

Articles

Cadmium Binding by Biological Ligands. 5. Solution Studies of Cadmium and Zinc Binding by Sulfhydryl Ligands *N,N'*-Dimethyl-*N,N'*-bis(2-mercaptoethyl)ethylenediamine and (2-Mercaptoethyl)amine

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Received November 27, 1991

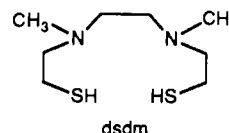
Extensive anaerobic alkalimetric titrations of cadmium and zinc with the tetradentate N_2S_2 -donor disulfhydryl ligand *N,N'*-dimethyl-*N,N'*-bis(2-mercaptoethyl)ethylenediamine (dsdm) and of zinc with the monosulfhydryl *NS*-donor (2-mercaptoethyl)amine in aqueous solutions have revealed polynuclear complexes with cadmium but not with zinc. Nearly 1600 pH measurements (glass electrode, 25 °C, 0.2 M (KNO_3) ionic strength), in 39 separate titrations, were collected with a computer-controlled titrator. The solutions consisted of 1–8 mM cadmium or zinc nitrate and a 1–8-fold excess in ligand. The functional form of the data was in agreement with the least-squares refined equilibrium models consisting of the following principal species: $Zn(dsdm)_2H_4^{2+}$ ($\log \beta = 53.9$), $Zn(dsdm)$ ($\log \beta = 19.3$); $Cd_4(dsdm)_4H_4^{4+}$ ($\log \beta = 109.7$), $Cd_3(dsdm)_3H_2^{2+}$ ($\log \beta = 77.8$), $Cd_2(dsdm)_2H^+$ ($\log \beta = 49.1$), $Cd(dsdm)$ ($\log \beta = 20.5$); $Zn(me)_2H_2^{2+}$ ($\log \beta = 30.6$), $Zn(me)_2H^+$ ($\log \beta = 24.9$), $Zn(me)_3H_2^+$ ($\log \beta = 39.4$), $Zn(me)_2$ ($\log \beta = 17.7$). Cd - $dsdm$ polynuclear species form between pH 3 and 6 but break down to the mononuclear 1:1 complex above pH 7. At pH 7, the dsdm ligand forms a more stable cadmium complex than does edta. Simulation calculations suggest that dsdm can effectively compete for cadmium binding with the sulfhydryl-rich protein metallothionein.

Introduction

Potential low-level chronic cadmium intoxication is a health concern, since the toxic metal ion from industrial pollution sources can be absorbed easily through the roots of important food crops, resulting in the uptake of the metal into major grains.² Cadmium has a tendency to be accumulated by mammals in the liver and kidneys, strongly bound to the sulfhydryl-rich protein metallothionein (MT).^{3,4} Effective chelates have not yet been found for the treatment of chronic cadmium intoxication.

Metallothionein usually incorporates 3 zinc and 4 cadmium ions/mol, in two clusters of tetrahedral thiolate binding sites.⁵ Chelating agents which can mimic the polysulfhydryl metal binding sites found in MT are anticipated to be effective antidotes for both acute and chronic cadmium intoxication. The efficacy of chelants in part seems to depend directly on the value of the stability constant of the cadmium complexes.⁶

The adjacent thiol groups from the several peptide residues -cys-X-cys- found in MT form a dithiol binding environment. The synthetic dithiol ligand *N,N'*-dimethyl-*N,N'*-bis(mercaptoethyl)ethylenediamine (dsdm), whose iron, zinc, and cobalt complexes have been studied extensively by Lippard and co-workers,⁷⁻¹³ may serve as a model for some aspects of cadmium binding in MT.



We report here the determination of the cadmium and zinc stability constants of dsdm and those of the zinc complexes with the monosulfhydryl analog, (2-mercaptoethyl)amine (mea). This work is part of a continuing investigation of the cadmium binding by ligands of biological interest and has included studies of penicillamine,¹⁴ (mercaptoethyl)amine,¹⁵ apo-metallothionein (pH 6 and 7),¹⁶ and the disulfhydryl ligand 2,3-dimercapto-1-propanesulfonic acid (dmpps).¹⁷

Experimental Section

Reagents. All titration solutions were prepared and stored under N_2 in a rigorously maintained inert-atmosphere glovebox (Vacuum Atmospheres) and were free of detectable O_2 and CO_2 . The preparation and standardization of HNO_3 , KOH , ethylenediamine (used for electrode

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calibration), and zinc and cadmium stock solutions have been described elsewhere.¹⁴⁻¹⁸

N,N'-Dimethylethylenediamine and ethylene sulfide were purchased from Aldrich and used without further purification. Toluene was dried over calcium hydride and distilled directly into the reaction flask when needed. Proton NMR (Bruker 360) spectra were taken of neat material, using TMS as reference. Infrared spectra were taken of neat material on KBr pellets.

