where N_o (~400) is the number of pH measurements in the nine metal-containing titration sets. Superscript calc denotes pH calculated as a function of total reagent concentrations and a particular equilibrium model. The weighting scheme used (Table II) has been described elsewhere [27, 28]. The goodness-of-fit, GOF, is defined as

$$GOF = \sqrt{S/(N_o - N_v)} \qquad (7)$$

where N_v refers to the number of constants.

Results

Bjerrum Plots

The presence of polynuclear and/or protonated metal–ligand complexes can be demonstrated using Bjerrum plots. In the *absence* of polynuclear and protonated (ternary) complexes, $\bar{n}$, the Bjerrum formation function (see Table II), refers to the average number of bound ligands per metal ion, and is only a function of the free ligand concentration. From the plot of $\bar{n}$ *vs.* pL, it is possible to determine approximate values of the stepwise metal binding constants by the half-$\bar{n}$ method [29]. Under such conditions the plots are independent of the total metal or ligand concentrations.

When polynuclear and/or ternary complexes are present, the Bjerrum plots are no longer independent of the total concentrations. Figure 1 clearly demonstrates the presence of total metal (Fig. 1a) and total ligand (Fig. 1b) dependence in the Bjerrum plots for the Cd^{2+}/mea system.

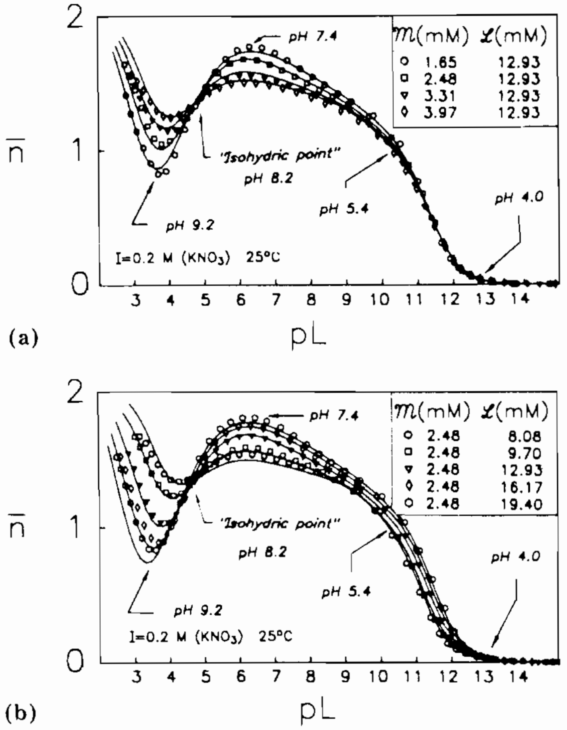

Fig. 1. Bjerrum formation plots. (a) Total metal concentration varied. (b) Total ligand concentration varied. Solid lines were calculated using the equilibrium constants in Table III.

A rare feature in functionally-complicated plots is a point of reversal of concentration dependences, called an 'isohydric point' [30–33]. We have seen such a point in the Cd^{2+}/penicillamine (pen) system (at pH ~ 5) and we see such a point in the present system, at pL ~ 5 (pH ~ 8). Although such cross-over points have been compared to spectroscopic isosbestic points [30–32], the analogy can be misleading in three-component (metal, ligand, hydrogen) equilibrium systems. In the Cd^{2+}/pen system, such a point corresponds to maximum degree of condensation of polynuclear complexes. In the present system, as shall be elaborated below, the isohydric point appears to be associated with the maximum degree of protonation of mononuclear metal complexes.

Other unusual features in the Bjerrum plots for the Cd^{2+}/mea system are the minimum points at pL ~ 4 (pH ~ 9); these puzzled us for some time. A decrease in $\bar{n}$ with *decreasing* pL has never been reported in the literature, to our knowledge. The feature in our curves is reproducible: the curves denoted by squares in Fig. 1a and by triangles in Fig. 1b are duplicate titrations and are superimposable. Unsuspected systematic errors in total concentrations cannot explain the 'dip' features; we were unsuccessful in removing the feature by assuming different total concentrations (offset by 10%).

