Indian Journal of Chemistry
Vol. 29A, October 1990, pp. 1008-1011

Metal ion mediated intramolecular interactions of nucleosides with amino acids—Influence of stacking interactions

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Received 26 December 1989; revised 2 March 1990; accepted 23 April 1990

Interaction of bivalent (Cu, Ni, Zn, Co, Mg and Ca) metal ions with purine nucleosides (inosine and xanthosine) and amino acids (alanine, phenylalanine and tryptophan) has been investigated by potentiometric *p*H measurements at 35°C and 0.10 mol dm^{-3} (KNO_3) ionic strength. Extra stabilization has been expressed in terms of $\Delta \log K$ values. The stacking interaction observed with different amino acids and nucleosides has been discussed. Further, the influence of exocyclic substituent of xanthosine on the stabilities is explained. Various parameters have been evaluated to rationalise the stacking interactions. No appreciable interaction is observed with Ca(II) and Mg(II).

The study of intramolecular interactions in a system containing a metal ion and two different or same ligands has gained significance because of the similar interactions occurring in many biological processes[1-8]. Metal ions stabilize, destabilize or modulate such processes by introducing conformational changes through electronic effects like 'stacking'[3] and 'hydrophobic'[4,5] effects.

In order to identify these factors, an attempt has been made in the present paper to study the ternary complexes of Cu(II), Ni(II), Co(II), Zn(II), Mg(II) and Ca(II) with inosine, xanthosine and biologically important amino acids like alanine, phenylalanine and tryptophan. Since xanthosine differs from inosine by way of having an additional exocyclic substituent at C(2) position, this study provides an opportunity to assess the influence of this substituent. 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

Inosine, xanthosine, alanine, phenylalanine and tryptophan were obtained from Sigma Chemical Company (USA). For every titration, fresh solid ligand was weighed out into the reaction cell to avoid possible concentration effects. Transition metal ions and alkaline earth metal ions were of AnalaR grade and were standardised volumetrically by titration with the disodium salt of EDTA in the presence of a suitable indicator as outlined by Schwarzenbach[10]. Carbonate free sodium hydroxide was prepared and standardized by titration with potassium hydrogen phthalate[11].

The experimental method consisted of a potentiometric titration of ligands in the absence and presence of various metal ions at 35 ± 0.1°C and 0.10 *M* ionic strength with standard NaOH solution. Other details can be found elsewhere[7].

Calculations

The acid dissociation constants of purine nucleosides and amino acids calculated by the computer program PKAS[12] are presented in Table 1.

All the binary and ternary stability constants were calculated by using the computer program BEST[13]. BEST was also used to generate the complete species distribution curves at various *p*H values.

Results

Bivalent metal binary systems

The formation constants for the 1:1 metal-inosine, metal-xanthosine and metal-amino acids have been remeasured under identical conditions and summarized in Table 1.

Bivalent metal ternary systems

(i) *M(II)-xanthosine-amino acid* (1:1:1) *system*: The mixed ligand titration curve of Cu(II)-xanthosine-alanine shows an inflection at $m = 2$, indicating simultaneous release of two protons from the system. Accordingly, it was assumed that a 1:1:1 mixed ligand complex formed in the buffer region between $m = 0$ and $m = 2$. The constant K^{M}_{MLA} was calculated and the values are presented in Table 2. The titration curves for Ni(II), Zn(II) and Co(II) resulted in an inflection at $m = 1$ followed by buffer region. It was confirmed on the basis of the data of 1:1 M(II)-xanthosine sys-

Table 1—Ionisation constants* and corresponding binary stability constants* of the ligands
[Temp. = 35°C, μ = 0.10 mol dm^{-3} (KNO_3)]

Metal ion	M-Ino (pKa = 8.46) K^{M}_{ML}	M-Xan (pKa = 5.31 & pK_{2a} = > 12) $K^{M}_{M(HL)}$	M-Ala (pKa = 9.42) K^{M}_{MA}	M-Phe (pKa = 9.01) K^{M}_{MA}	M-Tryp (pKa = 9.12) K^{M}_{MA}
Cu(II)	3.90	2.37	7.93	8.05	8.02
Ni(II)	2.71	2.01	5.47	5.17	5.67
Zn(II)	2.31	1.43	4.98	4.94	5.04
Co(II)	2.01	1.62	4.50	4.24	4.54

*Constants are accurate to ±0.03 log K units.

