

Interaction of metal ions with cytidine and amino carboxylic acids

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Abstract. The interaction of Cu(II), Ni(II), Zn(II), Co(II), Mg(II) and Ca(II) ions with cytidine and amino carboxylic acids (EDTA, NTA and IMDA) in a 1:1:1 ratio have been investigated by potentiometric equilibrium measurements at 35°C and 0.10 M (KNO₃) ionic strength. These investigations were undertaken to assess the influence of the secondary ligands on the structure and stability of the 1:1 metal-cytidine system. The stabilities of ternary and binary complexes are compared and stabilization factors are explained.

Keywords. Cytidine; divalent metal ions; amino carboxylic acids; ternary complexes; stability constants; steric factors.

1. Introduction

The interaction of metal ions with nucleosides and nucleotides, both in binary and ternary systems, have attracted the attention of many scientists throughout the world (Izatt *et al* 1971; Eichhorn 1973; Martin *et al* 1973; Sigel 1973; Taqui Khan and Rabindra Reddy 1973; Marzilli 1977; Martin and Mariam 1979; Rabindra Reddy *et al* 1979; Orenberg *et al* 1980; Rabindra Reddy and Harilatha Reddy 1983). This is mainly due to the fact that metal ions are known to effect the structure of nucleic acids (Eichhorn 1973). Since nucleotides and nucleosides constitute the backbone structure of nucleic acids, any information on the interactions of metal ions with these ligands (nucleosides and nucleotides) is worthwhile. In the present manuscript, ternary complexes of Cu(II), Ni(II), Zn(II), Co(II), Mg(II) and Ca(II) with cytidine and amino carboxylic acids, disodium salts of ethylene diamine tetraacetic acid (Na₂EDTA), nitrilotriacetic acid (NTA) and iminodiacetic acid (IMDA), have been investigated. Earlier work on the ternary complexes of cytidine was confined mainly to Th(IV) and UO₂(II) (Ramalingam and Krishnamoorthy 1982) and EPR and electron absorption studies on the interaction of Cu(II)-glycylglycine complexes (Deshpande *et al* 1983). In a recent article (Rabindra Reddy and Malleswar Rao 1985) the significance of secondary ligands in the structure and stability of metal cytidine complexes in solution has been emphasized. The results obtained from this investigation are compared with literature data to get a clear idea about the influence of secondary ligands on the stabilities of 1:1 metal-ligand complexes. The study of ternary com-

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plexes with diverse groups of secondary ligands, we hope, will lead to a better understanding of the metal-nucleoside interactions in solution.

2. Experimental

Cytidine, IMDA and NTA were obtained from Sigma Chemical Company (USA) and Na₂EDTA was obtained from Glaxo Laboratories, India. For every titration, fresh solid ligands were weighed out into the reaction cell to avoid possible hydrolysis. Transition and alkaline earth metal ions [Cu(II), Ni(II), Zn(II), Co(II), Mg(II) and Ca(II)] were of AnalaR grade and were standardized volumetrically by titration with the disodium salt of ethylene diamine tetraacetic acid in the presence of a suitable indicator as outlined by Schwarzenbach (1957). Carbonate free sodium hydroxide was prepared by the method of Schwarzenbach and Biedermann (1948).

The experimental method employed consisted of potentiometric titration of the ligands with standard sodium hydroxide solution, in the absence and presence of the above mentioned metal ions. The ionic strength was kept constant by using 0.10 M (KNO₃) as the supporting electrolyte and relatively low concentrations of the ligand and metal ion (1×10^{-3} M). During the course of the titration a stream of nitrogen was passed over the solution to eliminate the adverse effect of atmospheric carbon dioxide.

All measurements were made at $35^\circ \pm 0.1^\circ\text{C}$. Equimolar ratios of metal and ligands were employed in the investigation. Further details are given in our earlier work (Rabindra Reddy *et al* 1984).

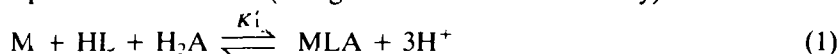
3. Calculations

3.1 Dissociation constants

The acid dissociation constants of the primary ligand cytidine and the secondary ligands Na₂EDTA, NTA and IMDA were calculated by the usual algebraic method.