Synthesis of *N,N'*-Dimethyl-*N,N'*-bis(2-mercaptoethyl)ethylenediamine. The ligand was synthesized by a slightly modified version of the methods reported in the literature.^{7,8,10} Ethylene sulfide (0.35 mol, 20.8 g) in 50 mL of toluene was added dropwise over 1 h to a refluxing solution of *N,N'*-dimethylethylenediamine (0.21 mol, 18.2 g) in 350 mL of toluene. Nitrogen gas was introduced through the top of the reflux condenser to minimize air oxidation of the ligand. Refluxing was continued for 6 h. The resulting solution was washed twice with 100 mL of water. The organic phase was separated, dried over magnesium sulfate, and filtered. Rotary evaporation under high vacuum removed toluene and unreacted dimethylethylenediamine. Yield based on ethylene sulfide: 0.15 mol (88%).

The proton NMR spectra of the ligand showed a thiol peak at δ 1.90 ppm (s, 2 H), a methyl peak at 2.20 ppm (s, 6 H), and methylene peaks at 2.40–2.94 ppm (m, 12 H), in good agreement with the literature.⁷

Comparison of the infrared data of the product with that reported⁷ showed good correlation in the split C–H stretching band (for methyl and methylene groups) centered at 2875 cm^{-1} , a S–H stretching band at 2550 cm^{-1} , and several C–C stretching and C–H bending bands between 1050 and 1460 cm^{-1} .

Titration Methods. Acidified solutions containing variable amounts of metal ion and ligand were prepared from the stock solutions and titrated with standard KOH, using a computerized titrator.¹⁴ Nearly 1600 pH measurements were recorded in 39 titrations. During all titrations (which were conducted in a capped cell outside the glovebox), premoistened N_2 was passed gently over the solutions. The temperature was maintained at 25 °C. Each titrated solution contained enough KNO_3 to maintain the ionic strength roughly constant at 0.2 M.

Methods of Calculation. The extensive FORTRAN library of routines, STBLTY,¹⁹ was used to reduce the data, develop the equilibrium model, and refine the equilibrium constants.

The dsdm $\text{p}K_a$ s were estimated from data sets 9–18 (dsdm, 3.2–8.8 mM; HNO_3 , 9.3–21.0 mM) using Bjerrum plots,^{20,21} \bar{n}_H vs pH, where \bar{n}_H refers to the average number of dissociable protons bound to the ligand at a given pH:

$$\bar{n}_H = ([\text{HNO}_3] - [\text{KOH}] + N'L - [\text{H}^+] + K_w/[\text{H}^+])/L$$

L refers to the total ligand concentration, K_w is the ionization constant of water (1.78×10^{-14} at 25 °C, 0.2 M ionic strength), and N' is the number of dissociable protons contributed to the solution by the ligand.

The Bjerrum metal–ligand formation function, \bar{n} , ideally refers to the average number of bound ligands per metal ion:

$$\bar{n} = \{L - ([\text{HNO}_3] - [\text{KOH}] + N'L - [\text{H}^+] + K_w/[\text{H}^+])/\bar{n}_H'\}/M$$

In this expression (metal–ligand case), \bar{n}_H' (which has the same meaning as \bar{n}_H) is calculated from the ligand $\text{p}K_a$ s at a given pH.²¹ M refers to the total metal ion concentration. From the plot of \bar{n} vs $-\log[\text{dsdm}^{2-}]$,²¹ it is possible to determine approximate values of the stepwise metal binding constants by the half- \bar{n} method.²⁰

Refinement of the equilibrium model had the objective of making as close a match as possible between the calculated dependent variables pH and those which were measured. The devised Gauss–Newton nonlinear weighted least squares refinement procedure specifically minimized the function

$$S = \sum_{N_0} (\text{pH}^{\text{obsd}} - \text{pH}^{\text{calcd}})^2 / \sigma^2$$

where N_0 is the number of points considered in the refinement and σ^2 are

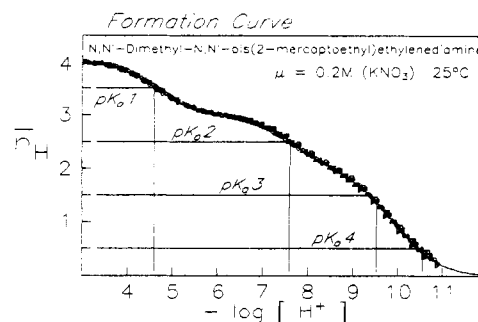


Figure 1. Ligand formation curves of dsdm.

