

## Interaction of metal ions with uridine 5'-monophosphate: Existence of possible intramolecular interactions

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Received 5 August 1991; revised and accepted 14 April 1992

Interaction of  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{La}^{3+}$ ,  $\text{Pr}^{3+}$ ,  $\text{Nd}^{3+}$ ,  $\text{Gd}^{3+}$  and  $\text{Dy}^{3+}$  with 5'-uridine monophosphate (5'-UMP) as the primary ligand and glycine (gly), histidine (histi) and histamine (hista) as secondary ligands in 1:1 and 1:1:1 ratio has been investigated potentiometrically at 35°C and 0.10 M ( $\text{KNO}_3$ ) ionic strength. The stability constants of binary (1:1) and ternary (1:1:1) systems are compared to assess the influence of charge of the metal ions and the nature of secondary ligands on the structure and stabilities of these complexes. The stabilisation of the ternary complexes is measured in terms of  $\Delta \log K$  which is the difference between the stabilities of binary and ternary complexes in solution. Based on geometry of metal-5'-UMP complexes, coordination number of the metal ions concerned and the steric factors, it has been concluded that O(4) is also involved in coordination in addition to the known phosphate binding in binary complexes. However, in ternary systems only phosphate is involved in metal coordination.

Nucleic acid-protein interactions constitute one of the important natural interactions. Therefore, a study of these interactions at monomer level is worthwhile as they provide good models for these biological interactions. In the present study we have investigated the interactions of 5'-uridine monophosphate (5'-UMP) with  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{La}^{3+}$ ,  $\text{Pr}^{3+}$ ,  $\text{Nd}^{3+}$ ,  $\text{Gd}^{3+}$  and  $\text{Dy}^{3+}$  in the absence and presence of secondary ligands glycine, histidine and histamine to assess the influence of secondary ligands on the structure and stability of 1:1 metal-5'-UMP complexes.

Earlier work in solution of 5'-UMP relates to the study of its interaction with  $\text{Cu}^{2+}$  (ref. 1, 2) and  $\text{Mn}^{2+}$  at neutral pH by NMR spectroscopy<sup>3</sup>. It was concluded, based on preferential line broadening, that in addition to phosphate coordination there is some metal ion-base interaction. Similar observations were made earlier in the case of  $\text{Mg}^{2+}$  complexes<sup>4</sup>. However, earlier potentiometric studies<sup>5,6</sup> did not confirm the metal ion-base interactions though studies with 5'-UTP showed a lowering in the  $pK$  value for N(3) in the presence of  $\text{Cu}^{2+}$  (ref. 7).

A more clear picture on the bonding modes of 5'-UMP emerged from the X-ray crystallographic data, though it conflicted with the solution studies. For example, Goodgame *et al.*<sup>8</sup> have shown in the case of  $\text{Co}^{2+}$ : 5'-UMP system that only phosphate is involved in metal coordination. Fischer and

Bau<sup>9</sup> have reached the same conclusions with a ternary system. Aoki<sup>10</sup> and other workers<sup>11</sup> confirmed bonding by phosphate only in  $\text{Cd}^{2+}$  complexes.

Recent studies involving the preparation and characterization of  $\text{Ni}^{2+}$  (ref. 12) and  $\text{Cr}^{3+}$  (ref. 13) complexes at neutral pH indicate direct bonding through phosphate group with additional interaction with base through C(4)O. The non-involvement of N(3) in metal coordination when there is a metal ion-base interaction may be due to the presence of a proton at this site under the experimental conditions employed. However,  $\text{CH}_3\text{Hg}^{2+}$  is shown to bind exclusively to N(3) of the base<sup>14-16</sup>.

Thus, it is clear that so far no attempt has been made to study the metal ion interaction with 5'-UMP in the basic region where N(3)-H dissociates making the site competitive with other potential binding sites in the ligand for metal coordination.

Therefore, it was thought important to investigate the metal ion interaction with 5'-UMP both in binary and ternary systems in the pH range 4.5-10 to identify the binding modes which are highly pH dependent. We have extended these studies to trivalent lanthanons to verify the effect of size and charge of the metal ions on the stability of these complexes. The structures of primary and secondary ligands are depicted in Chart 1 for ready reference.

