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Quantitative aspects of intramolecular interactions in ternary complexes of purine nucleotides and amino acids

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Interaction of bivalent metal ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) with purine nucleotides (5'- guanosinemono-phosphate and 5' – inosinemonophonsphate) and amino acids (alanine, phenylalanine and tryptophan) in 1:1 and 1:1:1 ratios has been investigated by potentiometric *p*H measurements and formation constants in various systems are determined by computer program. The extra stabilization is expressed in terms of $\Delta\log K$. The hydrophobic interactions associated with different amino acids and nucleotides have been identified with parameters like $\Delta\Delta\log K$, K_L % of $(MLA)_{st}$, and ΔG^0. The *p*H profiles of various species are generated in order to identify the stable species.

The report of intramolecular stacking interactions between the purine moiety of adenosine 5' -triphosphate (ATP^{4-}) and the aromatic rings of 2,2' -bipyridine (Bpy) in ternary M (Bpy) $(ATP)^{2-}$ complexes[1] has led to spurt in the investigations[2-5] of such interactions in solution. It was also observed that the degrees of intramolecular stacking were high in system containing nucleotides[6]. The metal ions stabilise, destabilise or modulate such processes by introducing conformational changes through electronic effects[7,8]. Hence it was thought important to investigate the metal ion mediated intramolecular interactions of nucleotides and amino acids.

In the present paper a detailed study on the interactions of Co(II), Ni(II), Cu(II) and Zn(II) with purine nucleotides viz. GMP, IMP (5' -monophates of guanosine and inosine respectively) and amino acids [alanine (Ala), phenylalanine (Pha), tryptophan (Trp)] are reported. Since GMP differs from IMP by way of having an additional exocyclic substituent amino (NH_2) at C(2) position, this study provides an opportunity to assess the influence of this substituent on the stabilities. Among the amino acids, alanine being the simplest was chosen as a reference to probe the effect of amino acid part on these interactions.

Materials and Methods

The disodium salts of 5' -GMP and 5' -IMP, ala, pha and trp were procured from Sigma Chemical Company, USA. For every titration fresh ligand solution was prepared. Stock solutions of analytically pure metal nitrates were prepared and their concentrations determined by EDTA titrimetry[9]. The experimental method[10] involved a potentiometric titration of ligands in the absence and presence of metal ions at 35.0.± 0.10°C against standard carbonate free NaOH[11]. The ionic strength was maintained constant at 0.10 *M* using KNO_3 (from BDH, Germany) as the supporting electrolyte using low concentration of the reactants (1×10^{-3} *M*). During the experiment oxygen free N_2 was passed through the titration cell to avoid the adverse effect of atmospheric CO_2. Each experiment was repeated at twice to get concurrent readings.

Calculations

The acid dissociation constants of various ligands determined by the computer program PKAS[12] are presented in Table 1.

Binary systems

In M(II) GMP/IMP (1 : 1) binary systems, different types of complexes were observed. Cu(II) Zn(II) form protonated complexes, their formation constants evaluated using Eq. (1). (charges are omitted)

$$M + H_2L \Leftrightarrow MHL + H^+ \qquad ...(1)$$

In the case of Co (II) -GMP/IMP and Ni (II) -GMP, the following equations were used.

Table 1 – Ionization constants* of the ligands and formation constants** of binary complexes of metal ions
Temp. = 35°C; I = 0.1*M* (KNO_3),

Metal ion	M-GMP (pK_a = 6.36, pK_{2a} = 9.36)		M-IMP (pK_a = 6.32, pK_{2a} = 8.93)			M-Ala[a] (pK_a = 9.42)	M-Pha[2] (pK_a = 8.98)	M-Trp[a] (pK_a = 9.14)
	K^M_{MHL}	K^{MHL}_{ML}	K^M_{MHL}	K^{MHL}_{ML}	K^M_{ML}	K^M_{MA}	K^M_{MA}	K^M_{MA}
Co(II)	2.65	3.97	2.51	3.85	-	4.42	4.24	4.41
Ni(II)	3.09	4.19	-	-	4.42	5.34	5.31	5.41
Cu(II)	3.80	-	3.68	-	-	7.93	7.95	8.03
Zn((II)	2.97	-	2.83	-	-	5.00	4.93	5.03

