Equilibrium and spectroscopic studies of ternary cadmium(II) and mercury(II) complexes with CMP and triamines

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EQUILIBRIUM AND SPECTROSCOPIC STUDIES OF TERNARY CADMIUM(II) AND MERCURY(II) COMPLEXES WITH CMP AND TRIAMINES

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Formation of ternary Cd(II) and Hg(II) complexes with cytidine 5′-monophosphate (CMP) and triamines has been studied. Complexes $M(CMP)(H_xPA)$ and $M(CMP)(PA)$ (M = Cd, Hg; PA = polyamine) were detected and overall stability constants and equilibrium constants for their formation determined. The mode of coordination in the complexes has been proposed on the basis of the equilibrium and ^{13}C, ^{31}P NMR and IR studies. In the Hg(II) systems, metalation involves the donor endocyclic N(3) atom, the CMP phosphate group and nitrogen donor atoms of PA. Relative to the Hg/CMP binary systems, the presence of a polyamine in ternary systems does not change the metal–nucleotide mode of coordination. In ternary systems including Hg(II) ions, the occurrence of noncovalent interactions has not been detected. Cd(II) ions form molecular complexes as well as protonated species. Introduction of a polyamine to the Cd/CMP system changes the coordination mode of the nucleotide. The phosphate group of CMP is inactive in binary complexes (metalation by the N(3) atom) but is involved in coordination in heteroligand species. In contrast to other polyamines studied, in the system including 1,7-diamino-4-azaheptane (*3,3-tri*), the phosphate group of CMP in Cd(CMP)(H*3,3-tri*) does not participate in metalation but is engaged in intramolecular noncovalent interactions that stabilize the complex.

Keywords: Cd(II); Hg(II); Cytidine 5′-monophosphate; Polyamines; Complexes

INTRODUCTION

Biogenic amines, present in all biological tissues [1–6] under physiological conditions, occur in protonated form and can be involved in noncovalent interactions with bioligands including nucleic acids. Reactions of polyamines with the phosphate groups of nucleic acids and/or donor endocyclic nitrogen atoms or oxygen atoms of purine or pyrimidine bases [7] play a significant role in genetic information transfer [8–10]. It has been noted that diseases such as cancer are often accompanied by an increase in a polyamine level in a cell. As shown in clinical studies, changes in concentration of polyamines may be helpful in diagnosis [5,11] or monitoring treatment [5,12–14].

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Metal ions present in living organisms significantly affect functions of particular components of the biological system [15–17], including transcription and replication of nucleic acids [18–20]. While interacting with bioligands, metal ions induce changes in their conformation [21,22], and can be viewed as factors interfering in reactions involving molecular complex formation [23]. The high levels of heavy metals in the natural environment (mercury and cadmium are among the most dangerous heavy metals [24–27]) disturb the natural functions of compounds such as nucleic acids, and may lead to neoplasmic changes [24]. According to the theory of hard and soft acids and bases [28–30], Cd^{2+} and Hg^{2+}, and Pd^{2+} and Pt^{2+} ions, show the greatest affinity for the nitrogen atoms of purine or pyrimidine bases in biomolecules.

This paper represents a continuation of our earlier work on interactions between fragments of nucleic acids and polyamines, including metalation of these bioligands [31–33]. We present results of potentiometric and spectroscopic studies of reactions in ternary systems involving Cd(II) or Hg(II) ions, cytidine 5′-monophosphate (CMP) and triamines.