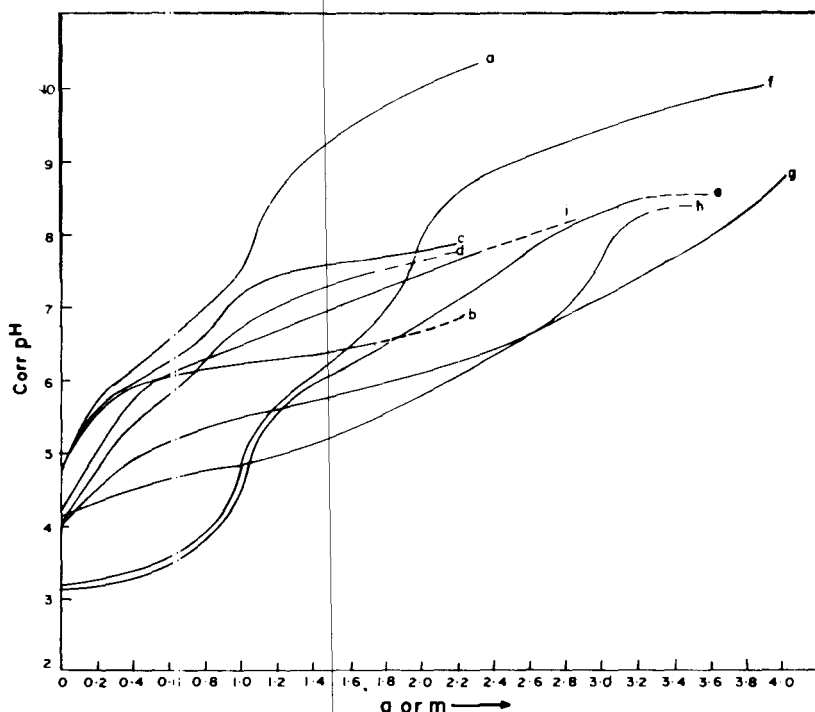


Fig. 1—Potentiometric titration curves for the interaction of 5'-UMP with bivalent and trivalent metal ions both in binary (1 : 1) and ternary (1 : 1 : 1) system at  $35 \pm 0.1^\circ\text{C}$   $I = 0.10 M$  ( $\text{KNO}_3$ ) [(a) Free 5'-uridine monophosphate; (b)  $\text{Cu(II)}:5'\text{-UMP}$  (1 : 1); (c)  $\text{Zn(II)}:5'\text{-UMP}$  (1 : 1); (d)  $\text{La(III)}:5'\text{-UMP}$  (1 : 1); (e)  $\text{Ni(II)}:5'\text{-UMP}:\text{glycine}$  (1 : 1 : 1); (f)  $\text{Mg(II)}:5'\text{-UMP}:\text{glycine}$  (1 : 1 : 1); (g)  $\text{Cu(II)}:5'\text{-UMP}:\text{histamine}$  (1 : 1 : 1); (h)  $\text{Co(II)}:5'\text{-UMP}:\text{histidine}$  (1 : 1 : 1); (i)  $\text{La(III)}:5'\text{-UMP}:\text{histidine}$  (1 : 1 : 1)].