Table 2—Ternary constants* (1:1:1) of the metal complexes of the nucleotides with amino acids
[Temp. = 35°C; μ = 0.10 mol dm^{-3} (KNO_3)]

Metal ion	M:Ino:Ala (1:1:1)		M:Ino:Phe (1:1:1)		M:Ino:Trypt (1:1:1)		M:Xan:Ala (1:1:1)		M:Xan:Phe (1:1:1)		M:Xan:Tryp (1:1:1)	
	K^{M}_{MLA}	K^{MA}_{MAL}	K^{M}_{MLA}	K^{MA}_{MAL}	K^{M}_{MLA}	K^{MA}_{MAL}	K^{M}_{MLA}	K^{ML}_{MLA}	K^{M}_{MLA}	K^{ML}_{MLA}	K^{M}_{MLA}	K^{ML}_{MLA}
Cu(II)	—	3.81	—	4.33	—	4.39	10.06	—	10.75	—	10.86	—
Ni(II)	8.07	—	8.32	—	8.76	—	—	5.18	—	5.46	—	5.99
Zn(II)	7.23	—	7.83	—	7.97	—	—	4.82	—	5.31	—	5.59
Co(II)	6.39	—	6.63	—	6.96	—	—	4.24	—	4.56	—	4.96

*Constants are accurate to ±0.06 log K units.

tem that only binary complex was formed in the buffer region between $m = 0$ and $m = 1$. Therefore, the formation of a ternary complex was assumed only between the buffer region $m = 1$ and $m = 2$. All the constants (K^{ML}_{MLA}) so calculated are listed in Table 2.

The behaviour of the other amino acid ternary systems was exactly similar to that observed for corresponding M(II)-xanthosine-alanine systems described above (Table 2).

(ii) *M(II)-inosine-amino acid* (1:1:1) *system*: The mixed ligand titration curves of Cu(II) with inosine and alanine (1:1:1) showed an inflection at $m = 1$ indicating the formation of a 1:1 Cu(II)-alanine complex and this was confirmed by comparing data in this region with that for the 1:1 Cu(II)-alanine system. The constant (K^{MA}_{MLA}) was calculated in the buffer region between $m = 1$ and $m = 2$ and the values are presented in Table 2. In the case of Ni(II), Zn(II) and Co(II), it was assumed that a ternary complex was formed in the buffer region between $m = 0$ and $m = 2$. The assumption of the simultaneous formation of ternary complex is justified based on the pKa values of the participating ligands (Table 2). This was further confirmed by plotting the percentage of various species present in solution versus pH.

The other amino acid ternary systems behaved in a manner exactly similar to that observed for the corresponding M(II)-inosine-alanine (1:1:1) systems.

Discussion

The stability constants pertaining to the interaction of nucleosides and amino acids with various metal ions are compiled in Tables 1 and 2. Although the stability constants for some of the binary systems were reported[14,15], we have remeasured them in order to gather the information under identical experimental conditions. A good agreement was found between our values and literature data.

It can be seen from Table 2 that the ternary stability constants exhibit the following order with respect to amino acids: tryptophan > phenylalanine > alanine.

It is well established that in 1:1 binary systems these amino acids behave as bidentate ligands involving carboxylate (COO^-) and amino (NH_2) groups in metal coordination. In nucleosides O(6) and N(7) atoms are involved in metal binding[16-18]. Based on this data, it is assumed that similar type of bonding exists in ternary systems also.