EXPERIMENTAL

1,8-Diamino-4-azaoctane (spermidine, *Spd*) was purchased from Merck, and diethylenetriamine (*dien*), 1,6-diamino-3-azahexane (*2,3-tri*) and 1,7-diamino-4-azaheptane (*3,3-tri*) from Aldrich. Appropriate nitrates were prepared by dissolving in water a proper amount of free amine and adding an equimolar amount of HNO_3. The white precipitate obtained was recrystallized, washed with methanol, and dried in a desiccator over P_4O_{10} or in air. Nitrates of polyamines were subjected to elemental analysis and results (C, N, H) were in agreement with theoretically calculated values ($\pm 0.5\%$). CMP (as acid) was purchased from Sigma. Cadmium(II) and mercury(II) salts used were $Cd(NO_3)_2 \cdot 4H_2O$ (from Merck) and $Hg(NO_3)_2 \cdot H_2O$ (from Aldrich). In order to avoid hydrolysis, an accurately determined amount of HNO_3 (taken into account in computer analysis) was added to the stock solution of $Hg(NO_3)_2$. The concentration of Hg(II) was determined by titration with NaCl solution using diphenylcarbazone as indicator, while Cd(II) was determined complexometrically, using EDTA and pyrocatechol violet as indicator. Potentiometric studies were performed on a DTS Radiometer 800 Multi-Titration System with a GK-2401C electrode calibrated in terms of hydrogen ion concentration [34] and using borax (pH 9.225) and phthalate (pH 4.002) buffers. Concentrations of ligands in the titrated systems varied from 1.3×10^{-3} to 2.6×10^{-3} M, and concentration of metal ions from 1.3×10^{-3} to 1.7×10^{-3} M; the ratio of M : L : L′ (M = metal, L = nucleotide, L′ = PA) in the samples studied was 1 : 1 : 1 and 1 : 2 : 2. Potentiometric titrations were performed at ionic strength $\mu = 0.1$ M (KNO$_3$) at $T = 20 \pm 1°$C under helium, using CO_2-free NaOH solution (about 0.2 M) as titrant. Addition of NaOH solution did not change the ionic strength, because the measurements were performed, starting from fully protonated polyamines, so that $-NH_x^+$ cations were replaced by equivalent amounts of Na^+. Calculations were performed using 100–150 points for each run, taking into account only that part of the titration curve corresponding to no precipitation in the system. Selection of models and determination of protonation constants of ligands and stability constants of complexes were made using the SUPERQUAD program [35], whereas distribution of particular forms was determined by the HALTAFALL program [36].

Selection and verification of models was performed as described previously [37]. Samples for NMR and IR studies were prepared by dissolving appropriate amounts of ligand and metal nitrates in D_2O and adjusting the acidity by addition of NaOD and DNO_3, correcting pH-readings (using pH meter N517 from Mera-Tronik), according to the formula $pD = pH_{readings} + 0.40$ [38]. The ligand concentration in the samples for NMR studies was 0.1 M. The metal ion to ligand ratio varied from 1:10:10 to 1:100:100. ^{13}C NMR spectra were recorded on a Varian Gemini 300VT spectrometer in the range 20 to 170 ppm, using dioxane as internal standard. ^{31}P NMR spectra were recorded on a Varian Unity 300 spectrometer using H_3PO_4 as standard ($H_3PO_4 : D_2O = 1:10$). IR measurements were carried out using a Bruker IFS-113v spectrophotometer with a KRS5-50 cell. The ligands studied are shown below.
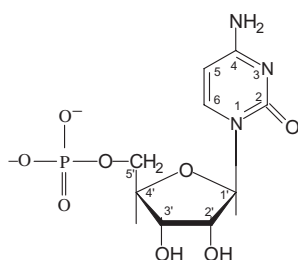
Polyamines
1,5-diamino-3-azapentane (*dien*)
1,6-diamino-3-azahexane (*2,3-tri*)
1,7-diamino-4-azaheptane (*3,3-tri*)
1,8-diamino-4-azaoctane (*Spd*)

RESULTS AND DISCUSSION

A computer analysis of the pH-metric titration data was made taking into account earlier determined (under identical experimental conditions) protonation constants of the bioligands, stability constants of complexes formed in binary systems, and stability constants of the mercury(II) and cadmium(II) hydroxocomplexes $Hg(OH)^+$, $Hg(OH)_2^0$, $Cd(OH)^+$ and $Cd(OH)_2^0$ [39]. As reported previously [39,40], no reduction of Hg(II) to Hg(I) was observed.

Hg(II)/CMP/Triamine Systems

Table I presents overall stability constants ($\log\beta$) and equilibrium constants ($\log K_e$) of complex formation in ternary systems of Hg(II) including CMP and triamines (PA). As follows from computer analysis of potentiometric data, formation of the mono-protonated species Hg(CMP)(H*dien*), Hg(CMP)(H*2,3-tri*), Hg(CMP)(H*3,3-tri*) and Hg(CMP)(H*Spd*) is observed from pH of about 4.5, 4.5, 6 and 6, respectively. The complexes bind about 30%, 40%, 65% and 60% (respectively) metal ions at the pH where they dominate (an example is shown in Fig. 1a,b).