* accurate to ±0.02 *pK* units; **accurate to ± 0.03 log *K* units,
[a]see ref. 17.

$$M + H_2L \Leftrightarrow MHL + H^+ \quad ...(2)$$

$$MHL \Leftrightarrow ML + H^+ \quad ...(3)$$

For Ni(II)-IMP systems, however, the Eq. (4) was used.

$$M + H_2L \Leftrightarrow ML + 2H^+. \quad ...(4)$$

The formation constants for M(II)- amino acid (AA) systems were determined using Eq. (5).

$$M + HA \Leftrightarrow MA + H^+ \quad ...(5)$$

Ternary systems

The formation constants of Cu (II)/Zn (II) -GMP/IMP-AA were determined using Eq. (6).

$$M + H_2L + HA \Leftrightarrow M(HL)A + 2H^+ \quad ...(6)$$

In the case of Ni (II)-GMP-AA system and Co(II)-GMP/IMP-AA systems, Eq. (7) was employed.

$$M + H_2L \Leftrightarrow MHL + H^+$$
$$MHL + HA \Leftrightarrow MLA + 2H^+ \quad ....(7)$$

However, for Ni (II)-IMP-AA system Eq. (8) was employed.

$$M + H_2L + HA \Leftrightarrow MLA + 3H^+ \quad ...(8)$$

where M = metal ion, H_2L = GMP/IMP, HA = amino acids (AA).

All the formation constants were subjected to refinement considering all possible species present in the solution, i.e., H_2L^-, HL^{2-}, L^{3-}, HA, A^-, ML, ML_2, MA, MA_2, MAL excluding hydroxo and polynuclear species using computer program BEST[12]. The error limits in these constants were minimised (Sigma fit = 0.001 to 0.0001).

Best was also used to generate complete species distribution curves at various *p*H values.

Results and Discussion

The titration curve of free GMP (Fig. 1a) and free IMP showed inflection at a=1 (where a = moles of base added per moles of ligand) followed by buffer region, indicating the stepwise dissociation of its protons. Their pK_a and pK_{2a} are assigned to dissociations of phosphate secondary hydrogen and N(1) -H respectively. The amino acid pK_a corresponds to its amino (NH_2) group. The ionisation constants are included in Table 1.

M(II)-GMP (1:1) system

In the titration of Cu(II) and Zn(II) with GMP in an equimolar ratio, a precipitate invariably appeared before inflection could be reached. However, the calculations were performed taking experimental points much below the precipitation regions, a = 0-0.7, using Eq. (1)

The titration curve of Co(II) showed an inflection at a=1 (Fig.1b) followed by buffer region. Accord-

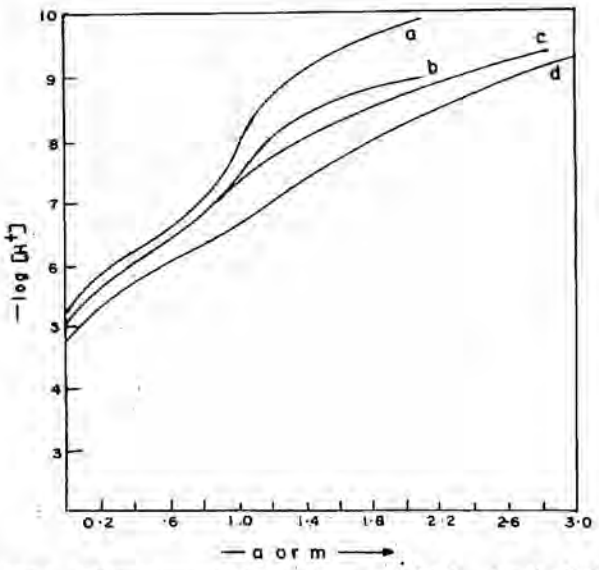

Fig 1 –Potentiometric titration curves at 35°C ; I = 0.10M (KNO_3)a = 5' – GMP; b = Co(II)-GMP (1:1); c = Co(II)-GMP-Ala (1:1:1); d = Ni(II)-GMP-Ala (1:1:1) and m = moles of base added per mole of metal ion.

ingly, it was assumed that a protonated and a normal complex formed in the buffer region between a = 0-1 and a = 1-2 respectively. The constants K^{M}_{MHL} and K^{MHL}_{ML} were determined using Eqns (2) and (3) respectively. They are listed in Table 1.Similar trends were obtained in the case of Ni (II) system also.