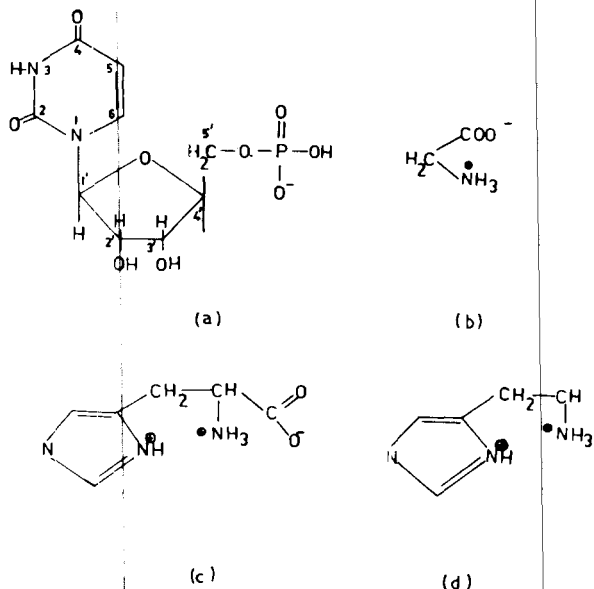


Chart 1—(a) 5'-Uridine monophosphate; (b) glycine; (c) histidine; and (d) histamine.

### Materials and Methods

5'-Uridine monophosphate (5'UMP), glycine (gly), histidine (histi) and histamine (hista) were obtained from Sigma Chemical Co. (USA). Transition and alkaline earth metal ions were of AR

Table 1—Ionisation constants\* of ligands

[Temp. =  $35 \pm 0.1^\circ\text{C}$ ;  $\mu = 0.10 M$  ( $\text{KNO}_3$ )]

Ligand	$pK_a$	$pK_{2a}$	$pK_{3a}$
5'-UMP	0.7**	6.34	9.53
Glycine	2.33	9.75	—
Histidine	6.00	9.00	—
Histamine	5.87	9.63	—

\*The constants are accurate to  $\pm 0.05 pK$  units.

\*\*From reference 21.

grade and all rare earth oxides were of Johnson Matthey's spectral grade.

The experimental method consisted of potentiometric titration of metal ions and 5'UMP in the absence and presence of secondary ligands glycine, histidine and histamine in 1 : 1 and 1 : 1 : 1 ratios respectively at  $35 \pm 0.1^\circ\text{C}$  with standard NaOH solution. The ionic strength was maintained constant by using  $0.10 M$  ( $\text{KNO}_3$ ) as the supporting electrolyte and relatively low concentrations of ligand and metal ion ( $1 \times 10^{-3} M$ ). During the course of the titrations a stream of nitrogen was passed over the solution to eliminate the adverse effect of atmospheric carbon dioxide,

Table 2—Stability constants\* of binary and ternary complexes of M(II) and 5'-UMP with glycine, histidine and histamine (1 : 1 : 1) systems

[Temp. = 35 ± 0.1°C;  $\mu = 0.10 M (KNO_3)$ ]

Metal ion	M <sup>2+</sup> : 5'UMP (1 : 1)			M <sup>2+</sup> : 5'UMP : gly			M <sup>2+</sup> : 5'UMP : histi			M <sup>2+</sup> : 5'UMP : hista		
	$K_{ML}^M$	$K_{M(HL)}^M$	$K_{ML}^{M(HL)}$	$K_{MAL}^{M(HA)}$	$K_{M(HA)HL}^M$	$K_{MAL}^{M(HA)HL}$	$K_{MAL}^M$	$K_{MA(HL)}^M$	$K_{M(HA)HL}^M$	$K_{MAL}^M$	$K_{MA(HL)}^M$	$K_{M(HA)HL}^M$
Cu(II)	6.57	—	—	13.51	—	—	15.77	—	—	15.86	—	—
Ni(II)	—	2.18	3.97	12.34	—	—	—	13.43	—	—	11.02	—
Zn(II)	—	2.23	4.94	12.92	—	—	—	11.33	—	—	10.70	—
Co(II)	—	2.06	3.87	11.32	—	—	—	11.66	—	—	10.94	—
Mg(II)	—	1.80	2.44	—	2.07	6.71	—	—	7.12	—	—	6.50
Ca(II)	—	1.70	2.30	—	2.04	6.69	—	—	7.05	—	—	6.42

\*The constants are accurate to ± 0.06 log *K* units

Table 3—Stability constants\* of binary and ternary complexes of M(III) and 5'-UMP with glycine, histidine, histamine (1 : 1 : 1) systems

[Temp. = 35 ± 0.1°C;  $\mu = 0.10 M (KNO_3)$ ]