Table I. Refined Equilibrium Constants: Zinc and Cadmium *N,N'*-Dimethyl-*N,N'*-bis(2-mercaptoethyl)ethylenediamine

equil expression	log constant ^a ± σ
$\text{dsdm}^{2-} + \text{H}^+ = \text{dsdmH}^-$	10.369 ± 0.010 ($\text{p}K_a$ 4) ^b
$\text{dsdmH}^- + \text{H}^+ = \text{dsdmH}_2$	9.465 ± 0.009 ($\text{p}K_a$ 3)
$\text{dsdmH}_2 + \text{H}^+ = \text{dsdmH}_3^+$	7.628 ± 0.008 ($\text{p}K_a$ 2)
$\text{dsdmH}_3^+ + \text{H}^+ = \text{dsdmH}_4^{2+}$	4.654 ± 0.009 ($\text{p}K_a$ 1)
$\text{Zn}^{2+} + 2\text{dsdmH}_2 = \text{Zn}(\text{dsdmH}_2)_2^{2+}$	14.14 ± 0.02^c {53.85} ^d
$\text{Zn}^{2+} + \text{dsdm}^{2-} = \text{Zn}(\text{dsdm})$	19.25 ± 0.03
$\text{Zn}(\text{dsdm}) = \text{Zn}(\text{dsdm})\text{OH}^- + \text{H}^+$	-10.17 ± 0.05 {9.08}
$\text{Cd}^{2+} + 2\text{dsdm}^{2-} + 6\text{H}^+ = \text{Cd}(\text{dsdmH}_3)_2^{4+}$	61.9 ± 0.1^e
$4\text{Cd}^{2+} + 4\text{dsdm}^{2-} + 4\text{H}^+ = \text{Cd}_4(\text{dsdmH})_4^+$	109.68 ± 0.07
$\text{Cd}^{2+} + 3\text{dsdm}^{2-} + 8\text{H}^+ = \text{Cd}(\text{dsdmH}_3)_2(\text{dsdmH}_2)^{4+}$	90.02 ± 0.06
$3\text{Cd}^{2+} + 3\text{dsdm}^{2-} + 2\text{H}^+ = \text{Cd}_3(\text{dsdmH})_2(\text{dsdm})^{2+}$	77.76 ± 0.04
$2\text{Cd}^{2+} + 2\text{dsdm}^{2-} + \text{H}^+ = \text{Cd}_2(\text{dsdmH})(\text{dsdm})^+$	49.14 ± 0.03
$2\text{Cd}^{2+} + 3\text{dsdm}^{2-} + 3\text{H}^+ = \text{Cd}_2(\text{dsdmH})_3^+$	73.07 ± 0.06
$\text{Cd}^{2+} + \text{dsdm}^{2-} = \text{Cd}(\text{dsdm})$	20.40 ± 0.02

^a 25 °C; 0.2 M KNO_3 . ^b $\text{p}K_a$ s: GOF = 1.84, data sets 9–18 (dsdm, 3.7–8.8 mM), pH 3–11, $N_0 = 339$. ^c Zn–dsdm constants: GOF = 1.94, data sets 19–29 (Zn^{2+} , 1.0–5.6 mM; dsdm, 4.1–12.9 mM), pH 2.6–9.5, $N_0 = 443$. ^d Constant in braces is in the log β form. ^e Cd–dsdm constants: GOF = 2.22, data sets 30–39 (Cd^{2+} , 1.0–8.3 mM; dsdm, 3.6–13.4 mM), pH 2–7, $N_0 = 288$.

the estimated variances.²² The “goodness-of-fit”, GOF, an index of how precisely a model predicts the observed data, is

$$\text{GOF} = [S/(N_0 - N)]^{1/2}$$

where N is the number of refined constants. GOF < 2 generally implies a good fit.^{14–17}

Development of the Equilibrium Model. The “species competition” bootstrap method of Sillen²³ was used to develop a minimum-set equilibrium model consistent with (and guided by) the functional dependence of the Bjerrum plots. An example of the process has been detailed elsewhere.¹⁷

Results

Ligand Bjerrum Plots and $\text{p}K_a$ s. The Bjerrum protonation curves (\bar{n}_H vs pH) are shown in Figure 1. The precise superposition of the 10 sets suggests the absence of complications such as ligand self-association, decomposition, or electrode malfunction. The dsdm constants, estimated from these curves (half- \bar{n}_H method²⁰) and subsequently refined, are listed in Table I.

