

## Synthesis and characterization of mixed ligand complexes of zinc(II) with cytidine and amino acids<sup>†</sup>

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Mixed ligand complexes of Zn(II) with cytidine and amino acids, L-alanine, L-phenylalanine and L-tryptophan have been synthesized and characterized by elemental analysis, conductivity data, electronic spectra, IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra. In these complexes, the nucleoside acts as a monodentate ligand involving only N(3) in metal coordination whereas the amino acids coordinate through carboxylate oxygen and amino nitrogen. The results drawn about binding sites of these octahedral complexes are compared with the results obtained from solution studies.

Nucleic acids and proteins recognise each other by very specific and selective interactions through amino acid side chains and nucleic acid constituents<sup>1,2</sup>. Most of these reactions are mediated through a metal ion resulting in the formation of mixed ligand complexes<sup>3,4</sup>. Study of these complexes has become necessary as they serve as models for many metalloenzyme reactions. Among the nucleic acid constituents - nucleosides - cytidine was considered simplest as far as its reactions with metals are concerned. However, even this nucleoside was found to exhibit the most complex behaviour as it was reported that the site of attachment of metal species to cytidine depends on the nature of metal ion. Hard metal ions bind to C-2, soft metal species to N-3 and zinc ion binds to both N-3 and O-2 (ref.5). Further, it was observed<sup>6</sup> that cytidine shows discriminating tendencies toward stacking interactions, a phenomenon of biological significance. In this paper, we report the synthesis and characterization of mixed ligand complexes of zinc with cytidine and various amino acids (L-alanine, L-phenylalanine and L-tryptophan). Based on IR, <sup>1</sup>H and <sup>13</sup>C NMR spectra, the binding modes were assigned. Cytidine acts as a monodentate ligand with exclusive N(3) binding whereas all the amino acids coordinate through carboxylate oxygen and amino nitrogen. The conclusions are compared with solution data for a more comprehensive understanding of these reaction sites specially at biological pH values.

### Materials and Methods

Cytidine (cyd), L-alanine (L-ala), L-phenylalanine (L-Phe) and L-tryptophan (L-Trypt) were obtained from Sigma Chemical Company (USA). Zinc chloride (BDH) was used as supplied. The <sup>1</sup>H NMR spectra were recorded at room temperature (20-25°C) on a Bruker AM 300/MHz pulsed FT NMR spectrometer in D<sub>2</sub>O which was also used as an internal standard. The proton decoupled <sup>13</sup>C NMR spectra were recorded at room temperature (20-25°C) on a Varian Unity-400 MHz and Varian Gemini-200 MHz spectrometer operating in FT mode with DSS ('0' ppm) (Sod-2, 2-dimethyl-2-silapentane-5-sulphonate) as an external reference. The IR spectra were recorded (in KBr discs) on infrared spectrophotometers; IR-435, Shimadzu in the 4000-400 cm<sup>-1</sup> region and Perkin Elmer FTIR. Far infrared spectra in the region 800-200 cm<sup>-1</sup> on Perkin Elmer 1430. The electronic spectra of the complexes were recorded in water on Shimadzu UV-160A, spectrophotometer. Conductivity measurements were performed using Digisun, Digital conductivity bridge (Model: DI-909) and a dip type cell calibrated with KCl solution. Metal analyses were obtained from microanalytical Perkin Elmer 240 C elemental analyser. The metal analysis were carried out on a AAS, Perkin-Elmer 2380.

### Synthesis of metal complexes

An aqueous solution containing 0.74 mmol of L-alanine and 0.73 mmol of cytidine were added simultaneously to another aqueous solution containing 0.81 mmol of zinc chloride; similarly the

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Table 1—Analytical and physical data of the complexes