Metal ion	M <sup>3+</sup> : 5'-UMP (1 : 1)		M <sup>3+</sup> : 5'-UMP : gly (1 : 1 : 1)		M <sup>3+</sup> : 5'-UMP : histd (1 : 1 : 1)		M <sup>3+</sup> : 5'-UMP : histm (1 : 1 : 1)	
	$K_{M(HL)}^M$	$K_{ML}^{M(HL)}$	$K_{M(HA)HL}^M$	$K_{M(HA)HL}^M$	$K_{M(HA)HL}^M$	$K_{M(HA)HL}^M$		
La(III)	3.59	5.47	3.64	8.35	7.72			
Pr(III)	3.65	5.80	3.91	8.62	7.91			
Nd(III)	3.78	5.94	3.98	8.73	8.05			
Gd(III)	3.89	6.05	4.00	8.92	8.12			
Dy(III)	4.33	6.29	4.52	9.06	8.49			

\*The constants are accurate to ± 0.06 log *K* units.

other experimental details can be found elsewhere<sup>17</sup>.

### Calculations

For the calculation of stability constants of bivalent and trivalent ternary systems suitable material balanced equation were set up and solved with the help of computer program BEST<sup>18</sup>. However, it was noticed that different types of interactions were observed with the metal ions under investigation.

The constants for transition metal-5'-UMP-glycine, histidine and histamine systems were calculated using equations from ref. 19. For Mg(II), Ca(II) and lanthanide systems the equations described in ref. 20 were used.

Experiments were conducted at three different concentrations to assess the influence of concentration on stabilities. Since there was no appreciable change in the stabilities it was assumed that the probability of formation of species other than those described above is remote. The possible hydrolysis of free metal ion over the experimental

pH range was ruled out by performing independent metal ion titrations and comparing the curves with those obtained for binary systems. Species distribution curves were generated using computer program BEST.

### Results and Discussion

#### (a) Metal : 5'-UMP : glycine (1 : 1 : 1) system

The mixed ligand titration curve for Ni<sup>2+</sup> system (Fig. 1e) shows an inflection at *m* = 1 followed by a buffer region and a precipitate around *m* = 3.5. In the buffer region *m* = 0-1, only 1 : 1 (metal : glycine) complex formation takes place. (The stability constant obtained in this buffer region is found to be similar to that of a simple 1 : 1 binary constant). The behaviour of rest of the metal ions was similar in this buffer region. Thus, the ternary complex formation takes place only after *m* = 1. Accordingly, it was assumed that the simultaneous dissociation of two protons takes place. Cu<sup>2+</sup>, Zn<sup>2+</sup> and Co<sup>2+</sup> behaved in a similar manner. The constants were calculated taking the ex-

Table 4— $\Delta \log K^*$  values for various metal-ligand (1 : 1 : 1) systems in solution

[Temp. =  $35 \pm 0.1^\circ\text{C}$ ;  $\mu = 0.10 \text{ M (KNO}_3\text{)}$ ]

Metal ion	M : 5'-UMP : gly (1 : 1 : 1)	M : 5'-UMP : histi (1 : 1 : 1)	M : 5'-UMP : histi (1 : 1 : 1)
Cu(II)	—	-0.82	-0.95
Ni(II)	—	+2.83	+2.48
Zn(II)	—	+2.75	+2.36
Co(II)	—	+2.82	+2.60
Mg(II)	+0.27	+0.95	+2.02
Ca(II)	+0.34	+0.94	+1.91
La(III)	+0.05	+1.35	+1.02
Pr(III)	+0.26	+1.49	+1.08
Nd(III)	+0.20	+1.47	+1.03
Gd(III)	+0.19	+1.68	+1.15
Dy(III)	+0.19	+0.97	+0.68

\*The values are calculated taking appropriate binary constants of secondary ligands from reference 26.

perimental points far below the precipitation region<sup>19</sup>. In the case of  $\text{Cu}^{2+}$  system the constant  $K_{\text{MAL}}^{\text{M(HA)}}$  was computed in the entire buffer region.

