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Quantitative study of hydrophobic interactions in ternary complexes involving nucleosides and amino acids

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Ternary complexes of Cu(II), Ni(II), Zn(II) and Co(II) metal ions with purine nucleosides [inosine(Ino) and xanthosine (Xan)] and aliphatic amino acids [alanine (ala), α-aminobutyricacid(aba), norvaline(norVal) and norleucine(norLeu)] have been studied in solution by potentiometric *p*H measurement at 35°C and 0.10 $M(KNO_3)$ ionic strength. The stabilization is expressed in terms of $\Delta\log K$. The influence of aliphatic amino acid side chain on the stability of the complexes is discussed. Related parameters like $\Delta\Delta\log K$, K_1, % of $(MLA)_{st}$ and $\Delta G°$ have been computed and discussed. Species distribution curves have also been obtained using the computer program BEST.

To a chemist, most of the biological systems are just mixed ligand complexes. One of the driving forces for such biological processes is ligand-ligand interactions. At biological *p*H, these interligand interactions determine the efficiency and specificity of such processes as intercalation occurring in proteins, enzymes, DNA and RNA[1]. To gather more information about these interactions which occur *in vivo*, the study of ternary complexes consisting of nucleosides and amino acids is helpful[2,3]. Mostly these interactions are of non-covalent nature[4]. Among these non-covalent interactions, the influence of aromatic ring stacking has been investigated extensively on the basis of equilibrium, CD and NMR studies[5-11] and enhancement in stability of the ternary systems has been attributed to the stacking interactions. In the present study an attempt is made to interpret the stabilization occurring in metal-purine nucleoside-aliphatic amino acid ternary systems as a result of hydrophobic interactions.

In proteins, the interactions of side chains of the amino acid residues result to give distinct structural features, due to hydrophobic interactions. For example, the enhanced rate of metalloporphyrin formation in the presence of amino acids is due to the hydrophobic interactions of the sidechain of the amino acid with porphyrin plane[12]. It has been suggested that these hydrophobic bonds may be responsible for the increase in stabilization with increase in chain length of the carboxylic acid dimers. This extra stabilization is measured in terms of the hydrophobicity of the amino acid. Glycine, being simple amino acid without side chain, has zero hydrophobicity. Amino acids with aromatic or bulky aliphatic side chains possess high hydrophobicity values, while those with small aliphatic side chains will have low hydrophobicity values[13].

Presently attempts have been made to quantify the hydrophobic interaction between an aliphatic and an aromatic group by choosing amino acids in such a way that the side chain varied in length from a methyl group as in l-alanine to an *n*-butyl group as in l-norleucine.

Materials and Methods

Inosine, xanthosine, alanine, α-aminobutyric acid, norvaline and norleucine 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 were of AnalaR grade and their solutions were standardized volumetrically by titration with the disodium salt of EDTA in the presence of a suitable indicator as outlined by Schwarzenbach[14].

The experimental method consisted of potentiometric titrations of ligands in the absence and presence of transition metal ions at 35° ± 0.1°C and 0.10 *M* ionic strength against standard carbonate-free NaOH solution. Other details can be found elsewhere[15].

Calculations

The acid dissociation constants of purine nucleosides and amino acids were calculated using the computer program PKAS[16] and are presented in Table 1.

All the binary and ternary stability constants were calculated using the computer program BEST[17].

Table 1—Ionization constants* and corresponding binary stability constants* of the ligands
[Temp. 35 ± 0.1°C; $\mu = 0.10\ M(KNO_3)$]

Metal ion	M-Ino ($pK_a = 8.46$)	M-Xan ($pK_a = 5.31$, $pK_{2a} = > 12$)	M-Ala ($pK_a = 9.42$)	M-Aba ($pK_a = 9.50$)	M-norVal ($pK_a = 9.57$)	M-norLeu ($pK_a = 9.59$)
	K^M_{ML}	K^M_{MHL}	K^M_{MA}	K^M_{MA}	K^M_{MA}	K^M_{MA}
Cu(II)	3.90	2.37	7.93	8.09	8.21	8.39
Ni(II)	2.71	2.01	5.47	5.22	5.36	5.48
Zn(II)	2.31	1.43	4.98	4.58	4.71	4.87
Co(II)	2.01	1.62	4.50	4.13	4.21	4.27

*Constants are accurate to ±0.03 log$K$ units.