Comparison of $\log K_e$ values for Hg(CMP)(H*2,3-tri*), Hg(CMP)(H*3,3-tri*) and Hg(CMP)(H*Spd*) complex formation ($\log K_e = 8.23$, 8.08 and 8.81, respectively) with $\log K_e$ for Hg(CMP)(*en*) and Hg(CMP)(*tn*) complexes ($\log K_e = 8.35$ and 8.26, respectively, Table I) implies involvement of two donor nitrogen atoms of PA in metalation. The value of $\log K_e$ characterizes the process of a polyamine bonding to the anchoring complex: Hg(CMP) + HPA $\rightleftharpoons$ Hg(CMP)(HPA) ($\log K_e = \log\beta_{Hg(CMP)(HPA)} - \log\beta_{Hg(CMP)} - \log\beta_{HPA}$). This mode of triamine interaction with the Hg(CMP) species

TABLE I Overall stability constants ($\log \beta$) and equilibrium constants ($\log K_e$) for complex formation in Hg(II), Cd(II)/CMP/triamine ternary systems and Hg(II) or Cd(II)/CMP/diamine ternary systems[a]

System	Species	$\log \beta$	$\log K_e$
Hg/CMP/*dien*	Hg(CMP)(*dien*)	21.60 (3)	9.80
	Hg(CMP)(H*dien*)	28.15 (5)	6.39
Hg/CMP/*2,3-tri*	Hg(CMP)(*2,3-tri*)	24.27 (7)	12.47
	Hg(CMP)(H*2,3-tri*)	30.43 (4)	8.23
Hg/CMP/*3,3-tri*	Hg(CMP)(H*3,3-tri*)	30.63 (7)	8.08
	Hg(CMP)(H*3,3-tri*)$_2$	46.69 (9)	
Hg/CMP/*Spd*	Hg(CMP)(*Spd*)	23.32 (5)	11.52
	Hg(CMP)(H*Spd*)	31.47 (5)	8.81
	Hg(CMP)(H$_2$*Spd*)	38.38 (6)	5.80
	Hg(CMP)(*Spd*)(OH)	12.65 (9)	
Cd/CMP/*dien*	Cd(CMP)(H*dien*)	17.89 (12)	5.53
Cd/CMP/*2,3-tri*	Cd(CMP)(H*2,3-tri*)	18.54 (9)	5.74
	Cd(CMP)(H$_3$*2,3-tri*)	32.18 (10)	3.98
Cd/CMP/*3,3-tri*	Cd(CMP)(H*3,3-tri*)	18.40 (9)	5.25
	Cd(CMP)(H$_3$*3,3-tri*)	34.93 (4)	3.68
Cd/CMP/*Spd*	Cd(CMP)(H$_2$*Spd*)	27.99 (7)	4.81
	Cd(CMP)(H$_3$*Spd*)	35.29 (5)	3.59
Hg/CMP/*en*[a]	Hg(CMP)(*en*)	20.15 (3)	8.35
Hg/CMP/*tn*[a]	Hg(CMP)(*tn*)	20.06 (7)	8.26
	Hg(CMP)(H*tn*)	28.18 (9)	5.80
	Hg(CMP)(H$_2$*tn*)	34.51 (12)	3.37
Hg/CMP/*Put*[a]	Hg(CMP)(H*Put*)	28.94 (8)	6.59
	Hg(CMP)(H$_2$*Put*)	35.36 (11)	3.38
	Hg(CMP)(H*Put*)$_2$	43.26 (11)	
Cd/CMP/*en*[a]	Cd(CMP)(H$_2$*en*)	22.79 (8)	2.82
Cd/CMP/*tn*[a]	Cd(CMP)(H$_2$*tn*)	25.70 (12)	3.96
Cd/CMP/*Put*[a]	Cd(CMP)(H$_2$*Put*)	26.10 (7)	3.52

[a]Ref. [41].