M (II)-IMP (1:1) system

The titration curves of Co(II), Cu(II), and Zn(II) in the presence of IMP are similar to those observed for corresponding M(II)-GMP system.

However, in the case of Ni (II) system, the formation of normal complex was considered in the buffer region between a=0–2, which gave constant value. The constant K^{M}_{ML} was determined by Eq. (4) and listed in Table 1.

M(II)-AA (1:1) system

The binary constants K^{M}_{MA} for Ala, Pha, and Trp were determined using Eq.(5) and are compiled in Table 1.

M(II)-GMP-AA (1:1:1) system

In Cu(II) and Zn(II) ternary systems, a precipitate appeared before m=2 (where m = moles of base added per moles of metal ion). However, calculation were performed taking experimental points well ahead of the precipitation region. The formation of a protonated ternary complex ($K^{M}_{M(HL)A}$) is assumed in the buffer region between m =0–2. The constants thus determined using Eq. (6) are presented in Table 2.

The titration curves of the mixed ligand systems Co(II)/Ni(II)-GMP-AA in an equimolar ratio showed an inflection at m=1 (Fig. 1c & d) indicating the formation of a (1:1) binary (K^{M}_{MHL}) complex in the buffer region between m=0–1. On comparison with 1:1 binary M-nucleotide and M-AA titration curves, it was observed that the titration curve for mixed ligand system coincided with that of M-nucleotide curve in this region. This confirms that there is no formation of ternary complex in the said region.

Table 2 — Formation constants* of (1:1:1) ternary complexes of nucleotides and amino acids with metal ions

(Temp. = 35°C; I = 0.1M (KNO_3)

Metal ion	M-GMP-Ala		M-GMP-Pha		M-GMP-Trp		M-IMP-Ala[a]		M-IMP-Pha[b]		M-IMP-Trp[c]	
	$K^{M}_{MA(HL)}$	K^{MHL}_{MLA}	$K^{M}_{MA(HL)}$	K^{MHL}_{MLA}	$K^{M}_{MA(HL)}$	K^{MHL}_{MLA}	$K^{M}_{MA(HL)}$	K^{MHL}_{MLA}	$K^{M}_{MA(HL)}$	K^{MHL}_{MHA}	$K^{M}_{MA(HL)}$	K^{MHL}_{MLA}
Co(II)	-	8.50	-	8.87	-	9.15	-	8.36	-	8.73	-	9.00
Ni(II)	-	9.62	-	10.14	-	10.38	-	-	-	-	-	-
Cu(II)	11.88	-	12.44	-	12.62	-	11.74	-	12.28	-	12.48	-
Zn(II)	8.16	-	8.70	-	8.88	-	8.01	-	8.53	-	8.72	-

For Ni(II) complexes : [(a) = K^{M}_{MAL} = 9.83, (b) = K^{M}_{MAL} =10.36, (c) K^{M}_{MAL} = 10.57];
*accurate to ±0.03 log K units

Table 3 – Intramolecular aromatic ring stacking in ternary complexes

Metal ion	Parameters associated with stacking	M:GMP:Ala	M:GMP:Pha	M:GMP:Trp	M:IMP:Ala	M:IMP:Pha	M:IMP:Trp
Co(II)	$\Delta\log K$	0.11	0.66	0.77	0.09	0.64	0.74
	$\Delta\Delta\log K_M$		0.55	0.66		0.55	0.65
	K_I		2.55	3.57		2.54	3.46
	% of $(MLA)_{st}$		71.83	78.11		71.75	77.57
	$-\Delta G$'s		3.24	3.89		3.24	3.83
Ni (II)	$\Delta\log K$	0.09	0.64	0.78	0.07	0.63	0.74
	$\Delta\Delta\log K_M$		0.55	0.69		0.56	0.67
	K_I		2.55	3.89		2.63	3.67
	% of $(MLA)_{st}$		71.83	79.55		72.45	78.58
	$-\Delta G$'s		3.24	4.07		3.30	3.95
Cu (II)	$\Delta\log K$	0.15	0.69	0.79	0.13	0.65	0.77