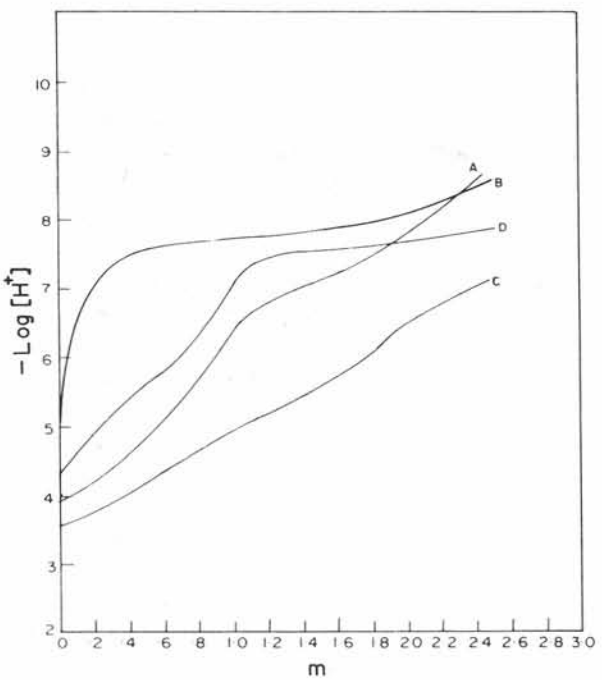

Fig. 1—Potentiometric titration curves of ternary systems (1:1:1) at 35°C and 0.1M (KNO_3) ionic strength strength. (A = Cu(II)-Ino-Ala; B = Zn(II)-Ino-Ala; C = Cu(II)-Xan-Ala; and D = Zn(II)-Xan-Ala)

BEST was also used to generate the complete species distribution curves at various pH values.

The stability constants of metal-inosine complexes were calculated using Eq. (1) (charges are omitted for clarity).

$$M + HL \rightleftharpoons ML \quad \ldots(1)$$

To calculate the stability constants of 1:1 protonated complexes of metal-xanthosine, Eq. (2) was employed.

$$M + HL \rightleftharpoons MHL \quad \ldots(2)$$

The stability constants of Cu-Xan-AA were calculated with the help of Eq. (3),

$$M + H_2L + HA \rightleftharpoons M(HL)A \quad \ldots(3)$$

For the other metal ions, viz. Ni(II), Zn(II) and Co(II), Eq. (4) was employed

$$M + H_2L \rightleftharpoons M(HL)$$

$$M(HL) + HA \rightleftharpoons M(HL)A \quad \ldots(4)$$

The stability constants of Cu-Ino-AA were calculated employing Eq. (5)

$$M + HA \rightleftharpoons MA$$

$$MA + HL \rightleftharpoons MAL \quad \ldots(5)$$

For the other metal ions, viz. Ni(II), Zn(II) and Co(II), Eq. (6) was employed.

$$M + HL + HA \rightleftharpoons MLA \quad \ldots(6)$$

where,

M = metal ion, H_2L = xanthosine, HL = inosine and HA = amino acids.

Results

(i) *M(II)-inosine (1:1) system*

The formation constants for the normal 1:1 metal-inosine complexes calculated using Eq. (1) are listed in Table 1.

(ii) *M(II)-xanthosine (1:1) system*

The constants for the monoprotonated 1:1 metal-xanthosine complexes calculated using Eq. (2) are given in Table 1.

(iii) *M(II)-amino acid (1:1) system*

The binary constants of alanine, α-aminobutyric acid, norvaline and norleucine were also calculated and are included in Table 1.