is supported by ^{13}C NMR analysis (Table II). For example, in the spectrum of the *Spd* complexes of MLL′H at pH 7.7, the rather small but systematic shifts in the signals from the carbon atoms C(1), C(3) and C(4), neighboring N_a and N_b (the shorter carbon chain), equal 0.061, 0.061 and 0.091 ppm, and point to participation of these nitrogen donors in interactions with Hg(CMP). No shift observed for the signal assigned to C(7), neighboring the terminal nitrogen N_c of spermidine, confirms that this amine group is blocked to metalation (Table II). These results indicate that in the monoprotonated Hg complexes of CMP with *Spd*, as well as with *2,3-tri* and *3,3-tri*, the {N3,O} chromophore is formed. On the other hand, a comparison of $\log K_e = 6.39$ for the H*dien* reaction Hg(CMP) + H*dien* $\rightleftharpoons$ Hg(CMP)(H*dien*) with the $\log K_e$ values for diamine complexes ($\log K_e = 5.80$ and 6.59 for Hg(CMP)(H*tn*) and Hg(CMP)(H*Put*), respectively; *tn* = 1,3-diaminopropane, *Put* = putrescine) (Table I), in which monofunctional coordination was established, implies an involvement of only one nitrogen atom of *dien* in coordination. A similar mode of PA interaction has also been found in diprotonated Hg(CMP)(H$_2$*Spd*) species. The equilibrium constant value for this species $\log K_e = 5.80$ is close to $\log K_e$ of Hg(CMP)(H*tn*) and Hg(CMP)(H*Put*) (5.80 and 6.59), in which the presence of the {N2,O} coordination set was established [41]. In the diprotonated complex, the interaction of protonated *Spd* with Hg(CMP) involves the N_c nitrogen atom of this polyamine, as indicated by
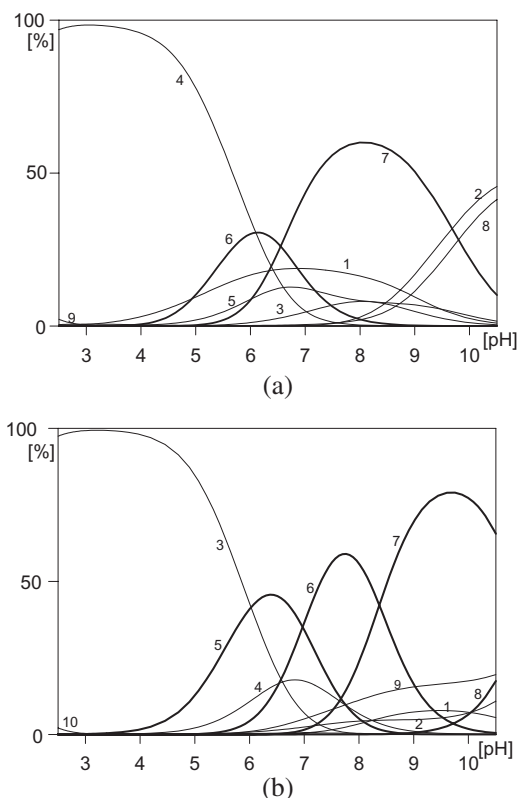
FIGURE 1 Distribution diagrams for the Hg(II)/CMP/*dien* and Hg(II)/CMP/*Spd* systems; percentages of the species refer to total metal. (a) Hg/CMP/*dien*: 1, Hg(*dien*); 2, Hg(*dien*)$_2$; 3, HgH(*dien*)$_2$; 4, Hg(CMP); 5, Hg(CMP)(OH); 6, Hg(CMP)(H*dien*); 7, Hg(CMP)(*dien*); 8, Hg(OH)$_2^0$; 9, Hg^{2+}; $C_{Hg} = 1.29 \times 10^{-3}$ M; $C_{CMP} = 1.33 \times 10^{-3}$ M; $C_{Spd} = 1.33 \times 10^{-3}$ M; (b) Hg/CMP/*Spd*: 1, Hg(*Spd*); 2, Hg(H*Spd*); 3, Hg(CMP); 4, Hg(CMP)(OH); 5, Hg(CMP)(H$_2$*Spd*); 6, Hg(CMP)(H*Spd*); 7, Hg(CMP)(*Spd*); 8, Hg(CMP)(*Spd*)(OH); 9, Hg(OH)$_2^0$; 10, Hg^{2+}; $C_{Hg} = 1.28 \times 10^{-3}$ M; $C_{CMP} = 1.29 \times 10^{-3}$ M; $C_{Spd} = 1.29 \times 10^{-3}$ M.

shifts in the signals assigned to the carbon atoms C(6) and C(7) neighboring the terminal amine group of the longer methylene fragment of the chain (0.106 and 0.056 ppm, Table II). This mode of interaction also follows from the analysis of log K_e values. Moreover, the pattern of changes in NMR spectra and results of the equilibrium study point to an involvement of the N(3) atom and phosphate group of the nucleotide in metalation (Table II).