FICS and ACSS Analysis

The results of the stoichiometric analysis are summarized in Fig. 2. Unlike the stoichiometric curves in the Cd^{2+}/pen system [1], those in the present study indicate the presence of predominantly mononuclear complexes, as shown by the solid circles ($\bar{e}_m'$) in Fig. 2a. The ligand ($\bar{e}_l'$) and hydrogen ($\bar{e}_h'$) coefficients are highly correlated in the pH 4–8 region. This suggested the presence of a series of $Cd(HL)_n^{2+}$ complexes. For pH > 8, the hydrogen ($\bar{e}_h'$) coefficients drop below unity while the ligand coefficients decrease less steeply to $\bar{e}_l' \sim 2$. This suggested the presence of the bis complex, CdL_2.

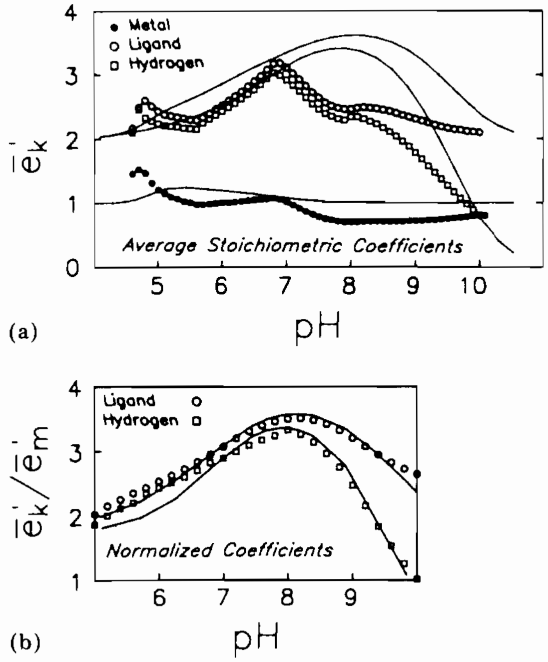

Fig. 2. Average stoichiometric coefficients of the metal-containing species. (a) Direct coefficients. (b) Normalized coefficients. The solid lines were calculated using the equilibrium constants in Table III.

The ACSS method used to construct the $\bar{e}_k'$ (k = m, l, or h) diagram (Fig. 2a) requires potentiometric data of the highest possible precision. Often, such diagrams show spurious effects, due to the propagation of experimental errors into the calculation of the Sillen sum, S. One such effect is the decrease in $\bar{e}_k'$ values in the pH 7–8 region, where $\bar{e}_m'$ falls to 0.7, which is not physically meaningful (in metal-*containing* species). Part of these spurious effects may be removed (with some loss of information, however) if one normalizes the curves by plotting ratios $\bar{e}_l'/\bar{e}_m'$ or $\bar{e}_h'/\bar{e}_m'$, as in Fig. 2b. These normalized coefficients refer to the number of bound ligands and hydrogens per metal ion, respectively, and are independent of the Sillen sum. Such plots for the Cd^{2+}/mea system (Fig. 2b) suggest that each Cd^{2+} binds more than three ligands and hydrogens, and are consistent with the formation of the tetrakis complex $Cd(HL)_4{}^{2+}$. Since the latter complex is largely protonated, values of $\bar{n}$ (Fig. 1) are expected to be much less than four. This emphasizes the need to exercise caution in interpreting Bjerrum plots and the benefits derived from stoichiometric analysis by the ACSS method.