Accordingly, it was assumed that the formation of ternary complex takes place in the buffer region between m=1–3. The constant $K^{MHL}{}_{MLA}$ was determined using Eq. (7). The data is included in Table 2.

M(II)-IMP-AA (1:1:1) systems

The titration curves of M(II)-IMP-AA systems were similar to those of corresponding M(II)-GMP-AA systems, except for Ni(II) systems where no inflection was observed. Accordingly, it was assumed that a normal ternary complex is formed in the buffer region between m=0–3. The constant $(K^{M}{}_{MAL})$ was determined using Eq. (8) and the data is presented in Table 2.

The dissociation constants and formation constants pertaining to the interaction of nucleotides and amino acids with various metal ions are compiled in Tables 1 and 2. Although the ionisation constants and formation constants for binary complexes of amino acids [13-18] and nucleotides [19-22] were reported, they were reevaluated to avoid possible errors in the evaluation of various parameters reported. In GMP and IMP, N(7) also acts as a potential metal binding site in addition to phosphate oxygen and N(1). However, N(7) was not considered in this work since the titration curves of binary and ternary systems overlapped with that of free ligand curve in the region of N(7) involvement. Similar observations were made earlier with other systems[19]. The interaction of metal ions with nucleotides is highly *p*H dependent viz. N(7) and phosphate oxygen are found to be more favoured sites in acidic medium and the preference for N(1) site increases as *p*H increases [23]. The metal ions under investigation show high selectivity in complexation with nucleotides which is evident from the type of complexes formed by Co(II), Ni(II), Cu(II) and Zn(II) metal ions. Amino acids usually act as bidentate ligands involving carboxylate .(COO^-) and amino (NH_2) groups, and similar type of bonding may also exist in the systems under consideration.

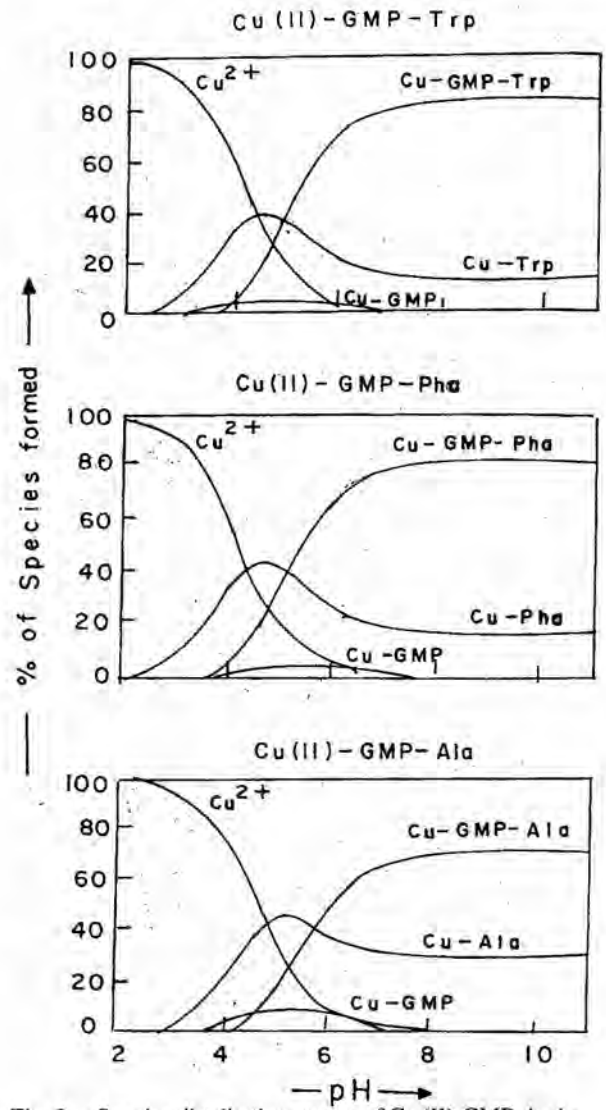

Fig 2 – Species distribution curves of Cu (II)-GMP-Amino acids (1:1:1) ternary systems.