With increasing pH, as a result of deprotonation of the above species, the hetero-ligand complexes Hg(CMP)(*dien*), Hg(CMP)(*2,3-tri*) and Hg(CMP)(*Spd*) are formed, binding a maximum of about 60%, 75% and 80% of metal ions at pH 8.0, 8.0 and 9.6, respectively (Fig. 1a,b).

Values of the equilibrium constants for Hg(CMP)(*2,3-tri*) and Hg(CMP)(*Spd*) formation, log $K_e = 12.47$ and 11.52, being significantly higher than those for parent Hg(II) complexes with diamines and monoprotonated complexes with triamines (Table I), indicate the involvement of all nitrogen atoms of these asymmetric ligands in coordination. This conclusion is confirmed by an analysis of spectroscopic results. For example, at pH 8.0, in Hg(CMP)(*2,3-tri*) spectra, the shifts in the signals from

TABLE II   Differences between ^{13}C NMR and ^{31}P NMR chemical shifts for the ligands in the Hg(II)/CMP/triamine systems in relation to metal-free systems (ppm)

System	pH	Cytidine 5′-monophosphate										Polyamine						
		C(2)	C(4)	C(5)	C(6)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	P	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
Hg/CMP/*dien*	6.0	0.083	0.026	0.009	0.072	0.282	0.045	0.111	0.175 0.297	0.226	0.365	0.197	0.021					
	8.0	0.117	0.153	0.046	0.126	0.206	0.008	0.134	0.283 0.405	0.472	0.122	0.045	0.516					
Hg/CMP/*2,3-tri*	5.5	0.136	0.201	0.095	0.104	0.327	0.118	0.137	0.046 0.049	0.063	0.389	0.141	0.015	0.334	0.365	0.505		
	8.0	0.148	0.253	0.022	0.220	0.287	0.102	0.096	0.007 0.001	0.152	0.092	0.263	0.312	0.084	0.138	0.081		
Hg/CMP/*3,3-tri*	8.0	0.093	0.134	0.022	0.132	0.374	0.014	0.188	0.277 0.036	0.230	0.115	0.665	1.590	0.490				
	9.6	0.060	0.015	0.031	0.015	0.167	0.105	0.091	0.091 0.091	0.167	0.089	0.046	0.243	0.009				
Hg/CMP/*Spd*	6.6	0.091	0.015	0.015	0.060	0.289	0.076	0.167	0.061 0.061	0.136	0.214	0.036	0.030	0.046	0.026	0.000	0.106	0.056
	7.7	0.136	0.045	0.016	0.031	0.273	0.015	0.183	0.167 0.167	0.227	0.129	0.061	0.000	0.061	0.091	0.046	0.025	0.000
	9.6	0.061	0.001	0.016	0.001	0.121	0.015	0.107	0.091 0.076	0.136	0.053	0.066	0.488	0.127	0.116	0.246	0.226	0.055

the carbon atoms of *2,3-tri* (neighboring nitrogen atoms N_a, N_b and N_c of the amine groups) C(1), C(2), C(3), C(4) and C(5) are 0.263, 0.312, 0.084, 0.138 and 0.081 ppm, respectively. At pH 9.6 in the Hg(CMP)(*Spd*) spectra (distribution in Fig. 1b), changes in the signals from the carbon atoms C(1), C(2), C(3), C(4), C(5), C(6) and C(7) are 0.066, 0.488, 0.127, 0.116, 0.246, 0.226 and 0.055 ppm, respectively (Table II). This pattern of signal shifts corresponds very well with the results of the equilibrium study. The shifts in the signals assigned to C(2) and C(4) atoms located in the vicinity of N(3) of the nucleotide (for example, in the Hg(CMP)(*2,3-tri*) species) at pH 8.0 are 0.148 and 0.253 ppm (Table II), and point to the involvement of the endocyclic N(3) atom in coordination. Moreover, the shifts in NMR signals from the C(5′) atom of ribose and in the ^{31}P signal (0.152 and 0.092 ppm, respectively) indicate participation of the phosphate group in coordination and point to the formation of an {N4,O} chromophore. In the IR spectrum, no changes were observed in the position of the $1650\,cm^{-1}$ band assigned to the stretching vibration of the C=O group of the nucleotide (as in the spectra of other Hg(II) and Cd(II) heteroligand complexes including CMP and triamines), which means that the oxygen atom from the carbonyl group is not involved in metalation. In view of the results of the equilibrium study for the *dien* system, the third nitrogen atom of the ligand in the Hg(CMP)(*dien*) complex is a poor donor, as can be concluded from a comparison of the equilibrium constants for analogous species of triamines. The value of $\log K_e = 9.80$ for Hg(CMP)(*dien*) is significantly lower than those for Hg(CMP)(*2,3-tri*) and Hg(CMP)(*Spd*) complexes, but it is higher than that for monoprotonated species of *2,3-tri*, *3,3-tri* and *Spd* (Table I).