M(II)-inosine-amino acid (1:1:1) system

The mixed ligand titration curve of Cu(II) with inosine and alanine (1:1:1) shows an inflection at m = 2 (Fig. 1) indicating the formation of a 1:1 Cu(II)-alanine complex and this was confirmed by comparing the data in this region with 1:1 Cu(II)-alanine system. The constant (K^{MA}_{MLA}) was calculated

Table 2—Stability constants* (1:1:1) of ternary complexes of M(II) with the nucleosides and amino acids
[Temp. = 35 ± 0.1°C; μ = 0.10 $M(KNO_3)$]

Metal ion	K^{M}_{MLA}				K^{MA}_{MAL}			
	M:Ino:Ala[a] (1:1:1)	M:Ino:Aba[b] (1:1:1)	M:Ino:nVal[c] (1:1:1)	M:Ino:nLeu[d] (1:1:1)	M:Xan:Ala[e] (1:1:1)	M:Xan:Aba[f] (1:1:1)	M:Xan:nVal[g] (1:1:1)	M:Xan:nLeu[h] (1:1:1)
Cu(II)	—	—	—	—	—	—	—	—
Ni(II)	8.07	8.01	8.22	8.29	5.18	5.32	5.50	5.68
Zn(II)	7.23	7.07	7.24	7.45	4.82	4.69	4.86	5.14
Co(II)	6.39	6.25	6.38	6.48	4.24	4.72	4.36	4.46

*Constants are accurate to ± 0.06 log K units.
For Cu(II) complexes:
((a) K^{MA}_{MAL} = 3.81; (b) K^{MA}_{MAL} = 4.01; (c) K^{MA}_{MAL} = 4.05; (d) K^{MA}_{MAL} = 4.09; (e) K^{MA}_{MLA} = 10.06; (f) K^{MA}_{MLA} = 10.58; (g) K^{MA}_{MLA} = 10.74; (h) K^{MA}_{MLA} = 10.97).

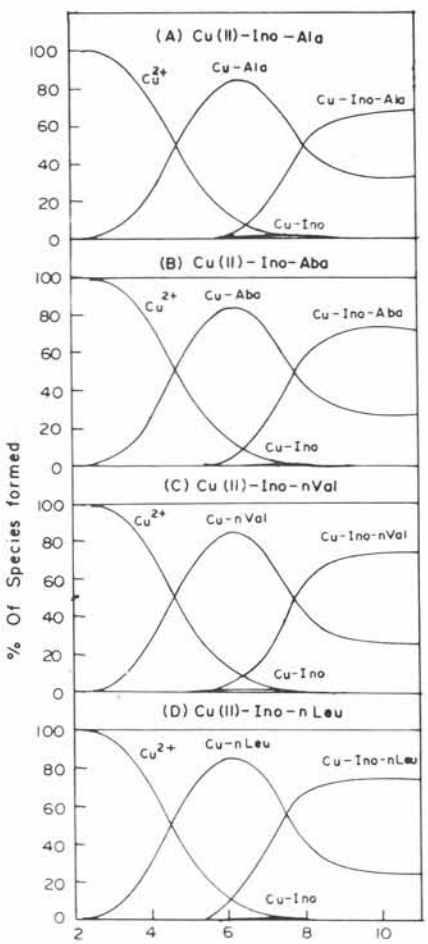

Fig. 2—Species distribution curves of Cu(II)-inosine-amino acids (1:1:1) ternary systems. (A = Cu(II)-Ino-Ala; B = Cu(II)-Ino-Aba; C = Cu(II)-Ino-nVal; D = Cu(II)-Ino-nLeu)

in the buffer region between m = 1 and m = 2 using Eq.(5) and the value is included in Table 2. In the case of Ni(II), Zn(II) and Co(II), it was assumed that ternary complexes were formed in the buffer region between m = 1 and m = 2 (Fig. 1). The assumptionof simultaneous formation of ternary complex is justified based on the pK_a values of the participating ligands. This is further confirmed by plotting the percentage of various species present in solution versus pH (Fig. 2). The constants (K^{M}_{MLA}) thus calculated are presented in Table 2.