Development and Refinement of the Equilibrium Model

The 'species competition' bootstrap method of Sillen [34] was used to develop an equilibrium model consisting of a minimum number of species and being consistent with the functional behavior of the Bjerrum plots and the ACSS stoichiometric plots. After a lengthy series of least-squares calculations, testing various combinations of likely species, we arrived at the equilibrium model consisting of $Cd(HL)_2{}^{2+}$, $Cd(HL)_3{}^{2+}$, $Cd(HL)_4{}^{2+}$, $Cd(HL)_2L^+$, CdL_2, $Cd_3(HL)_2L_2{}^{4+}$, and $Cd_3L_4{}^{2+}$. The equilibrium constants are listed in Table III. Using the pH data from sets 6–14 (Table I), the model converged at GOF = 1.73, which means that the calculated pH values, on the average, are within 0.04 pH units of the observed values. We consider this to be an excellent fit. The removal of the trinuclear species from the model increases the GOF by 0.22, which is significant. The removal of the $Cd(HL)_4{}^{2+}$ species from the model, however, increases the GOF value by ~2.

We included at various stages of refinement the following species: $Cd(HL)^{2+}$, CdL^+, $Cd(HL)L^+$, $Cd(HL)L_2$, $CdL_2(OH)^-$, and $Cd_3(HL)L_3{}^{3+}$. These species ultimately were rejected, on the basis of either increased GOF or large calculated estimated standard deviations (>0.5 log units) in the derived constants in question. The inclusion of the metal hydrolysis constants (as fixed contributions) for the species $CdOH^+$, Cd_2OH^{3+}, and $Cd_4(OH)_4{}^{4+}$ did not change the GOF.

Using the equilibrium constants from Table III, we were able to successfully simulate the Bjerrum curves, as is indicated by the solid curves in Fig. 1. Also, the normalized coefficients (Fig. 2b) were well reproduced.

Discussion

Mononuclear Complexes

Figure 3 shows the calculated distribution-of-species curves as a function of pH, at concentrations corresponding to sets 10 and 14 (Table I). A series of protonated complexes $Cd(HL)_n{}^{2+}$, n = 2–4, predominate at pH 5, 6, and 8, respectively. We could find no previous reports of these complexes in the literature. In our study, we could not find evidence for the monokis (n = 1) species. Felder *et al.* [7] reported log K′ = 5.1 for the formation of $Cd(HL)^{2+}$. Assuming this value is reliable, we calculate log K″ = 5.7 for the reaction $Cd(HL)^{2+} + HL = Cd(HL)_2{}^{2+}$. Since K′ < K″, the concentration of the monokis

TABLE III. Equilibrium Constants.

Cadmium 2-mercaptoethylamine			Cadmium D-penicillamine
Equilibrium expression	log K		log K Lit.
	This work	Lit.	
$LH + H^+ \rightleftarrows LH_2{}^+$	(pK_{a1}) 8.31(2)[a]	8.35,[b,c] 8.27,[d] 8.19,[e] 8.23(5)[f]	7.96(1)[g]
$L^- + H^+ \rightleftarrows LH$	(pK_{a2}) 10.78(5)	10.81,[b] 10.58,[d] 10.73[e]	10.72(1)[g]
$Cd^{2+} + 2HL \rightleftarrows Cd(HL)_2{}^{2+}$	10.73(2)	9.03[h]	12.51(7)[i]
$Cd(HL)_2{}^{2+} + HL \rightleftarrows Cd(HL)_3{}^{2+}$	4.75(3)		
$Cd(HL)_3{}^{2+} + HL \rightleftarrows Cd(HL)_4{}^{2+}$	3.42(4)		
$Cd(HL)_3{}^{2+} \rightleftarrows CdL(HL)_2{}^+ + H^+$	−8.03(5)		
$Cd^{2+} + 2L^- \rightleftarrows CdL_2$	17.10(7)	19.75[b]	20.27(2)[g], 20.68(6)[i]
$3Cd^{2+} + 4L^- + 2H^+ \rightleftarrows Cd_3L_2(HL)_2{}^{4+}$	59.77(9)		62.74(12)[g]
$3Cd^{2+} + 4L^- \rightleftarrows Cd_3L_4{}^{2+}$	48.12(11)		