A more comprehensive comparison could be made with the help of Δlog K values (the Δlog K being the difference between the binary and ternary constants i.e. Δlog K = log K^{MA}_{MAL} − log K^{M}_{ML} or log K^{ML}_{MLA} − log

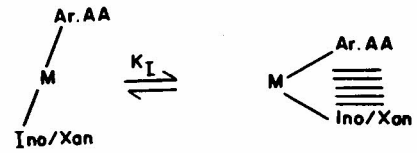
Table 3—Extent of the intramolecular aromatic-ring stacking in ternary complexes

Metal ion	Parameters associated with stacking	M:Ino:Ala	M:Ino:Phe	M:Ino:Tryp	M:Xan:Ala	M:Xan:Phe	M:Xan:Tryp
	$\Delta\log K$	−0.09	+0.43	+0.49	−0.24	+0.33	+0.47
	$\Delta\Delta\log K_M$		+0.52	+0.58		+0.57	+0.71
Cu(II)	K_I		2.31	2.80		2.71	4.12
	% of $(MLA)_{Cl}$		69.80	73.70		73.08	80.50
	$-\Delta G^\circ$		3.06	3.41		3.36	4.18
	$\Delta\log K$	−0.11	+0.34	+0.38	−0.29	+0.29	+0.32
	$\Delta\Delta\log K_M$		+0.45	+0.49		+0.58	+0.61
Ni(II)	K_I		1.81	2.09		2.80	3.07
	% of $(MLA)_{Cl}$		64.52	67.64		73.70	75.45
	$-\Delta G^\circ$		2.65	2.88		3.41	3.59
	$\Delta\log K$	−0.06	+0.58	+0.62	−0.16	+0.37	+0.55
	$\Delta\Delta\log K_M$		+0.64	+0.68		+0.53	+0.71
Zn(II)	K_I		3.36	3.78		2.38	4.12
	% of $(MLA)_{Cl}$		77.09	79.10		70.49	80.50
	$-\Delta G^\circ$		3.77	4.00		3.12	4.18
	$\Delta\log K$	−0.12	+0.38	+0.41	−0.26	+0.32	+0.42
	$\Delta\Delta\log K_M$		+0.50	+0.53		+0.58	+0.68
Co(II)	K_I		2.16	2.38		2.80	3.78
	% of $(MLA)_{Cl}$		68.38	70.49		73.70	79.11
	$-\Delta G^\circ$		2.94	3.12		3.41	4.00

K^M_{MA}). In Table 3 are given the $\Delta\log K$ values of various systems. The $\Delta\log K$ values of metal-nucleosides (inosine and xanthosine) with different amino acids increase in the order: alanine < phenylalanine < tryptophan, indicating the dependence of stabilization of ternary complexes on the aromatic ring size of secondary ligands. The negative $\Delta\log K$ values for alanine in both inosine and xanthosine systems can be explained on the basis of intramolecular interaction. Alanine being an aliphatic ligand cannot take part in stacking interactions. Thus, the ternary complexes of alanine are less stable compared to their binary counterparts.

In the case of phenylalanine and tryptophan, the $\Delta\log K$ values are positive indicating the stabilization of their ternary complexes in solution. This is due to the stacking interaction between the aromatic moieties of the amino acids and nucleosides. Phenylalanine forms less stable complexes compared to tryptophan, since the stabilities of the corresponding binary systems are almost the same, the extra stabilization in the tryptophan complexes clearly explains the influence of stacking. The indole moiety, being larger in size compared to phenyl ring can stack in a better way resulting in more stabilization.

It is also interesting to note here that the $\Delta\log K$ values for the inosine system are slightly higher than those of the xanthosine complexes. This may be due to the presence of an additional exocyclic donor group O(2) in xanthosine which may compete for metal coordination with the expected metal binding sites i.e. O(6) and N(7). Thus, though O(2) participation in ternary complexes is not favoured sterically, it may exert some influence on the conventional binding sites. This may be the reason for lower stabilization of xanthosine ternary complexes over inosine complexes[19].



(1)

In order to rationalise the stacking interaction, a few more quantities like $\Delta\Delta\log K$, K_I, % of $(MLA)_{St}$ and ΔG°_S have been evaluated. The $\Delta\Delta\log K$ is expressed as,

$\Delta\Delta\log K = \Delta\log K$ (M-Ino/Xan-Ar.AA system) − $\Delta\log K$ (M-Ino/Xan-Ala).