In order to assess the extra stabilization in ternary complexes, a quantity $\Delta\log K$ has been introduced. The $\Delta\log K$ is defined as :

$$\Delta\log K = \log\beta^{M}_{MLA} - \log K^{M}_{ML} - \log K^{M}_{MA}$$

The $\Delta\log K$ values of metal nucleotides with different amino acids (Table 3) increase in the order : Ala < Pha < Trp, indicating the dependence of stabilisation of ternary complex on the aromatic ring size of secondary ligands. The less positive or nearer to zero $\Delta\log K$ values for alanine in both GMP and IMP systems can be explained on the basis of intramolecular interaction. Alanine has limited tendency for hydrophobic interactions[24] and being an aliphatic ligand cannot take part in stacking interactions also.

In the case of phenylalanine and tryptophan the $\Delta\log K$ values are more positive indicating the stabilisation of their ternary complexes in solution. This is due to the stacking interaction between aromatic moieties of the amino acids and nucleotides [25-27]. Phenylalanine forms less stable complexes compared to tryptophan even though the stabilities of the corresponding binary systems are almost same. The extra stabilisation in the tryptophan complexes clearly emphasizes the influence of stacking in the latter. The indole moiety being larger in size compared to phenylalanine ring can stack in a better way resulting in more stabilisation.

It is also interesting to note that $\Delta\log K$ values for GMP systems are slightly higher than those of the IMP system. This may be due to the presence of an additional exocyclic amino group in GMP, which may exert an influence on the non-covalent interactions.

In order to rationalize the stacking interaction, additional parameters like $\Delta\log K$, K_1, % of $(MLA)_{st}$ and ΔG^{o}_{S} have been evaluated. The $\Delta\Delta\log K$ is expressed as,

$$\Delta\Delta\log K = \Delta\log K \text{ (M-GMP/IMP-Pha/Trp)} - \Delta\log K \text{ (M-GMP/IMP-Ala)}$$

Alanine is taken as a reference for zero based scale of stacking interaction and extent of stacking is computed for other systems.

Further, in solution intramolecular equilibrium may exist between two isomers i.e. open and stacked (or closed) respectively. K_1 is the dimensionless constant for intramolecular equilibrium which is independent of absolute concentration of ternary complexes and expressed as,

$$K_1 = [M(GMP/IMP)(AA)st]/[M(GMP/IMP)(AA)_{op}]$$

This can be calculated using the equation

$$K_1 = 10^{\Delta\Delta\log K} - 1$$

The percentage of stacked isomer could be calculated from K_1 values.

$$\% \text{ of } (MLA)_M = (K_1 / 1 + K_1) \times 100$$

The free energy (ΔG°_S) change in kJ/mol associated with stacking interaction in calculated from

$$\Delta G^{\circ}_S = -RT \ \Delta\Delta \log K$$

A detailed discussion of these parameters can be found elsewhere[28-32], and values of the above parameters are listed in Table 3. Though, the values are only rough estimates, they clearly show that the ternary complexes of tryptophan are more stabilized due to stacking interaction compared to the other amino acid systems studied. This is further reflected in species distribution curves of the systems shown in Fig. 2. For example, formation of the complex Cu-GMP-Trp reaches maximum (~84%) at physiological *p*H 7.5, followed by those of corresponding ternary complexes of phenylalanine (~82%) and alanine (~69%).

Thus, it is evident from the present investigation that intramolecular stacking interaction plays an important role in the formation and stabilizaton of ternary complexes of biomolecules in solution.

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