[a] The number in parentheses refers to the est. std. dev. in the least significant digit of the preceding quantity. [b] Ref. 8, ionic strength, $I = 0.15$ *M* (KNO_3). [c] Ref. 36, spectroscopic data. [d] Ref. 7; $I = 0.1$ *M* (KCl). [e] Ref. 37, $I = 0.1$ *M* (KNO_3). [f] Ref. 38; $I \rightarrow 0$, spectroscopic. [g] Ref. 1; $I = 0.2$ *M* (KNO_3). [h] Ref. 9, $I = 0.26$ *M*, definition of constant uncertain. [i] A. M. Corrie, M. D. Walker, and D. R. Williams, *J. Chem. Soc., Dalton, 1976*, 1012–1015; $I = 3$ *M* ($NaClO_4$).

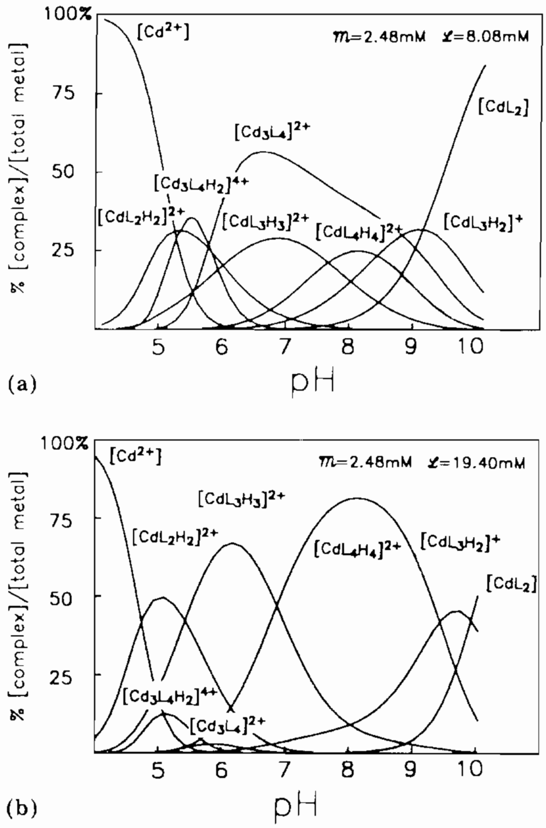

Fig. 3. Distribution of species as a function of pH.

complex under the excess-ligand conditions of our study is expected to be small, which is consistent with our not having detected its presence. Perrin and Sayce [16] reported examples of systems where the value of the second stepwise constant was greater than that of the first. They reasoned that sulfur accepts electron density from the metal ion by π bonding, thus facilitating the addition of the second sulfur ligand to the metal in a cooperative fashion. This view is not entirely consistent with the π-donor character of the thiolate group, however.

The tris complex undergoes a deprotonation to form the $Cd(HL)_2L^+$ complex at pH ~ 9. Presumably, coordination by the amine groups starts to take place for pH > 8. The onset of the chelate effect of the potentially bidentate ligand probably coincides with the release of monodentate-coordinated ligands in the tris and tetrakis complexes, as the bis complex CdL_2 forms at pH > 10. In the Cd^{2+}/pen system, the corresponding bis complex is more stable than the mea complex (Table III) and forms at lower values of pH.

The degree to which the chelate effect is suppressed, as judged by the predominance of the protonated complexes (both mononuclear and polynuclear) is surprising and has no precedence in the results of crystal structure determinations of cadmium complexes with sulfhydryl ligands [12]. It is likely a safe assumption that the site of protonation is the amine end of the ligand, in view of the infrared study of Shindo and Brown [11], the stability of the zwitterionic form of the ligand in the absence of metal ions [36–38], and the expected greater affinity of the 'soft' sulfur ion for the 'soft' (class b) metal ion such as Cd^{2+}, as compared to the affinity of the amine nitrogen, which is considerably 'harder' in character.