Cd(II)/CMP/Triamine Systems

Table I presents overall stability constants ($\log \beta$) and equilibrium constants ($\log K_e$) for complexes formed in ternary systems of Cd(II) with CMP and triamines. Starting from pH of about 4–5, the species Cd(CMP)(H₃*2,3-tri*), Cd(CMP)(H₃*3,3,-tri*) and Cd(CMP)(H₃*Spd*) are formed (ML‖‖‖L′ type; an example of distribution is given in Fig. 2a).

These adducts dominate at a pH of about 7, binding about 35% of metal ions. The $\log K_e$ values of their formation, 3.98, 3.68 and 3.59, respectively, are comparable with the $\log K_e$ for Cd(CMP)(H₂*tn*) and Cd(CMP)(H₂*Put*) species (3.69 and 3.52, respectively [41]), which suggests a similar mode of interaction and formation of molecular complexes. The metalation involves the N(3) atom as well as the phosphate group of the nucleotide, as has been found from analysis of NMR results (Table III). In the PA-free system the –O–PO₃ group does not participate in coordination [39].

Triamine is located in the outer coordination sphere (all donor nitrogen atoms of PA in the Cd(CMP)(H₃PA) adducts are protonated and blocked for metalation) and is involved in noncovalent interactions with the phosphate group of Cd(CMP), as is indicated by the ^{13}C and ^{31}P NMR results (Table III). For example, at pH 6, when Cd(CMP)(H₃*2,3-tri*) dominates, the shifts in the signals assigned to C(2), C(4), C(5′), and to the phosphorus atom are 0.492, 0.264, 0.207 and 0.532 ppm, respectively, while in the spectra of triamine shifts in the signals from C(1), C(2), C(3), C(4) and C(5) atoms are 0.044, 0.753, 0.247, 0.117 and 0.099 ppm, respectively.

Formation of molecular complexes is also confirmed by the coincidence of the titration curves obtained experimentally with those simulated by computer assuming adduct formation (with the use of the determined β values). There is agreement between the pH
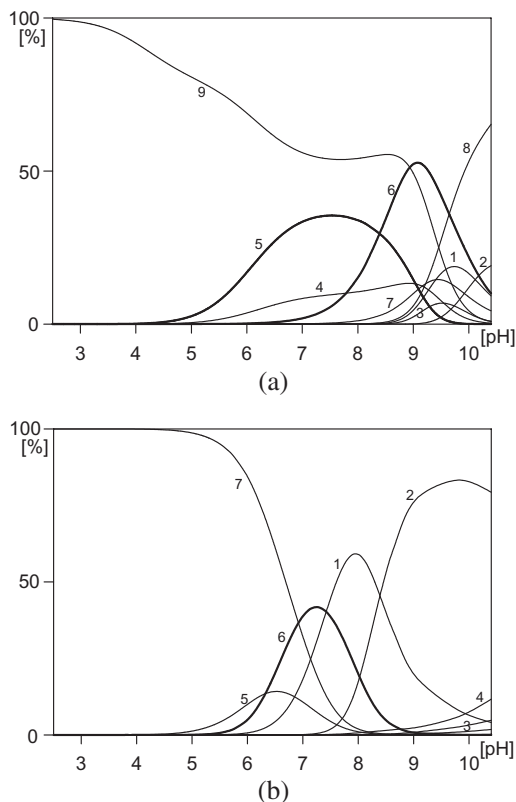
FIGURE 2   Distribution diagrams for the Cd(II)/CMP/*dien* and Cd(II)/CMP/*Spd* systems; percentages of the species refer to total metal. (a) Cd(II)/CMP/*Spd*: 1, Cd(*Spd*); 2, Cd(*Spd*)$_2$; 3, Cd(H*Spd*)$_2$; 4, Cd(CMP); 5, Cd(CMP)(H$_3$*Spd*); 6, Cd(CMP)(H$_2$*Spd*); 7, Cd(OH)$^+$; 8, Cd(OH)$_2^0$; 9, Cd^{2+}; $C_{Cd} = 1.72 \times 10^{-3}$M; $C_{CMP} = 3.46 \times 10^{-3}$ M; $C_{Spd} = 3.45 \times 10^{-3}$ M; (b) Cd(II)/CMP/*dien*: 1, Cd(*dien*); 2, Cd(*dien*)$_2$; 3, Cd(*dien*)$_3$; 4, Cd(*dien*)(OH); 5, Cd(CMP); 6, Cd(CMP)(H*dien*); 7, Cd^{2+}; $C_{Cd} = 1.38 \times 10^{-3}$ M; $C_{CMP} = 2.67 \times 10^{-3}$ M; $C_{dien} = 2.65 \times 10^{-3}$ M.