Cd–dsdm Bjerrum Plots. The presence of polynuclear complexes can be shown using Bjerrum plots. Without such species, \bar{n} is only a function of the free ligand concentration; ideally, the plots are *independent* of the total metal and ligand concentrations.

Inspection of Bjerrum plots for Cd–dsdm data sets 30–34 (Cd^{2+} , 1.0–8.3 mM; dsdm, 8.5 mM; HNO_3 , 30.0–37.8 mM) in Figure 2a decidedly shows pronounced dependence on the total metal concentration, as seen by the systematic spreading in the formation curves. In the low pH region (pH 2.7–3.6), curves associated with higher total metal concentrations appear to suggest more stable complexation, which is characteristic of the presence of

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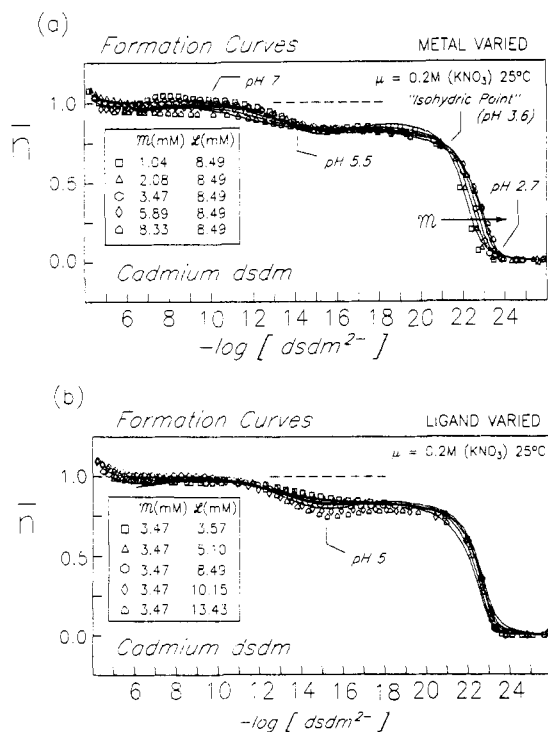


Figure 2. Cd-dsdm Bjerrum formation curves constructed from (a) data sets 30–34 (varied metal concentration) and (b) data sets 35–39 (varied ligand concentration). M and L refer to total metal and ligand concentrations.

polynuclear species. At pH 3.6, there is a reversal of this concentration dependence, pivoting about an "isohydric point",^{14,15} which marks the point of maximum condensation.^{24,25} Thus, polynuclear complexes begin to dissociate above pH 3.6.

With Cd-dsdm data sets 35–39 (Cd^{2+} , 3.5 mM; dsdm, 3.6–13.4 mM; HNO_3 , 20.9–50.1 mM), polynuclear species are not expected to produce total concentration dependence in Bjerrum plots. However, if protonated metal-ligand complexes are present, there will be systematic dependence in such curves.¹⁵ Such appears to be the case in the Cd-dsdm system, as shown by the curves in Figure 2b, particularly in the pH 5 region.

Note that in both types of Bjerrum plots for the cadmium system, there is a region of leveling at $n = 0.82$, pH 4.0–5.5. This feature does not show up in the zinc-dsdm system (Figure 3a,b).

Zn-dsdm Bjerrum Plots. Figure 3a shows the plots for the metal-varied series (sets 19–23: Zn^{2+} , 1.0–5.6 mM; dsdm, 7.4 mM; HNO_3 , 29.8–36.1 mM). Of note is the reverse metal dependence, in contrast to the case of Cd-dsdm. This is the first time such a reverse pattern has been reported, as far as we know. Polynuclear complexes would not explain the reverse pattern. The simple model we eventually developed (below) does predict the reverse pattern, both below pH 4 and near pH 5.

The ligand-varied series (sets 24–29: Zn^{2+} , 3.3 mM; dsdm, 4.1–12.9 mM; HNO_3 , 22.8–48.2 mM) formation curves are characteristic of protonated metal-ligand complexes, as shown in Figure 3b. The same general pattern appears at low pH in the Zn-meal (Figure 4b) and Cd-meal¹⁵ systems.