The final equilibrium model allows us to correlate the unusual features in the Bjerrum plots with the proposed species. The isohydric point at pH 8.2 coincides with the predominance of the tetra-protonated mononuclear complex (Fig. 3). The maximum spreading of Bjerrum curves at pH ~ 7 (pL ~ 6) takes place when the tris and tetrakis complexes are comparable in concentration. The maximum spread in the curves at pL ~ 4 (pH ~ 9) takes place when the tetrakis and the $CdL_3H_2^+$ species are comparable in concentration. The decrease in the formation curves in the pL 4–6 region is consistent with the loss of coordinated ligands which bind in a monodentate fashion and the onset of bidentate binding by *fewer* ligands. The spread in curves for pL 10–12 (pH < 5) in Fig. 2b is consistent with the protonated complex $Cd(HL)_2^{2+}$ being in equilibrium with free, uncoordinated metal ions. The lack of spread in the comparable region in Fig. 2a is consistent with the predominance of mononuclear complexes. The Cd^{2+}/pen system, which contains polynuclear complexes in greater abundance than the Cd^{2+}/mea system, shows dependence on the total metal concentrations in the high pL region [1].

Polynuclear Complexes

Figure 3 dramatically illustrates the effect of excess ligand on the suppression of polynuclear complexes, which prevail in the pH 5–7 region. The complexes $Cd(HL)_3^{2+}$ and $Cd_3L_4^{2+}$ (pH ~ 7) interchange in dominance dramatically as a function of ligand excess. The concentration ratio of the trinuclear to the mononuclear complexes is a rough measure of the prevalence of polynuclear complexes. Figure 4 illustrates the relationship as a function of total reagent concentrations. The sensitivity of formation of polynuclear complexes to the total concentrations suggests that one must exercise care in extending the results of the present study to solution studies at concentrations significantly different from those of the present study. For example, NMR studies frequently require metal concentrations greater than 10 m*M* [35]. The species found in the dilute solutions may not be present at higher concentrations. Likewise, conclusions suggested by crystallographic structure determinations about the nature of complexes present in solution must be flexible.

Implications with Regard to Metallothioneins

In the Cd^{2+}/pen system, the complexes are predominantly *anionic* and polynuclear in the neutral pH

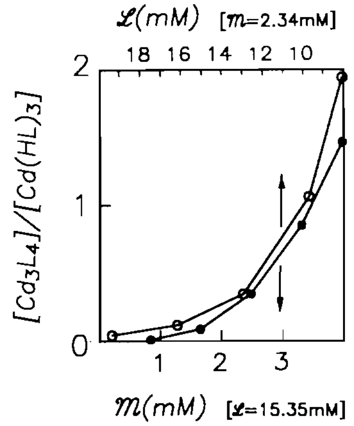

Fig. 4. Plot of the concentration ratio $[Cd_3L_4^{2+}]/[Cd(HL)_3^{2+}]$ as a function of the total metal and ligand concentrations.

region. The complexes in the present system are *cationic* and mononuclear predominantly. Thiolate ligands have a tendency to bridge metal ions, presumably due to electronic stabilization of the molecular orbitals by delocalization of the electron density among several positive metal ion centers. This tendency may be particularly favored in complexes which possess net negative charge, barring excessive charge or steric effects. In positively charged complexes, the electron-rich sulfur orbitals may be sufficiently stabilized by the net positive charge, without the formation of bridged metal complexes. The polynuclear complexes formed in the Cd^{2+}/mea system are less stable than those found in the Cd^{2+}/pen system (Table III).

Two strong metal binding residues in metallothioneins are the sulfhydryl (cysteine) and amine (lysine) groups. Since the number of sulfhydryl groups is greater than the number of amines, the coordination environments around the metal ions are likely anionic. Our simple model studies suggest this situation to favor the formation of polynuclear arrangements. At neutral pH, the lysine groups probably do not extensively coordinate the metal ions in the proteins, judging by the degree to which the mea and pen complexes remain protonated, even in spite of the potential chelate effect possessed by the model ligands.

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