3.2 Formation constants

To calculate the stability constants of the ternary complexes of Cu(II), Ni(II), Zn(II), Co(II), Mg(II) and Ca(II) with cytidine and Na₂EDTA in a 1:1:1 ratio the following equations were used (charges are omitted for clarity):



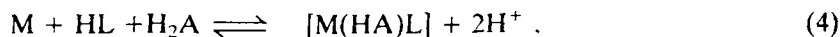
together with the related equilibria



$$K_{MLA}^M = \frac{T_M - [M]}{[M][L][A]}. \quad (3)$$

Equations (1)–(3) were also employed to calculate the stability constants of the ternary complexes of Cu(II), Ni(II), Zn(II) and Co(II) with cytidine-NTA and cytidine-IMDA.

For the ternary complexes of Mg(II) and Ca(II) with cytidine-NTA and cytidine-IMDA in a 1:1:1 ratio, (4)–(6) used were in the buffer region between $m = 0$ and $m = 2$.

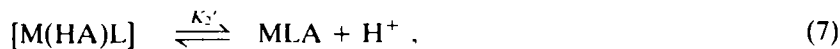


together with related equilibria



$$K_{M(HA)L}^M = \frac{T_M - [M]}{[M][L][A]} \quad (6)$$

In the buffer region between $m = 2$ and $m = 3$ the following equations were employed:



$$K_{MLA}^{M(HA)L} = \frac{T_M - [M]}{[M][A]} \quad (9)$$

In (8) and (9) 'M' equals M(HA)L. For (1)–(9), HL = monoprotonated cytidine, A = Na₂EDTA or NTA or IMDA (secondary ligands), T_M = total metal ion species present in solution.

The detailed calculations of these constants are described in our earlier work (Rabindra Reddy and Malleswar Rao 1985).

4. Results

The proton dissociation constants of the ligands cytidine, EDTA, NTA and IMDA are presented in table 1.

4.1 Metal:cytidine:Na₂EDTA system (1:1:1)

The mixed ligand titration curve of Cu(II)-cytidine-Na₂EDTA given in figure 1 (c) shows an inflection at $m = 3$ (where m is moles of base added per mole of metal ion), indicating simultaneous formation of a 1:1:1 mixed ligand complex in the entire buffer region between $m = 0$ and $m = 3$. The constant K_{MLA}^M was calculated with the help of (3). Similar trends were observed for other systems studied and the corresponding stability data are presented in table 2.

Table 1. Acid dissociation constants of ligands.
Temperature = 35°C; $\mu = 0.10 \text{ mol dm}^{-3}$ (KNO₃).

Ligand	pK_a	pK_{2a}	pK_{3a}	pK_{4a}
Cytidine	4.15 ± 0.04			
Na ₂ EDTA			6.12 ± 0.03	9.92 ± 0.03
NTA		2.35 ± 0.05	9.45 ± 0.05	
IMDA	2.45 ± 0.04	9.27 ± 0.04		