Zn-meal Bjerrum Plots. Parts a and b of Figure 4 show the formation curves to be similar to those found in the Cd-meal system.¹⁵ The spread near pH 8.6 is very unusual and has been explained with protonated mononuclear metal-ligand complexes.¹⁵

Equilibrium Models. Tables I and II list the refined constants and summarize the refinement characteristics for the three systems studied. The species proposed are those which best predict the

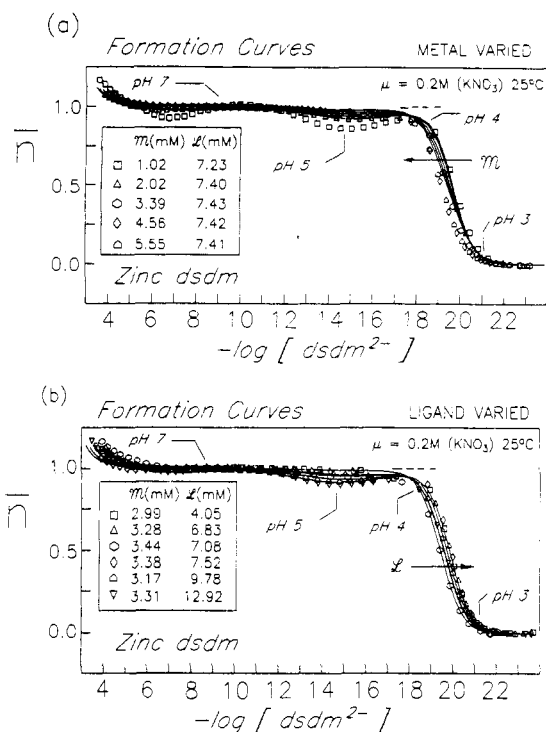


Figure 3. Zn-dsdm Bjerrum formation curves constructed from (a) data sets 19–23 (varied metal concentration) and (b) data sets 24–29 (varied ligand concentration).

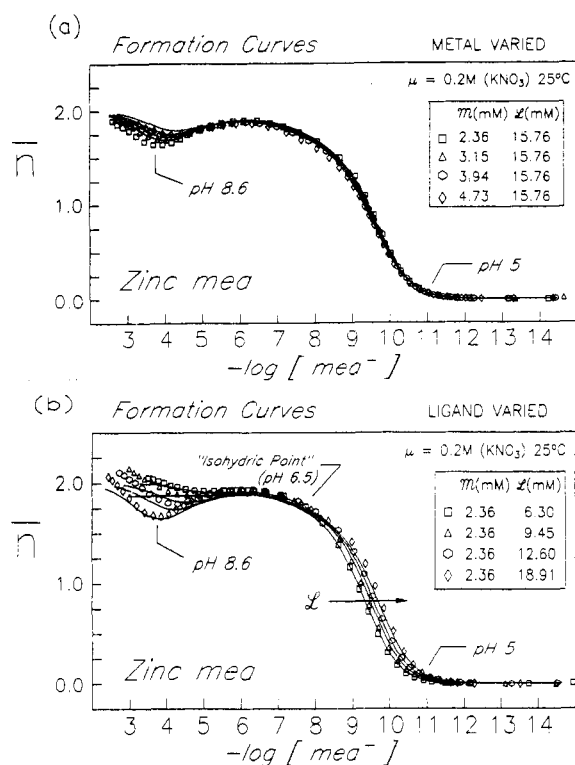


Figure 4. Zn-meal Bjerrum formation curves constructed from (a) data sets 1–4 (varied metal concentration) and (b) data sets 5–8 (varied ligand concentration).

Bjerrum plot functional dependencies and specifically minimize the difference between calculated and observed pH data.

Discussion

Cd-dsdm Equilibrium Model. The reactions between Cd^{2+} and dsdm produce a complicated assortment of species when the ligand-to-metal ratio is in excess of 1. Adequately designed potentiometric analysis is a superb technique for unraveling such

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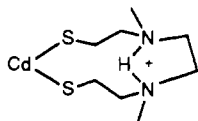
Table II. Refined Equilibrium Constants: Zinc (2-Mercaptoethyl)-amine

equil expression	log constant ^a ± σ
$\text{mea}^- + \text{H}^+ = \text{meaH}$	10.78 (pK _a 2) ^b
$\text{meaH} + \text{H}^+ = \text{meaH}_2^+$	8.31 (pK _a 1) ^b
$\text{Zn}^{2+} + 2\text{meaH} = \text{Zn}(\text{meaH})_2^{2+}$	9.10 ± 0.03 {30.66} ^c
$\text{Zn}(\text{meaH})_2^{2+} = \text{Zn}(\text{meaH})\text{mea}^+ + \text{H}^+$	-5.80 ± 0.04 {24.86}
$\text{Zn}(\text{meaH})\text{mea}^+ + \text{meaH} = \text{Zn}(\text{meaH})_2\text{mea}^+$	3.78 ± 0.03 {39.42}
$\text{Zn}(\text{meaH})\text{mea}^+ = \text{Zn}(\text{mea})_2 + \text{H}^+$	-7.15 ± 0.05 {17.71}