Alanine is taken as a reference for a zero-based

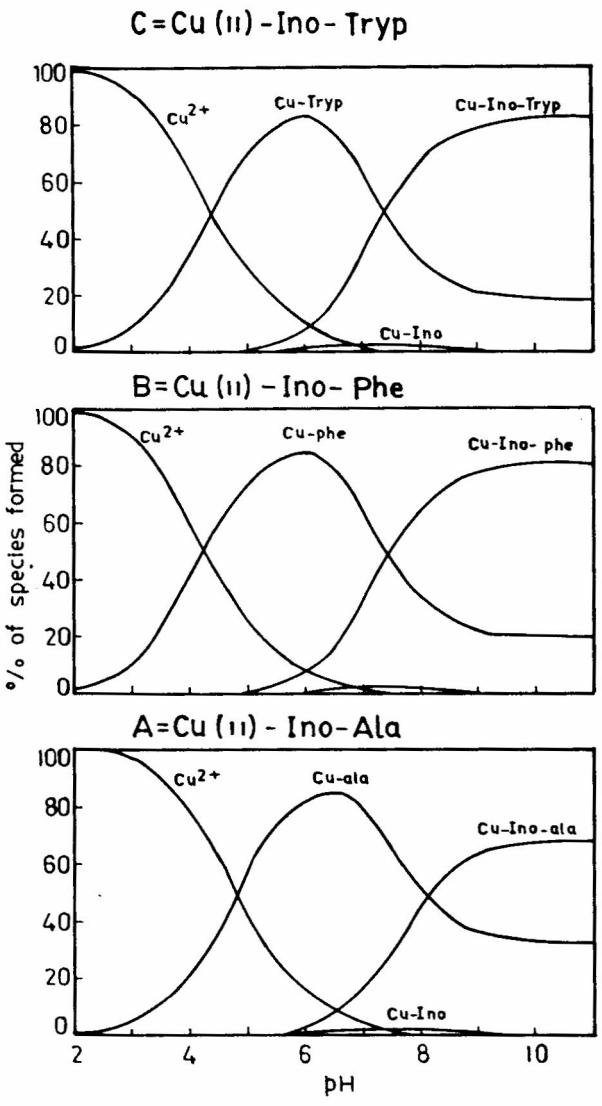


Fig. 1—Species distribution curves of the Cu-inosine-amino acids (1:1:1) ternary systems

scale of stacking interaction and extent of stacking is computed for other systems.

Further, in solution, intramolecular equilibrium may exist between two forms i.e. open and stacked respectively (Structure I).

K_I is the dimensionless constant for intramolecular equilibrium which is independent of absolute concentration of ternary complexes and expressed as,

$$K_I = [M(Xan)(AA)_{St}]/[M(Xan)(AA)_{Op}]$$

This can be calculated using the following equation,

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

The percentage of stacked isomer could be calculated from K_I values,

$$\% \text{ of } (MLA)_{St} = (K_I/1 + K_I)\ 100$$

The free energy change (ΔG°_S) associated with stacking interaction is calculated from $\Delta\Delta\log K$,

$$\Delta G^\circ_S = -RT\ \Delta\Delta\log K$$

A detailed discussion of these quantities can be found elsewhere[3,4,9,20].

All the above parameters have been listed in Table 3. This data clearly show that the ternary complexes of tryptophan are more stabilized due to stacking interaction compared to other amino acids studied. This is further reflected in species distribution curves of the systems studied (Fig. 1). For example, formation of the complex Cu-Ino-tryptophan is highest ($\sim 55\%$) at physiological *p*H 7.5 followed by that of corresponding ternary systems of phenylalanine and alanine ($\sim 50\%$ and $\sim 29\%$ respectively).

Acknowledgement

One of the authors MRPR thanks the CSIR, New Delhi, for the award of a senior research fellowship.

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