range of curve divergence (when the adduct formation is not taken into account) and the pH range of adduct formation.

An increase in the number of donor nitrogen atoms in the polyamine molecule does not significantly affect the stability of the adduct studied (Table I). The equilibrium constant of formation of, for example, Cd(CMP)(H$_3$*2,3-tri*), log $K_e = 3.98$, is higher than that (log $K_e = 2.82$) of the Cd(CMP)(H$_2$*en*) complex (in which the spectroscopic results exclude the involvement of oxygen atoms of the phosphate group in metalation) and indicates a participation of the nucleotide phosphate group in the interactions.

The stoichiometry of monoprotonated species Cd(CMP)(H*dien*) (an example of distribution is given in Fig. 2b), Cd(CMP)(H*2,3-tri*) and Cd(CMP)(H*3,3-tri*) suggests the involvement of two nitrogen atoms of the triamine in metalation. This mode of interaction is confirmed by changes in the NMR. For a complex including the asymmetric *2,3-tri* ligand, shifts in the signals (at pH 8.0) from C(1), C(2), C(3), C(4) and C(5) atoms of the amine are 0.094, 0.140, 0.101, 0.040 and 0.009 ppm, respectively, confirming the involvement of two triamine nitrogen atoms (N$_a$ and N$_b$) in metalation and formation of a five-membered chelate ring. The changes in the NMR signals

TABLE III   Differences between ^{13}C NMR and ^{31}P NMR chemical shifts for the ligands in the Cd(II)/CMP/triamine systems in relation to metal-free systems (ppm)

| System	pH	Cytidine 5′-monophosphate										Polyamine						
		C(2)	C(4)	C(5)	C(6)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	P	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
Cd/CMP/*dien*	7.5	0.195	0.229	0.073	0.196	0.211	0.040	0.086	0.177	0.068	0.130	0.065	0.206					
Cd/CMP/*2,3-tri*	6.0	0.492	0.264	0.077	0.310	0.589	0.005	0.220	0.289	0.207	0.532	0.044	0.753	0.247	0.117	0.099		
	8.0	0.163	0.213	0.041	0.196	0.308	0.040	0.135	0.242	0.149	0.107	0.094	0.140	0.101	0.040	0.009		
Cd/CMP/*3,3-tri*	9.0	0.011	0.144	0.066	0.201	0.318	0.037	0.083	0.103	0.066	0.041	0.348	0.924	0.200				
Cd/CMP/*Spd*	7.0	0.220	0.162	0.049	0.049	0.067	0.073	0.143	0.265	0.408	0.145	0.064	0.002	0.072	0.080	0.047	0.055	0.056
	9.0	0.061	0.052	0.014	0.006	0.168	0.092	0.102	0.104	1.190	0.096	0.108	0.241	0.088	0.072	0.068	0.055	0.064
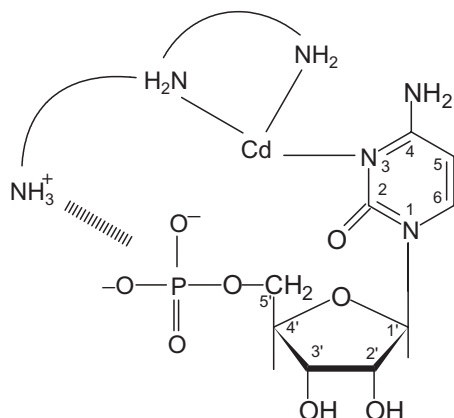
FIGURE 3   Tentative mode of interaction in the Cd(CMP)(H*3,3-tri*) complex.