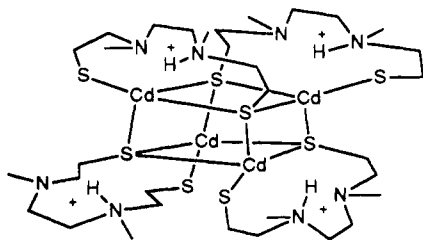
^a 25 °C; 0.2 M ionic strength (KNO₃). GOF = 1.86, data sets 1–8 (Zn²⁺, 2.4–4.7 mM; mea, 6.3–18.9; HNO₃, 5.9–8.4 mM), pH 3.2–9.5, N₀ = 257. ^b Reference 15. ^c Constants in braces are in the log β form.

multiple, overlapping, stoichiometries. Although the analysis is not itself a structural probe, the stoichiometries of principle species determined by the technique can sometimes suggest possible structures for the complexes. One can apply structural “rules” based on crystallographic, NMR, and spectroscopic studies of cadmium complexes with sulfur and nitrogen ligands. Generally, cadmium shows a strong preference for tetrahedral coordination with thiolate residues, cadmium binds to sulfur more strongly than to nitrogen, an –SH group deprotonates before it binds to cadmium, a protonated ammonium group cannot bind to cadmium without shedding its proton, and chelation stabilizes metal complexes with multidentate ligands. More specifically, dsdm can act both as a S₂-bidentate ligand (e.g., Fe(NO)₂(dsdm))¹³ and as a S₂N₂-tetradentate chelate (e.g., Zn(dsdm)ZnCl₂)⁸. A discussion based on some of these “rules” follows.

Minor mononuclear species Cd(dsdm)₂H₆⁴⁺ and Cd(dsdm)₃H₈⁴⁺ form in the pH interval 2.5–6.0. All the other complexes, with the exception of Cd(dsdm), are polynuclear. Figure 5, based on equimolar ligand and metal concentrations, shows the distribution of the polynuclear species more obviously. The first to form is a tetrameric species, peaking in concentration at pH 3.4. A symmetrical structure for the complex would have each ligand monoprotonated. Given the low pH and the likelihood of a protonated nitrogen atom, the ligand may act as a bidentate chelate. Baltusis et al.¹³ have shown precedence for such bidentate coordination in Fe(NO)₂(dsdm). We may propose that the ligand binds cadmium in a similar fashion:



The five-membered hydrogen-bonded ring above is inferred by analogy to a similar six-membered ring observed by Lippard's group.¹³ Such a hydrogen bond could assist in bringing the thiolate portions of the ligand into close proximity. There are several ways that four Cd(dsdm)H⁺ units can oligomerize to form a tetramer. If one assumes that cadmium has tetrahedral thiolate coordination, the highly symmetrical structure shown as follows may be proposed:



It would have pseudo-*D*_{2d} symmetry. Examples of cubane-like structures with other metal thiolate complexes are known, where thiol ligands serve as bridging and terminal groups. In metallothionein⁵ the A cluster has a less symmetric, adamantane-like

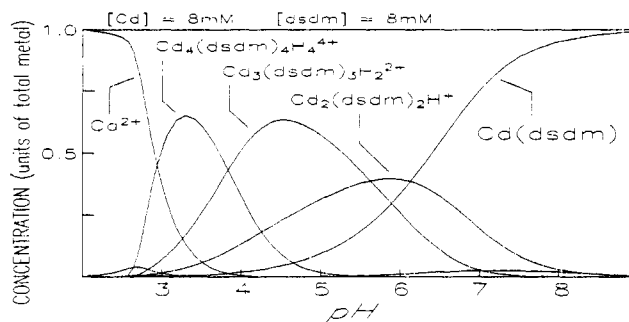
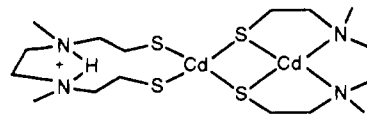


Figure 5. Distribution of species curve for Cd–dsdm in equimolar metal and ligand concentration solution. The unlabeled species is Cd(dsdm)₂H₆⁴⁺. The concentrations at the top of the drawing refer to the total reactant concentrations.

tetrameric structure (involving 11 thiolates), exhibiting only terminal and doubly-bridging thiolate groups.