Complex	Found (Calcd), %				$\Lambda_M$ ohm <sup>-1</sup> cm <sup>-1</sup> mol <sup>-1</sup> (in DMSO)
	C	H	N	M	
[Zn(cyd)(L-ala)(H <sub>2</sub> O) <sub>2</sub> Cl]	31.68	4.99	12.27	14.46	026
[Zn C <sub>12</sub> H <sub>23</sub> N <sub>4</sub> O <sub>9</sub> Cl]	(31.95)	(5.104)	(12.43)	(14.51)	
[Zn(cyd)(L-Phe)(H <sub>2</sub> O) <sub>2</sub> Cl]	40.90	5.09	10.69	12.19	025
[Zn C <sub>18</sub> H <sub>27</sub> N <sub>4</sub> O <sub>9</sub> Cl]	(41.01)	(5.13)	(10.63)	(12.41)	
[Zn(cyd)(L-Trypt)(H <sub>2</sub> O) <sub>3</sub> Cl]	40.96	5.08	12.08	11.28	072
[Zn C <sub>20</sub> H <sub>30</sub> N <sub>5</sub> O <sub>15</sub> ]	(41.11)	(5.14)	(11.99)	(11.19)	

Table 2—Some characteristics IR bands (cm<sup>-1</sup>)

Ligand/Complex	$\nu(\text{NH}_2)$ (Cyd)		$\nu(\text{NH}_2)$ (AA)		$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{COO}^-)$		Non-ligand bands			
	asy	sy	asy	sy			asy	sy	$\nu(\text{H}_2\text{O})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$	$\nu(\text{M-Cl})$
Free cytidine [C <sub>9</sub> H <sub>13</sub> N <sub>3</sub> O <sub>5</sub> ]	3450 s	3350 s	—	—	1660 s	1605 s	—	—	—	—	—	—
Free L-alanine [C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub> ]	—	—	—	—	—	—	1597 m	1412 s	—	—	—	—
[Zn(cyd)(L-ala)(H <sub>2</sub> O) <sub>2</sub> Cl]	3500 sh	3350 s	3175 s	3100 sh	1660- 1680 sh	1495 s	1635 s	1395- 1400 s	925 w	560 m	420 m	310 m
Free L-Phenylalanine [C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub> ]	—	—	—	—	—	—	1625 m	1410 s	—	—	—	—
[Zn(cyd)(L-Phe)(H <sub>2</sub> O) <sub>2</sub> Cl]	3350- 3450	—	3200 m	3100 sh	1710 1720	1490 1500	1640 m	1380- 1400	850 w	545 br	410 m	295 m
[Zn C <sub>18</sub> H <sub>27</sub> N <sub>4</sub> O <sub>9</sub> Cl]	s,br,asy,sy bands are merged)											
Free L-tryptophan [C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> ]	—	—	—	—	—	—	1665 s	1410 s	—	—	—	—
[Zn(cyd)(L-Trypt)(H <sub>2</sub> O) <sub>3</sub> Cl]	3300- 3450	—	3200 sh	3100 m	1720	1490 sh	1680 sh	1380- 1400 m	850 & 890	530 br	410 br	—
[Zn C <sub>20</sub> H <sub>30</sub> N <sub>5</sub> O <sub>15</sub> ]	(s,br,asy,sy bands are merged)											

br = broad, sh = shoulder, s = strong, m = medium, w = weak.

aqueous solution containing 0.74 mmol of L-phenylalanine and 0.74 mmol of cytidine were added simultaneously to another aqueous solution containing 0.77 mmol of zinc chloride finally an aqueous solution containing 0.73 mmol of L-tryptophan and 0.73 mmol of cytidine were added simultaneously to another aqueous solution containing 0.74 mmol of zinc chloride.

The above three types of mixtures were refluxed on a heating mantle upto 10-30h during which the colourless solutions changed to golden yellow colour (ppt not obtained), which provided physical evidence for the complex formation. The solutions were filtered and the filtrates were concentrated to half of the original volumes and kept *in vacuo* for ~ two months. Beautiful golden yellow coloured compounds were obtained which

were filtered and purified by recrystallisation from a mixture of solvents of methanol-ethyl acetate in 2:8 ratio. The purity of these compounds was established by TLC in various solvent systems.

#### Solution studies

The experimental method consisted of potentiometric titration of the free ligands and of solutions containing metal ion nucleoside (cytidine) and amino acids (L-alanine, L-phenylalanine and L-tryptophan) in a 1:1:1 ratio at 35 ± 0.1°C with standard sodium hydroxide solution. For binary systems, a 1:1 ratio was maintained. The experimental conditions maintained were the same as those described earlier<sup>7</sup>.