However,  $\text{Mg}^{2+}$  (Fig. 1f) and  $\text{Ca}^{2+}$  show an additional inflection at  $m=2$  followed by a buffer region. The constant  $K_{\text{M(HA)HL}}^{\text{M(HA)}}$  was computed in the buffer region  $m=1-2$  (ref. 21).

For  $\text{M}^{3+} : 5\text{'-UMP} : \text{glycine}$  systems, an inflection was obtained at  $m=1$  followed by a precipitate at  $m=1.8$ . The probability of formation of various complexes  $K_{\text{MAL}}^{\text{M(HA)}}$ ,  $K_{\text{MA(HL)}}^{\text{M(HA)}}$ ,  $K_{\text{ML(HA)}}^{\text{M(HA)}}$ ,  $K_{\text{M(HA)HL}}^{\text{M(HA)}}$  was assumed and based on comparison with binary data, pH range, behaviour of similar hard metal ions and species distribution curves it was confirmed that the last probability is the most predominant one.

#### (b) Metal : 5'-UMP : histidine/histamine (1 : 1 : 1) system

It was assumed, based on titration curve of  $\text{Cu}^{2+} : 5\text{'-UMP} : \text{histamine}$  (Fig. 1g), that a simultaneous release of protons takes place and the constant  $K_{\text{MAL}}^{\text{M}}$  was calculated<sup>11</sup>.  $\text{Cu}^{2+}$  forms similar complexes with histidine also.

The mixed ligand titration curve of  $\text{Co}^{2+} : 5\text{'-UMP} : \text{histidine}$  (Fig. 1h) shows an inflection at  $m=3$ . Solid phase appears after this inflection. The constant  $K_{\text{MA(HL)}}^{\text{M}}$  was computed in the buffer region  $m=0-3$ <sup>19</sup>.  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  systems showed similar behaviour. The behaviour of these metal ions was similar with histamine also.

The mixed ligand titration curves of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  show an inflection at  $m=2$  followed by precipitation. The formation of protonated complex  $K_{\text{M(HA)HL}}^{\text{M}}$  was assumed in the region  $m=0-2$ .

In (Fig. 1i) is given the titration curve of  $\text{La}^{3+} : 5\text{'-UMP} : \text{histidine}$ . A precipitate appeared around  $m=2$  for all the trivalent ternary systems. The formation of  $K_{\text{M(HA)HL}}^{\text{M}}$  was assumed based on similar reasons as cited in section (a).

The  $pK$  values of 5'-UMP and various secondary ligands are presented in Table 1.

The stability constants of binary (1 : 1)  $\text{M}^{2+} : 5\text{'-UMP}$  complexes are listed in Table 2. The protonated constants are in fair agreement with the recently reported values<sup>21</sup>. However, the normal 1 : 1 metal : 5'-UMP constants are reported for the first time. Further, the protonated constants are comparable with the corresponding constants of metal phosphate complexes<sup>21</sup> indicating exclusive phosphate interactions in this buffer region. X-ray crystallographic studies also support this view<sup>8</sup>. However, the data for the normal 1 : 1 complexes suggest the possibility of an intramolecular interaction. This is contrary to the literature reports<sup>21</sup> (i.e., intramolecular interactions are absent in pyrimidine nucleotides). When the stability constants of the corresponding (M-RibMP)<sup>21</sup>, (M-Uridine)<sup>19</sup> and (M-UMP) systems are compared, it is found that the (M-UMP) constants are comparable neither to (M-uridine) nor to (M-RibMP) constants. Had there been no intramolecular interactions in 5'UMP, the data would have been close to (M-RibMP) data if only phosphate binding is envisaged or to (M-uridine) data if only base binding is involved. Therefore, it is proposed that an intramolecular interaction exists in these systems. Then the question arises about the possible binding modes in the molecule. In basic medium the N(3) site is deprotonated