assigned to C(2) and C(4) atoms, adjacent to N(3), as well as that of the signal originating from C(5′) of ribose and the phosphorus atom of CMP, prove the involvement of the N(3) donor and phosphate group of the nucleotide in metalation (Table III). Similarly, as in the molecular complexes, in MLL′H species the value of the equilibrium constant for reaction of monoprotonated triamine ($\log K_e = 5.74$) with the anchoring Cd(CMP), higher than that of binary Cd(*tn*) complex formation ($\log K_e = 3.98$, {N2} coordination set [39]), points to the involvement of the phosphate group in the interaction (a chromophore {N3,O} occurs). A similar mode of coordination is also observed in the Cd(CMP)(H*dien*) complex. On the other hand, in the species Cd(CMP)(H*3,3-tri*), the phosphate group is not involved in coordination. Changes in the signals from C(5′) and the phosphorus atoms of CMP in the Cd/CMP/*3,3-tri* system (0.066 and 0.041 ppm, respectively) are much smaller than the analogous shifts observed in systems including *dien* and *2,3-tri* (Table III). The value of the equilibrium constant, $\log K_e = 5.25$, characterizing the attachment of H*3,3-tri* to the Cd(CMP) anchor, higher than those for binary species Cd(*tn*) and Cd(H*3,3-tri*) formation ({N2} chromophore; $\log K_e = 3.98$ and 4.69, respectively [39]), suggests the involvement of the phosphate group in noncovalent interactions, which stabilize the complex. However, this interaction results in a low efficiency of the phosphate group in metalation in Cd(CMP)(H*3,3-tri*), as has been discussed above (Fig. 3).

In the spectra of the diprotonated Cd(CMP)(H$_2$*Spd*) complex (it dominates at pH 9, Fig. 2a, binding about 50% of metal ions), changes in the signals assigned to C(1) and C(2) atoms of *Spd* located near the terminal N$_a$ atom are equal to 0.108 and 0.241 ppm, and are systematically higher than the shifts of signals assigned to the other carbon atoms, indicating involvement in the coordination of only one nitrogen atom of this bioamine (Table III). This conclusion corresponds to the stoichiometry of the complex – two nitrogen donor atoms are blocked for metalation. An analysis of the NMR results and the results of the equilibrium studies point to coordination via the N(3) atom and the phosphate group of the nucleotide. The value of $\log K_e = 4.81$ characterizing the formation of Cd(CMP)(H$_2$*Spd*) species, being higher than $\log K_e = 3.53$ describing the formation of Cd(*Cyd*)(H$_2$*Spd*) complex [42], additionally testifies to the involvement of the phosphate group of CMP in metalation.

CONCLUSIONS

In all species formed in the Hg(II)/CMP/PA systems, the endocyclic nitrogen atoms N(3) and phosphate groups from the nucleotide as well as nitrogen donor atoms of PA are involved in metalation. In MLL′-type species, the {N4,O} coordination set occurs (all nitrogen atoms from the polyamine take part in coordination). However, the efficiency of *dien* for metalation in the observed ternary complexes is significantly lower compared to the longer triamines. This can be explained as a result of lower basicity of *dien* as well as formation of a different type of ring (a structural factor). This conclusion is important for discussion of differences in the activity of biogenic amines in relation to their shorter homologues. Relative to the Hg/CMP binary systems, the presence of a polyamine in ternary systems does not change the metal–nucleotide mode of coordination. On the other hand, in the ternary Cd(II)/CMP/PA systems, the presence of the polyamine changes the coordination character of the nucleotide. The phosphate group, inactive in the binary complex Cd(CMP), is involved in metalation in the ternary complexes. However, the *3,3-tri* ligand has no effect on the mode of interactions in the ternary systems relative to that in the binary ones, which confirms the role of the structural factor (the polyamine length) on the coordination efficiency of the O–PO$_3$ group. The noncovalent interaction of the uncoordinated phosphate group with a protonated $-NH_3^+$ group of the polyamine, observed in Cd(CMP)(H*3,3-tri*) species, enhances complex stability. In Cd(CMP)(H$_3$PA) molecular complexes of the ML$^{||||}$L′ type, polyamines lie in the outer coordination sphere (protonated amine groups are blocked for metalation) and unexpectedly take part in noncovalent interactions with the metalated phosphate group of the nucleotide in Cd(CMP). This type of adduct is not formed in the system with *dien*, which once more emphasizes the role of the structural factor in these processes and explains the lower efficiency of the ligand relative to that of biogenic amines.

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