The ionization constants of various ligands were calculated using the computer program

Table 3—<sup>1</sup>H NMR chemical shifts at neutral pH of cytidine and amino acids in the absence and presence of zinc(II)

Ligand/Complex	Amino acids	Pyrimidine nucleoside	
		-CH-	
		C <sub>5</sub> H	C <sub>6</sub> H
Cytidine [C <sub>9</sub> H <sub>13</sub> N <sub>3</sub> O <sub>5</sub> ]	—	6.05(d)	7.86(d)
L-Alanine [C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub> ]	3.76(q)	—	—
[Zn(cyd)(L-ala)(H <sub>2</sub> O) <sub>2</sub> Cl] [Zn C <sub>12</sub> H <sub>23</sub> N <sub>4</sub> O <sub>9</sub> Cl]	3.67(q)	6.07(d)	7.89(d)
L-Phenylalanine [C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub> ]	3.97(t)	—	—
[Zn(cyd)(L-Phe)(H <sub>2</sub> O) <sub>2</sub> Cl] [Zn C <sub>18</sub> H <sub>27</sub> N <sub>4</sub> O <sub>9</sub> Cl]	3.87(t)	6.062(d)	7.88(d)
L-Tryptophan [C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> ]	4.03(t)	—	—
[Zn(cyd)(L-Trypt)(H <sub>2</sub> O) <sub>3</sub> Cl] [Zn C <sub>20</sub> H <sub>30</sub> N <sub>5</sub> O <sub>15</sub> ]	3.88(t)	5.99(d)	7.81(d)

Solvent = D<sub>2</sub>O (values are given in ppm), d = doublet, t = triplet, q = quartet.

Table 4—<sup>13</sup>C NMR chemical shifts at neutral pH of cytidine and amino acids in the absence and presence of zinc(II)

Ligand/Complex	Amino acid			Nucleotide moiety		
	COO <sup>-</sup>	C	C(2)	C(4)	C(5)	C(5)
Cytidine [C <sub>9</sub> H <sub>13</sub> N <sub>3</sub> O <sub>5</sub> ]	—	—	151.5	162.5	98.5	147.5
L-Alanine [C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub> ]	176.8	51.0	—	—	—	—
[Zn(cyd)(L-ala)(H <sub>2</sub> O) <sub>2</sub> Cl] [Zn C <sub>12</sub> H <sub>23</sub> N <sub>4</sub> O <sub>9</sub> Cl]	169.6	52.79	155.58	168.69	92.87	144.45
L-Phenylalanine [C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub> ]	170.0	55.5	—	—	—	—
[Zn(cyd)(L-Phe)(H <sub>2</sub> O) <sub>2</sub> Cl] [Zn C <sub>18</sub> H <sub>27</sub> N <sub>4</sub> O <sub>9</sub> Cl]	165.56	39.69	159.64	168.44	92.88	146.17
L-Tryptophan [C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> ]	174.40	54.93	—	—	—	—
Zn(cyd)(L-Trypt)(H <sub>2</sub> O) <sub>3</sub> Cl [Zn C <sub>20</sub> H <sub>30</sub> N <sub>5</sub> O <sub>15</sub> ]	168.79	48.16	154.0	168.5	93.00	144.80

PKAS<sup>8</sup>. The formation constant values were subjected to refinement using the computer program BEST<sup>9</sup>. BEST was also used to generate the complete species distribution curves of various pH values. The refinement of the stability constants of ternary systems was done by considering all possible species present in the solution, i.e. HL<sup>+</sup>, HA, L<sup>-</sup>, L, A<sup>-</sup>, ML, ML<sub>2</sub>, MA, MA<sub>2</sub> and MAL. The error limits in these constants were minimised (sigma fit = 0.001).

### Results and Discussion

The analytical and conductivity data of the complexes are presented in Table 1. The analytical data corresponds with metal: cyd: AA ratio of

1:1:1 and with two moles of water per mole of Zn(II) in complexes 1 and 2 and three moles of water in complex 3. The conductivity values in DMSO/H<sub>2</sub>O correspond to 1:1 electrolyte for complex 3 while those for 1 and 2 show that they are non-electrolytes<sup>10</sup>. The electronic spectral absorption bands and their assignments for the complexes are discussed. The complexes of L-ala, L-Phe and L-Trypt show absorption bands in the range 271-275 nm which are assignable to LMCT bands.