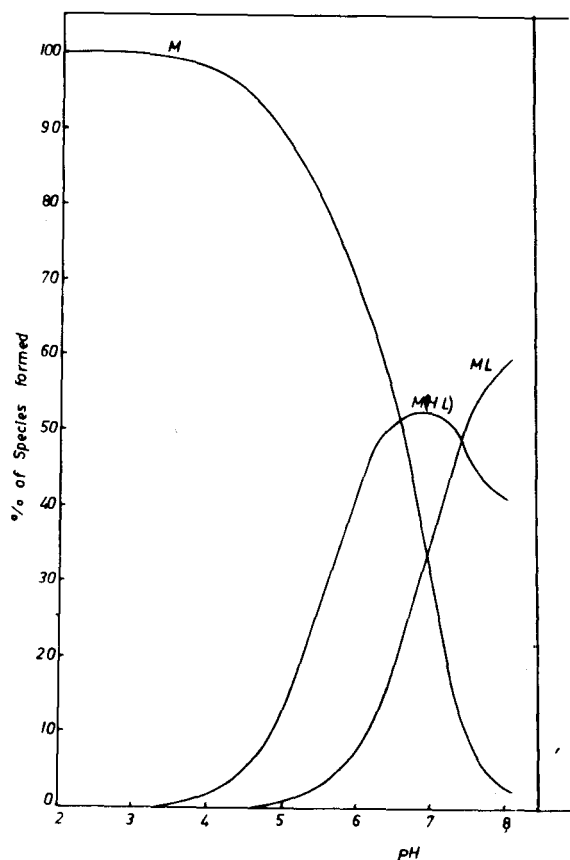


Fig. 2—Species distribution curve of  $\text{La}^{3+} : 5'\text{-UMP}$  (1 : 1) binary system

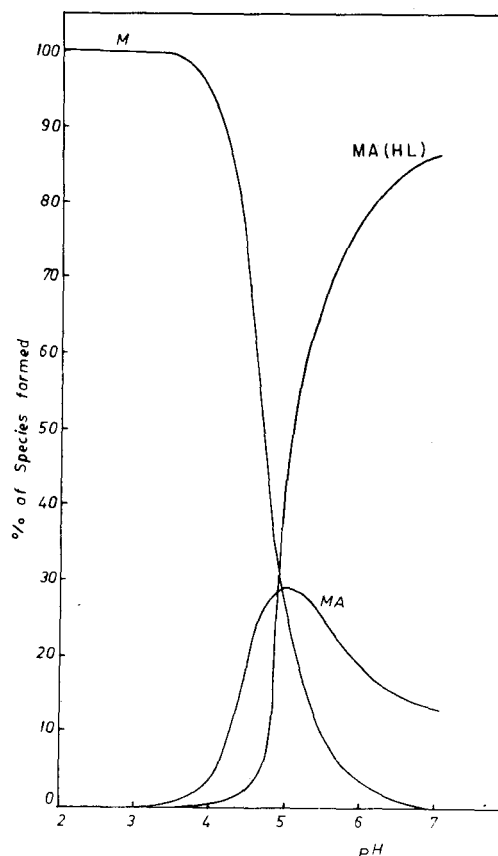


Fig. 3—Species distribution curve of  $\text{Ni}^{2+} : 5'\text{-UMP} : \text{histidine}$  (1 : 1 : 1) system

consequently a metal ion can bind to N(3) or O(4). However, O(4) may be preferred over N(3) because of its high charge density. Since there is no evidence of metal ions forming simultaneous innersphere complexes with nucleotide monophosphates there exists a possibility of phosphate involvement through a water molecule in addition to O(4) coordination. Similar observations were made earlier<sup>12,13</sup>.