As pH is raised, the tetramer dissociates into the trimer Cd₃(dsdm)₃H₂²⁺, whose concentration maximizes at pH 4.5 (Figure 5). This is not as symmetrical a molecule as the tetramer, since one of the ligands completely deprotonates.

As pH is further raised, the dimer Cd₂(dsdm)₂H⁺ predominates near pH 6 (Figure 5) and coexists extensively with the monomer Cd(dsdm). Extending the arguments proposed above, one can imagine the structure for the dimer to be a mixture of tetradentate and bidentate coordination.



The monomer Cd(dsdm) predominates in concentration above pH 6. The Zn(dsdm)ZnCl₂ structure reported by Hu et al.⁸ suggests that the ligand can act as a tetradentate chelate. We would expect that cadmium is likewise coordinated by dsdm, although there would be expected distortions, since the cadmium ion is larger than the zinc ion.

Zn–dsdm Equilibrium Model. In stark contrast to the cadmium system, zinc complexation with dsdm is unexpectedly very simple. No polynuclear species were found. At low pH (3.5–5), the bis complex Zn(dsdm)₂H₄²⁺ forms. As pH is raised, it dissociates into the Zn(dsdm) complex, which is undoubtedly a tetrahedral S₂N₂-donor species.⁸

For many guessed species compositions, the GOF remained above 5. With the bis complex, the fit precipitously improved as the GOF dropped below 2. No additional species lowered the GOF any further. Furthermore, the bis complex correctly predicts the anomalous metal dependence in the Bjerrum plots (Figure 3a).

The basis for the simplicity of coordination with zinc may be related to the stronger affinity that zinc has for nitrogen coordination compared with cadmium. That, coupled with the smaller size of zinc, allows zinc to displace more effectively the amine protons, inducing the ligand to wrap around the metal with little strain. The 1:1 complex has little tendency to oligomerize since the metal ion is coordinatively saturated. The larger cadmium ion would be expected to generate some strain upon tetradentate coordination. Hu and Lippard⁹ have shown that some of the strain can be relieved in the slightly larger ligand, *N,N'*-dimethyl-*N,N'*-bis(mercaptoethyl)-1,3-propanediamine.

Zn–mea Equilibrium Model. That zinc has a slightly higher affinity for nitrogen coordination than does cadmium is substantiated in the present study. The log β constant for Zn(mea)₂, 17.7 (Table II), is higher than the constant for Cd(mea)₂, 17.1.¹⁵ (The general β “stability” constant expression is $aM + bL + cH = M_aL_bH_c$, where L is the fully deprotonated form of the ligand.) Conversely, cadmium has a higher affinity for thiol coordination

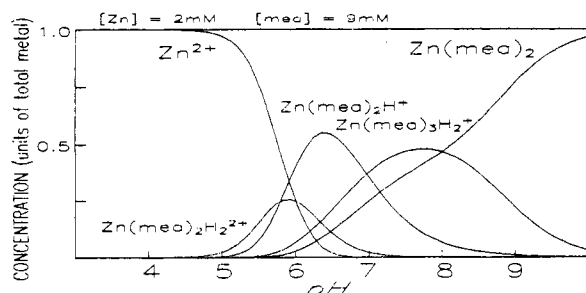


Figure 6. Distribution of species curves: Zn-mea.

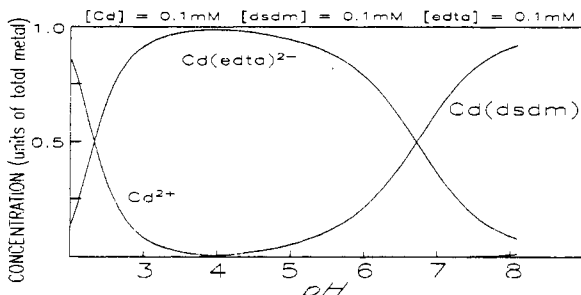


Figure 7. Distribution of species curves: edta in competition with dsdm for cadmium binding.

than zinc: $\log \beta$ of $\text{Cd}(\text{meaH})_2^{2+} = 32.3^{15}$ and $\log \beta$ of $\text{Zn}(\text{meaH})_2^{2+} = 30.6$ (Table II).

The formation curves for the Zn-mea system (Figure 4) are very similar to those of Cd-mea.¹⁵ Our initial equilibrium model thus consisted of the species found in the latter system. Those species which would not refine with the zinc data were dropped from further consideration. The species which successfully refined are listed in Table II; their distribution as a function of pH is shown in Figure 6.