The infrared spectra (Table 2) of these ternary complexes in comparison with free cytidine and respective free amino acid show characteristic band positions, band shifts and band intensities which can

be correlated to monodentate cytidine binding and bidentate amino acids chelation besides metal binding through water molecules and chloride is also evidenced by the IR spectra. The characteristic IR bands of free cytidine corresponding to  $\nu$   $\text{NH}_2$  anti sym and sym and  $\nu$   $\text{C}=\text{O}$  are shown in the spectra of the complexes without any negative shifts, thus ruling out their participation in coordination. Nominal upward shifts in these vibrational frequencies are presumed to be the consequence of involvement of cytidine in coordination through a different coordination site, probably the azomethine nitrogen site. There is a considerable shift in  $\nu$   $\text{C}=\text{N}$  ( $\Delta \nu_{\text{C}=\text{N}} \sim 115 \text{ cm}^{-1}$ ) corresponding with the N(3) of cytidine. This unambiguously suggests coordination through the respective nitrogen.

As regards chelation through amino acids, the IR spectra exhibit significant features in  $\nu$   $\text{NH}_2$  and  $\nu$   $\text{COO}^-$  regions. It is worthwhile to mention here that the free amino acids exist as Zwitter ions ( $\text{NH}_3^+$  A.A.  $\text{COO}^-$ ) and the IR spectra of these cannot be compared entirely with those of metal complexes as amino acids in metal complexes do not exist as Zwitter ions; particularly free amino acids with  $\text{NH}_3^+$  functions show  $\nu$   $\text{NH}_3^+$  in the range of  $3130\text{-}3030 \text{ cm}^{-1}$ . In the complexes,  $\text{NH}_3^+$  gets deprotonated and binds to metal through neutral  $\text{NH}_2$  group. The transformations of  $\text{NH}_3^+$  to  $\text{NH}_2$  must result in an upward shift in  $\nu$   $\text{NH}_2$  and free amino acids. At isoelectric point, they must show  $\nu$   $\text{NH}_2$  in the region  $3500\text{-}3300 \text{ cm}^{-1}$ . In the present complexes, the IR spectra show characteristic bands in the region  $3200\text{-}3100 \text{ cm}^{-1}$  which is lower in comparison with free  $\nu$   $\text{NH}_2$ . Hence, it can be concluded that nitrogen of the amino group is involved in coordination. The IR spectra show strong evidences in support of the involvement of carboxylate group in coordination. In comparison with free amino acids, the  $\nu$   $\text{COO}^-$  (asy) shows positive shifts and  $\nu$   $\text{COO}^-$  (sym) record negative shifts which are conformationary evidences in support of monodenticity<sup>11</sup>.

Thus it may be concluded that amino acids behave as monobasic bidentates in these complexes involving amino nitrogen and carboxylate oxygen in coordination. This spectra show additional features which can be correlated with coordination through water molecules and chlorides as the spectra show broad strong band in the region  $3500\text{-}3100 \text{ cm}^{-1}$  which broadly coincide with  $\nu$ -OH of water molecules. These broad bands show distinct structures which correspond to  $\nu$ - $\text{NH}_2$  of cytidine and amino acids as discussed earlier. The

Table 5—Solution data\* pertaining to the interaction of zinc(II) with cytidine and various amino acids  
[Temp =  $25^\circ\text{C}$ ;  $\mu = 0.10 \text{ M}$  ( $\text{KNO}_3$ )]

Ligand/Complex	$pK_a$	Stability constants of binary complexes	Stability constants of ternary complexes
Cytidine [ $\text{C}_9\text{H}_{13}\text{N}_3\text{O}_5$ ]	4.28	2.68	—
L-Alanine [ $\text{C}_3\text{H}_7\text{NO}_2$ ]	9.61	5.05	4.98
L-Phenylalanine [ $\text{C}_9\text{H}_{11}\text{NO}_2$ ]	8.85	4.74	5.11
L-Tryptophan [ $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$ ]	9.39	5.16	6.18

\*The constants are accurate upto  $\pm 0.04 \text{ pK}$  and  $\log K$  units.