The behaviour of other amino acid ternary systems is similar to that observed for corresponding M(II)-inosine-alanine (1:1:1) systems. The constants for these systems are also presented in Table 2.

M(II)-xanthosine-amino acid (1:1:1) system

The mixed ligand titration curve of Cu(II)-xanthosine-alanine (Fig. 1) 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 is formed in the buffer region between m = 0 and m = 2. The constant K^{M}_{MLA} was calculated with the help of Eq. 3 and the value is presented in Table 2. The titration curves for Ni(II), Zn(II), Co(II) resulted in an inflection at m = 1 (Fig. 1) followed by buffer region. It was confirmed on the basis of the data of 1:1 M(II)-xanthosine system that only binary complex has been formed in the buffer region between m = 0 and m = 1. Therefore the formation of a ternary complex has been 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 is similar to that of corresponding M(II)-xanthosine-alanine systems described above. The constants for these systems are also presented in Table 2.

Discussion

The dissociation constants of nucleosides, amino acids and their corresponding binary constants are

Table 3—Extent of intramolecular hydrophobic interaction in ternary complexes

Metal ion	Parameter	M-Ino-Ala	M-Ino-Aba	M-Ino-nVal	M-Ino-nLeu	M-Xan-Ala	M-Xan-Aba	M-Xan-nVal	M-Xan-nLeu
Cu(II)	$\Delta \log K$	−0.09	0.11	0.15	0.19	−0.24	0.12	0.16	0.21
	$\Delta\Delta \log K$		0.20	0.24	0.28		0.36	0.40	0.45
	K_1		0.58	0.73	0.90		1.29	1.51	1.82
	% of $(MLA)_{st}$		36.90	42.46	47.52		56.35	60.19	64.52
	$-\Delta G^\circ$		1.18	1.41	1.65		2.12	2.36	2.65
Ni(II)	$\Delta \log K$	−0.11	0.08	0.15	0.20	−0.29	0.10	0.14	0.20
	$\Delta\Delta \log K$		0.19	0.26	0.31		0.39	0.43	0.49
	K_1		0.54	0.81	1.04		1.45	1.69	2.09
	% of $(MLA)_{st}$		35.43	45.05	51.02		59.26	62.85	67.64
	$-\Delta G^\circ$		1.12	1.53	1.82		2.30	2.53	2.89
Zn(II)	$\Delta \log K$	−0.06	0.18	0.22	0.27	−0.16	0.11	0.15	0.27
	$\Delta\Delta \log K$		0.24	0.28	0.33		0.27	0.31	0.43
	K_1		0.73	0.90	1.13		0.86	1.04	1.69
	% of $(MLA)_{st}$		42.46	47.52	53.23		46.30	51.02	62.85
	$-\Delta G^\circ$		1.41	1.65	1.94		1.59	1.82	2.53
Co(II)	$\Delta \log K$	−0.12	0.11	0.16	0.20	−0.26	0.10	0.15	0.19
	$\Delta\Delta \log K$		0.23	0.28	0.32		0.36	0.41	0.45
	K_1		0.69	0.90	1.08		1.29	1.57	1.81
	% of $(MLA)_{st}$		41.12	47.52	52.14		56.35	61.10	64.52
	$-\Delta G^\circ$		1.35	1.65	1.88		2.12	2.41	2.65

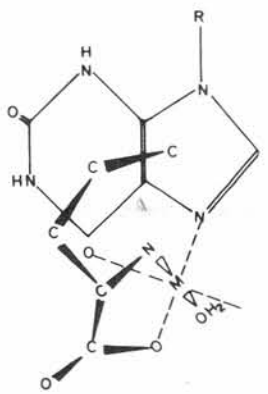

Fig. 3—Tentative and simplified structure of M(II)-Xan-nVal (1:1:1) ternary complex

remeasured under identical conditions and are included in Table 1.

The ternary constants, presented in Table 2, show increased stabilization in the following order: ala < α-aminobutyric acid < norvaline < norleucine.