No evidence was found for the tetrathiolate species $\text{Zn}(\text{meaH})_4^{4+}$. Such a species predominates with cadmium, which is consistent with the higher affinity for sulfur coordination shown by cadmium. Also, there was no tendency for zinc species to oligomerize, compared with cadmium.

Relative Binding Strengths. The 2,3-dimercapto-1-propane-sulfonic acid (dmeps) bis complex is a very stable species, as shown by simulated competitive binding with edta and metallothionein.¹⁷ It was thus of interest to see if dsadm was a stronger cadmium binder than dmeps. We first tested how well dsadm competes with edta for cadmium binding. We calculated the distribution of species in a solution containing cadmium and comparable amounts of dsadm and edta (known for its ability to bind cadmium strongly). Figure 7 shows that edta ($\log \beta$ of $[\text{Cd}(\text{edta})] = 16.36^{26}$) is able to suppress effectively the formation of polynuclear complexes of dsadm but not the 1:1 complex. At pH 7, 40% of the cadmium is tied up by $\text{Cd}(\text{edta})^{2-}$ and 60% is in the form of $\text{Cd}(\text{dsadm})$. At higher pH, the dsadm complex predominates.

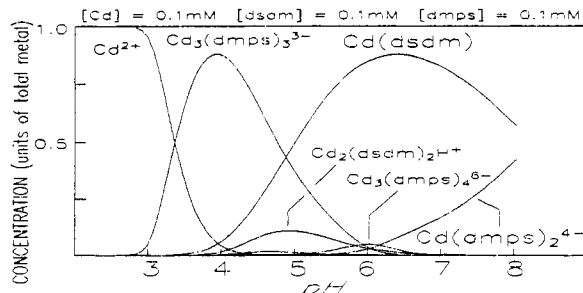


Figure 8. Distribution of species curves: dmeps in competition with dsadm for cadmium binding.

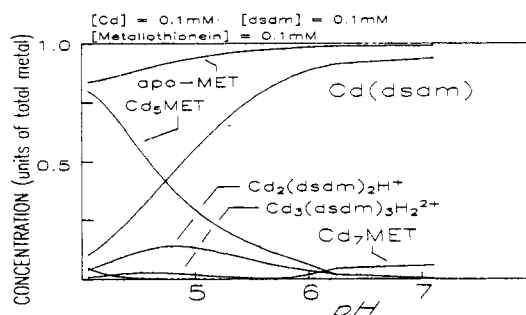


Figure 9. Distribution of species curves: metallothionein (0.1 mM) in competition with dsadm (0.1 mM) for cadmium (0.1 mM) binding.

We compared the relative strengths of dsadm to dmeps in a similar fashion. Figure 8 shows a distribution of species for a solution containing 0.1 mM Cd^{2+} , dsadm^{2-} , and dmeps^{3-} . To answer the question of which ligand is the stronger cadmium binder, one must specify the pH. Near neutral pH (5–8) dsadm is decidedly the stronger chelant. However, above pH 8.3, dmeps forms the more stable complex. The thiol groups of dsadm are more acidic (due to the electrostatic effect of positively charged nitrogen atoms in the ligand) than those of dmeps. Complexation takes place at lower pH for dsadm. At best, however, the cadmium is coordinated by two sulfur atoms in $\text{Cd}(\text{dsadm})$. It is the preference of cadmium for thiolate coordination (over nitrogen) that kicks $\text{Cd}(\text{dmeps})_2^{4-}$ into predominance at higher pH, since the metal ion finds itself bound by four sulfur atoms.

The most interesting comparison is that with metallothionein. We used the pH 6 constants for metallothionein from ref 16: $\log \beta$ of $[\text{MT-H}_{24}^{4+}] = 209.7$, $\log \beta$ of $[\text{Cd}_7\text{-MT-H}_4^{2-}] = 156$, and $\log \beta$ of $[\text{Cd}_5\text{-MT-H}_{13}^{3+}] = 191$. When a solution consisting of 0.1 mM Cd^{2+} , 0.1 mM apo-MT, and 0.1 mM dsadm^{2-} is allowed to react, the predicted distribution of species is shown in Figure 9. Near the neutral pH (5–8) dsadm is able to sequester most of the cadmium ions. The balance tips particularly in favor of dsadm at pH 6–7.

Comparisons such as these show that at pH 7 the dsadm ligand is able to bind cadmium more strongly than any other ligand so far reported.

(26) Martell, A. E.; Smith, R. M. *Critical Constants*; Plenum Press: New York, 1974; Vol. 1.