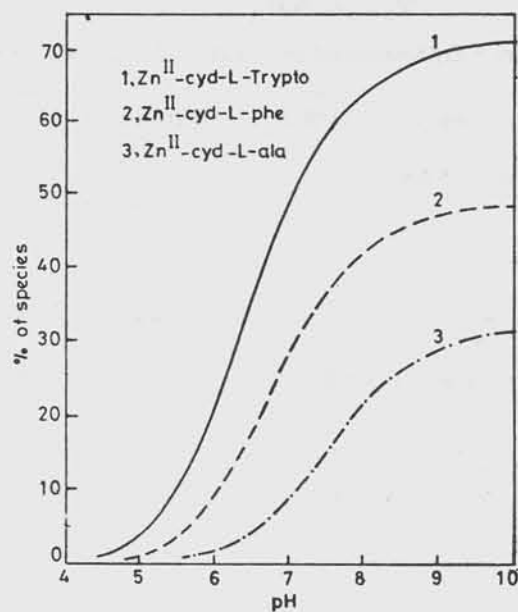


Fig. 1—Species distribution curves of 1:1:1 zinc(II)-cytidine-amino acid systems at  $25^\circ\text{C}$ ,  $\mu = 10 \text{ M}$  ( $\text{KNO}_3$ )

presence of coordinated water is further confirmed by non-ligand bands observed in the range  $925\text{-}850 \text{ cm}^{-1}$  due to rocking mode of coordinated water. Other low intensity bands observed in far IR region, in the range  $295\text{-}560 \text{ cm}^{-1}$  are due to  $\nu(\text{M}-\text{Cl})$ ,  $\nu(\text{M}-\text{O})$  and  $\nu(\text{M}-\text{N})$  (ref. 11) stretchings. In complex 3, no evidence was found for the coordination of chloride ion.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments at neutral pH are summarized in Table 3 and 4. They were identified by the literature assignments<sup>12-14</sup>.

The shifts in the H(5) and H(6) resonances of cytidine in the complexes indicate the involve-

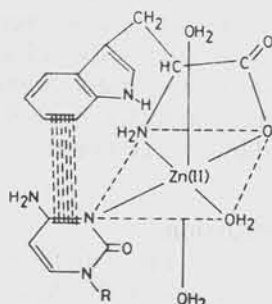
ment of N(3) in metal coordination. The 'CH' resonances of amino acids were shifted to upfield in metal complexes compared to free amino acids since zinc will cause deprotonation of  $\text{NH}_3$  proton on binding. The magnitude of the shifts indicate the involvement of carboxylate groups in metal coordination.

The downfield shifts of C(2) and C(4) resonances and upfield shifts of C(5) and C(6) resonances in  $^{13}\text{C}$  spectra of cytidine in all the complexes investigated confirm the exclusive participation of N(3) in metal binding. This is in agreement with the earlier observations made by Marzilli *et al.*<sup>6</sup> that  $^{13}\text{C}$  NMR provides definite evidence regarding N(3), O(2) binding depending on the direction of the shift of C(2) resonance. In the case of amino acids, the  $\alpha$ -carbon and carboxylate carbon resonances are significantly shifted in metal complexes thus providing definite evidence for the involvement of carboxylate and amino groups in zinc coordination.

Based on these observations, an octahedral geometry may be proposed for the complexes.

#### Solution studies

The species distribution curves for various systems under investigation based on Table 5 are given in Fig.1. It can be seen from the figure that the percentage of formation of various ternary complexes vary from 30-70% from alanine to tryptophan systems in the order: L-alanine < L-phenylalanine < L-tryptophan indicating the dependence of stabilities on the aromatic ring size. This is in agreement with conclusions reached by Leporati and Odani *et al.*<sup>15,16</sup>. It is reasonably established<sup>17-19</sup> that apart from various factors like nature of the metal ion, the ligand and geometry of the metal complex etc., the stacking interaction (wherever possible) seems to be most effective in



Tentative structure of zinc(II)-cytidine-tryptophan showing stacking interaction, (R=ribose)

enhancing the stability of the ternary complexes in solution. Thus, L-alanine, being an aliphatic ligand cannot participate in stacking interactions, resulting in the least percentage of the formation of ternary species in solution. However, in the case of L-phenylalanine and L-tryptophan, the aromatic moiety of these ligands are oriented in such a way that these are exactly parallel to cytidine ring, which is perpendicular to metal plane. This results in favourable stacking interactions as shown in structure I and hence the higher percentages of formation of ternary complexes in solution. In conclusion, it is evident from this investigation that cytidine, which was considered as the simplest example of metal ion nucleic acid interactions, proved to be discriminatory even in its binding to zinc.

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