Amino acids usually act as bidentate ligands involving carboxylate (COO^-) and amino (NH_2) and it is well established that purine nucleosides bind with metal using O(6) and N(7) sites, in solution[19-22]. Based on the experimental data, it is assumed that similar type of bonding may exist in the systems under consideration. A schematic representation showing the mode of bonding in ternary complex is given in Fig. 3.

The $\Delta \log K$ values are evaluated from the binary and ternary stability data determined in this investigation. In the case of metal-nucleoside-aliphatic amino acid systems, the $\Delta \log K$ values are found to be less positive in comparison to those of corresponding ternary systems with aromatic amino acids[11]. This suggests that the extent of interaction is weaker than stacking interaction[23]. This is in agreement with the theoretical observation about the qualitative assessment of intramolecular interactions which follow the order: aliphatic-aliphatic < aliphatic-aromatic < aromatic-aromatic. Among aliphatic amino acids, alanine shows negative $\Delta \log K$ value, indicating little contribution towards complex stabilization. As we go from alanine to norleucine through α-aminobutyric acid and norvaline (Table 2), $\Delta \log K$ shows an increase. This can be explained on the basis of 'hydrophobic interaction' phenomenon. In aqueous solution, the intramolecular hydrophobic ligand-lignad interaction in mixed ligand complexes emerges from the lipophilicity of one

part of the ligand and hydrophilicity of remainder of the complex. This unique organizing force[24] is based on repulsion by the solvent thus making hydrophobic, non-polar side chains align to give hydrophobic interaction. Such dependence of intramolecular interaction on solvent was studied and effect of adding organic solvents to aqueous solution was established[25]. Change in the water activity proportionately decreases the intramolecular interaction.

In the case of alanine, the methyl group is too small to allow any interaction with the aromatic rings of nucleosides resulting in negative $\Delta\log K$ values. However, with the increase in chain length, such as propyl and butyl groups of norvaline and norleucine respectively, there is better hydrophobic interaction as reflected in their $\Delta\log K$ values. This is depicted schematically in Fig. 3 for norvaline.

The differences in the stabilities of inosine and xanthosine ternary complexes can be attributed to the presence of additional exocyclic group [O(2)] on the xanthosine which renders it less effective for binding[11].

Since the alanine ternary systems with the methyl group contribute very little, if any, towards intramolecular interaction, we can evaluate a new parameter, $\Delta\Delta\log K$, to quantify the hydrophobic interactions. This can be obtained by deducting the $\Delta\log K$ value of other amino acid ternary systems from the corresponding alanine ternary systems as shown in Eq. (7)

$$\Delta\Delta\log K = \Delta\log K^{M}_{M\text{-Ino/Xan-AA}} - \Delta\log K^{M}_{M\text{-Ino/Xan-Ala}} \quad \ldots (7)$$

These values given in Table 3 further illustrate the dependence of stabilization on the chain length of amino acid.

Further these complexes exist in solution in two different forms, i.e. 'stacked' and 'open', in equilibrium with each other which can be expressed in terms of a dimensionless constant, K_I, which is given by Eq. (8)

$$K_I = \frac{[M(Ino/Xan)(AA)]_{st}}{[M(Ino/Xan)(AA)]_{op}} \quad \ldots (8)$$

The values of K_I are tabulated in Table 3. Norvaline and norleucine have higher K_I values thus accounting for more 'stacked form' species. These results suggest that the stacked form stabilizes the ternary systems in solution.

The K_I value in turn allow the calculation of the percentage of the 'stacked' species (see Eq. 9),

$$\%\text{ of } (MLA)_{st} = (K_I/1 + K_I)100 \quad \ldots (9)$$

Though the values are rough estimates only, they follow regular trend which correlates with our experimental results. The species distribution curves shown in Fig. 2 also give parallel evidence. For example, the percentage of stacked species in Cu-Ino-norleucine is the highest (55%) at physiological *p*H 7.5 followed by corresponding species of norvaline (40%), α-aminobutyric acid (37%) and alanine (29%).

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