The stability constants pertaining to the interaction of trivalent lanthanons and 5'-UMP are presented in Table 3. It can be seen from the table that the stability constants of 1 : 1  $\text{M}^{3+} : 5'\text{-UMP}$  are greater than those of the corresponding bivalent metal ions. This may be due to the higher value of charge/radius ratio of the former metal ions. The higher ratio of lanthanons permits a closer approach of ligands and better electrostatic interaction resulting in the greater stability of these complexes. This is further reflected in their species distribution curves. The species distribution curve of  $\text{La}^{3+} : 5'\text{-UMP}$  (Fig. 2) shows the formation of the complex to the extent of 52%; in contrast, only 40% of the species formed with the

bivalent metal ions suggesting that the lanthanons bind more effectively with phosphate group. This is in agreement with the known view that lanthanides bind exclusively to phosphate group in 5'-UMP<sup>22</sup>. In the basic region the data suggest that there is an additional interaction with base moiety of 5'-UMP. Based on the conclusions reached in the case of lanthanide : uracil complex<sup>23</sup>, it is inferred that these metal ions coordinate through phosphate oxygens with an additional interaction of O(4) through bridged water molecules.

The ternary constants of various systems are given in Tables 2 and 3. It can be seen from the tables that different types of complexes are formed in the case of both bivalent and trivalent metal ions. This specificity does not confine to any particular metal ion or ligand but varies with metal ion to metal ion and ligand to ligand.

The closeness in the ternary stability constants of  $\text{Cu}^{2+} : 5'\text{-UMP} : \text{histidine/histamine}$  systems suggests that histidine is acting like histamine. The corresponding  $\Delta \log K$  values (the  $\Delta \log K$  is the difference between the binary and ternary constants) are presented in Table 4. The negative

$\Delta \log K$  values indicate the amount of destabilization in ternary systems. This suggests that the 5'-UMP is bonded through the base moiety along with indirect phosphate chelation as the direct phosphate involvement would have resulted in the positive  $\Delta \log K$  values as it favours stacking interactions.

$Ni^{2+}$ ,  $Zn^{2+}$  and  $Co^{2+}$  form monoprotonated ternary complexes whereas  $Mg^{2+}$  and  $Ca^{2+}$  form diprotonated complexes with histidine and histamine. In these cases the experimental  $pH$  range was restricted to neutral  $pH$  owing to the formation of a precipitate in the region of base  $pK$ . Therefore, in the above systems the 5'-UMP coordinates only through the phosphate group in addition to the secondary ligand binding. This is clearly evident from the species distribution curve of  $Ni^{2+}$ :5'-UMP:histidine system (Fig. 3) where the formation of the protonated complex reaches a maximum of 85% at  $pH$  7. This type of interaction favours stacking as base moiety is free to stack with the imidazole moieties of the histidine or histamine. This is further reflected in the positive  $\Delta \log K$  values of these systems (Table 4).

However, different types of complexes have been observed in the case of  $M^{2+}$ :5'-UMP:glycine systems resulting in the evaluation of  $\Delta \log K$  values only for  $Mg^{2+}$  and  $Ca^{2+}$ . These values are positive though lower in magnitude compared to those for histidine and histamine systems, which indicates that the ternary complex formation is favoured due to less steric hindrance and the participation of mixed N/O donor atoms.

These specific and selective interactions were also observed in case of trivalent lanthanons. The trivalent lanthanons form diprotonated ternary complexes with 5'-UMP and histidine or histamine, similar to those observed in the case of  $Mg^{2+}$  and  $Ca^{2+}$  ternary complexes. This suggests that the mode of bonding is similar in these systems, i.e., 5'-UMP coordinates only through phosphate. This is in accord with the HSAB<sup>24</sup> principle.

However, the differences in the magnitude of the  $\Delta \log K$  values observed are due to the differences in the stabilities of binary<sup>25</sup> and ternary complexes of respective bivalent and trivalent metal ions.

The  $\Delta \log K$  values for the  $M^{3+}$ :5'-UMP:glycine system are less positive compared

to those for histidine and histamine system. This is in line with the expected interactions of glycine, histidine and histamine ligands where the latter two are known to participate in stacking interactions, thus enhancing the stability of the ternary complexes.

Finally, it is clear from this investigation that the metal ion interaction with pyrimidine nucleotides is highly  $pH$  dependent and also varies with metal ion to metal ion and ligand to ligand indicating their high specificity.

### Acknowledgement

The financial assistance from the UGC, New Delhi [Scheme No. F. 12-87/84 (SR-III)], is gratefully acknowledged.

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