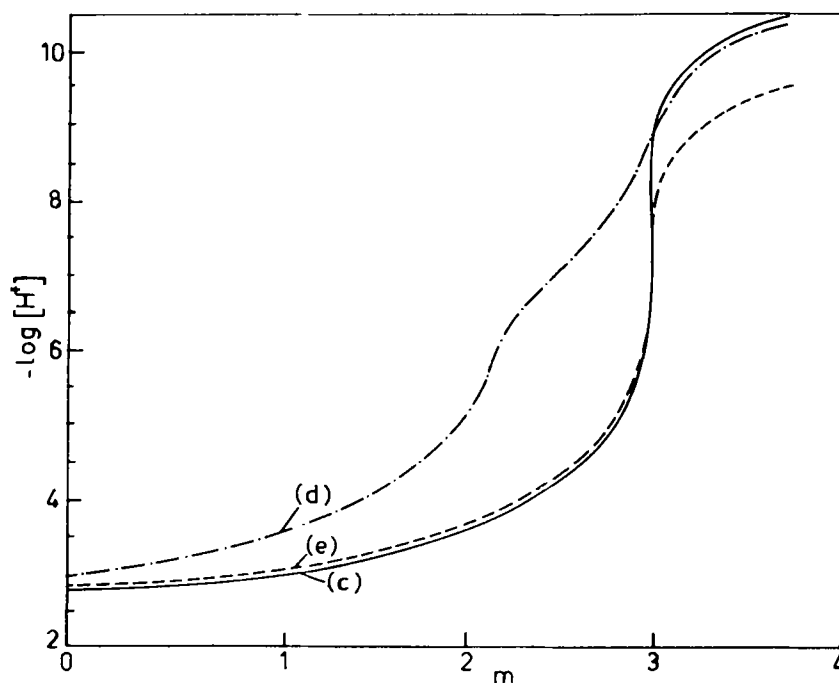


Figure 1. Potentiometric titration curves for Cu(II) : cytidine : Na₂EDTA, Cu(II) : cytidine : NTA and Mg(II) : cytidine : NTA in a 1:1:1 ratio at 35°C and 0.10 M (KNO₃) ionic strength. c = Cu(II) : cytidine : Na₂EDTA; d = Mg(II) : cytidine : NTA; e = Cu(II) : cytidine : NTA; m = moles of base added per mole of metal ion.

Table 2. Stability constants* of the ternary complexes of cytidine with Na₂EDTA in a (1:1:1) ratio.

Temperature = 35°C; $\mu = 0.10 \text{ mol dm}^{-3}$ (KNO₃)

Metal ion (II)	Metal:cytidine [†] (1:1) K_{ML}^M	Metal:Na ₂ EDTA (1:1) K_{MA}^M	Metal:cytidine:Na ₂ EDTA (1:1:1) K_{MLA}^M
Cu	3.19	13.80	18.59
Ni	2.94	13.45	17.93
Zn	2.60	13.34	17.62
Co	2.74	13.39	17.78
Mg	2.42	9.43	13.98
Ca	2.47	10.18	14.69

*The constants are accurate upto ± 0.05 ;

†The values are from Khan and Raju (1981).

4.2 Metal:cytidine:NTA systems (1:1:1)

The mixed ligand titration curve of Cu(II) with cytidine and NTA in a 1:1:1 ratio is given in figure 1 (e), resulting in an inflection at $m = 3$, indicating the simultaneous formation of a 1:1:1 mixed ligand complex in the entire buffer region between $m = 0$ and $m = 3$. The constant K_{MLA}^M was calculated with the help of (3). Similar

results were obtained for Ni(II), Zn(II) and Co(II) and the constants are listed in table 3.

However, in the case of other metal ions [Mg(II) and Ca(II)], an inflection was obtained at $m = 2$ [figure. 1(d)] indicating the formation of a monoprotonated complex in the region between $m = 0$ and $m = 2$. The constant $K_{MLA}^{M(HA)L}$ was also calculated in the buffer region between $m = 2$ and $m = 3$ with the help of (9). All the constants thus calculated are listed in table 3.

4.3 Metal:cytidine:IMDA system (1:1:1)

This system is exactly similar to the metal:cytidine:NTA system. The constants thus calculated are presented in table 4.

5. Discussion

The dissociation constants of various ligands are listed in table 1. In tables 2, 3 and 4 the stability constants of the binary and ternary complexes are compiled. Although the stability constants for the interaction of Cu(II), Ni(II), Zn(II), Co(II), Mg(II) and Ca(II) with amino carboxylic acids are available in the

Table 3. Stability constants* of the ternary complexes of cytidine with NTA in a (1:1:1) ratio.

Temperature = 35°C; $\mu = 0.10 \text{ mol dm}^{-3}$ (KNO₃)

Metal ion (II)	Metal:NTA (1:1)			Metal:cytidine:NTA (1:1:1)		
	K_{MHA}^M	K_{MA}^{MHA}	K_{MA}^M	$K_{M(HA)L}^M$	$K_{MLA}^{M(HA)L}$	K_{MLA}^M
Cu			11.82			15.86
Ni			11.20			15.25
Zn			10.91			15.16
Co			10.77			15.06
Mg	3.94	6.72		8.43	5.95	
Ca	3.79	6.45		8.28	6.07	

* The constants are accurate up to ± 0.04 .

Table 4. Stability constants* of the ternary complexes of cytidine with IMDA in a (1:1:1) ratio.

Temperature = 35°C; $\mu = 0.10 \text{ mol dm}^{-3}$ (KNO₃)

Metal ion (II)	Metal:IMDA (1:1)			Metal:cytidine:IMDA (1:1:1)		
	K_{MHA}^M	K_{MA}^{MHA}	K_{MA}^M	$K_{M(HA)L}^M$	$K_{MLA}^{M(HA)L}$	K_{MLA}^M
Cu			10.28			15.18
Ni			8.17			13.20
Zn			7.24			12.80
Co			6.92			12.59
Mg	2.71	2.65		8.21	3.30	
Ca	2.42	1.74		7.78	2.51	

* The constants are accurate up to ± 0.04 .

literature, we have remeasured these constants as it is preferable to determine the binary and ternary constants under identical experimental conditions. Otherwise, the experimental differences might show up in $\Delta \log K$ values, which are the differences between the overall stability constants of both 1:1:1 ternary stability constants and corresponding 1:1 binary constants leading to errors in interpretation. However, our values agree well with those reported in the literature (Rajan and Martell 1964).

In table 5 are given the $\Delta \log K$ values for various metal ligand systems. It can be seen from the tables that the $\Delta \log K$ values are positive for all the systems studied which indicates that the formation of ternary complexes is preferred to that of binary complexes. These enhanced stabilities of ternary systems are due to the tendency of secondary ligands to form chelate rings. When the $\Delta \log K$ values of these systems are compared (EDTA, NTA and IMDA) it is found that the metal-cytidine-IMDA system has more positive $\Delta \log K$ values compared to other systems. These trends can be explained on the basis of steric factors. The less bulky IMDA will have least steric hindrance compared to NTA and EDTA resulting in the formation of more stable ternary complexes in solution. Based on our observation we proposed a tentative structure for metal-cytidine-IMDA system

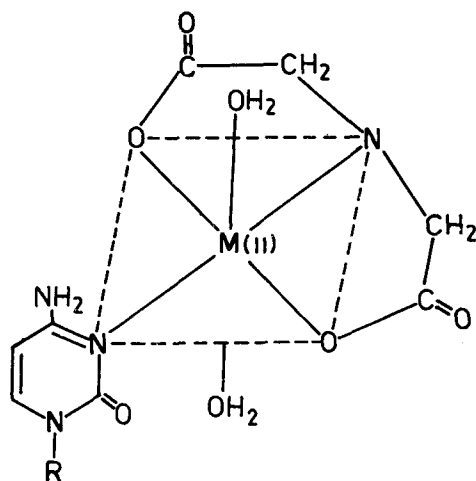


Figure 2. Tentative structure of 1:1:1 M(II) : cytidine : IMDA. R = Ribose.

Table 5. $\Delta \log K$ values for various metal-ligand systems in solution. Temperature = 35°C; $\mu = 0.10 \text{ mol dm}^{-3}$ (KNO_3).

Metal ion (II)	Metal:cytidine:IMDA (1:1:1)	Metal:cytidine: Na_2EDTA (1:1:1:1)	Metal:cytidine:NTA (1:1:1)
Cu	+1.71	+1.60	+0.85
Ni	+2.09	+1.54	+1.11
Zn	+2.96	+1.68	+1.65
Co	+2.93	+1.65	+1.48
Mg	+3.08	+2.13	+2.07
Ca	+2.89	+2.04	+2.02

(figure 2). It is of interest and importance to compare the $\Delta \log K$ values obtained for the interaction of metal-cytidine with biologically important secondary ligands—histidine and histamine (Rabindra Reddy and Malleswar Rao 1985) with corresponding values with amino carboxylic acids. The metal-cytidine-histidine and metal-cytidine-histamine form more stable complexes as is evident from their more positive $\Delta \log K$ values in spite of the fact that the amino carboxylic acids have larger numbers of donor groups. These results clearly suggest that the stacking interactions, which are expected to occur between two aromatic moieties of the ligands, seem to be the most effective phenomenon that contribute to the extra stabilization found in ternary complexes. This observation is in line with the results obtained earlier on the nucleoside complexes (Rabindra Reddy *et al* 1985) and their related systems (Rabindra Reddy and Madhusudhan Reddy 1986).

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