

Influence of non-coordinated aliphatic side chains of amino acids on the hydrophobic interactions involving purine nucleotide complexes

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Abstract. Interaction of bivalent Co, Ni, Cu and Zn metal ions with purine nucleotides (5'-guanosine monophosphate and 5'-inosine monophosphate) and aliphatic amino acids (alanine, α -aminobutyric acid, norvaline and norleucine) has been investigated in solution by potentiometric pH measurements at 35°C and 0.10 M (KNO₃) ionic strength. The stability constants of binary (1:1) and ternary (1:1:1) complexes of various systems were determined. The stabilization of the ternary complexes is measured in terms of $\Delta \log K$, which is the difference between the stabilities of binary and ternary complexes. The influence of aliphatic amino acid side chains on hydrophobic interactions in these systems were identified and rationalised. Species distribution curves were generated using a computer program in order to identify the stable species at biological pH.

Keywords. Hydrophobic interactions; stability constants; $\Delta \log k$; nucleotides; amino acids.

1. Introduction

In view of its importance in biological systems metal ion mediated interactions involving nucleic acids and amino acids have gained much attention during the last few decades¹⁻⁴. One of these, the hydrophobic interactions⁵ lead to structural changes⁶ that are uniquely suited for the organization of living organisms, for example, in the folding of proteins to produce three dimensional structures⁷. In order to understand these hydrophobic interactions, several model studies have been initiated⁸⁻¹³. In continuation of these efforts, in this manuscript the influence of non-coordinated aliphatic side chains of amino acids on the hydrophobic interactions involving purine nucleotide complexes have been investigated. The ligands and metal ions involving in this study are 5'-monophosphates of guanosine and inosine, aliphatic amino acids - alanine- α -aminobutyric acid, norvaline, norleucine and Co(II), Ni(II), Cu(II) and Zn(II).

2. Experimental

The disodium salts of 5'-guanosinemonophosphate (5'-GMP) and 5'-inosine monophosphate (5'-IMP), alanine (Ala), α -aminobutyric acid (Aba), norvaline (nVal) and norleucine (nLeu) were obtained from Sigma Chemical Company, USA. For every titration, fresh solid ligand was weighed out into the reaction cell to avoid any possible

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concentration effects. Transition metal nitrates were of AnalaR grade (BDH) and their solutions were standardized volumetrically by titration with the disodium salt of EDTA, in the presence of a suitable indicator as outlined¹⁴.

The experimental method consisted of a potentiometric titration of ligands in the absence and presence of metal ions at $35^\circ \pm 0.1^\circ\text{C}$ against standard carbonate free NaOH¹⁵. The ionic strength was maintained at 0.10 M using KNO₃ (BDH, Germany) as the supporting electrolyte and relatively low concentrations of the reactants (1×10^{-3} M). During the course of titration a stream of O₂ free N₂ was passed through the reaction cell to avoid the adverse effect of atmospheric CO₂. Each experiment was repeated twice to get concurrent readings. Other details can be found elsewhere¹⁶.

3. Calculations

The ionisation constants of various ligands were determined by computer program PKAS⁷ using experimental data. The formation constants were determined by setting up appropriate material balanced equations as dictated by the experimental evidence.

3.1 Binary systems

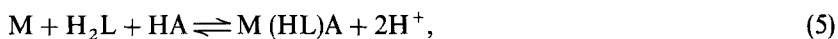
For the determination of binary stability constants of nucleotides and amino acids with various metal ions, the following equations were employed (charges are omitted for clarity),



The protonated stability constants (1:1) of nucleotides with Cu(II) and Zn(II) metal ions were evaluated using (1) whereas, (1) and (2) were employed for Co(II)-GMP/IMP and Ni(II)-GMP systems. However, for Ni(II)-IMP (1:1) system (3) was employed. In the case of M(II)-AA system the stability constants were determined with the help of (4).

3.2 Ternary systems

For the determination of ternary stability constants (1:1:1) of various metal ions with nucleotides and amino acids, the following equations were set up as dictated by the trends of the titration curves,



Equation (5) was used for the determination of protonated ternary stability constants (1:1:1) of Cu(II)/Zn(II)-GMP/IMP-AA systems. In the case of Co(II)-GMP/IMP-AA and Ni(II)-GMP-AA systems (6) was employed. However, for Ni(II)-IMP-AA system (7) was used 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 ,

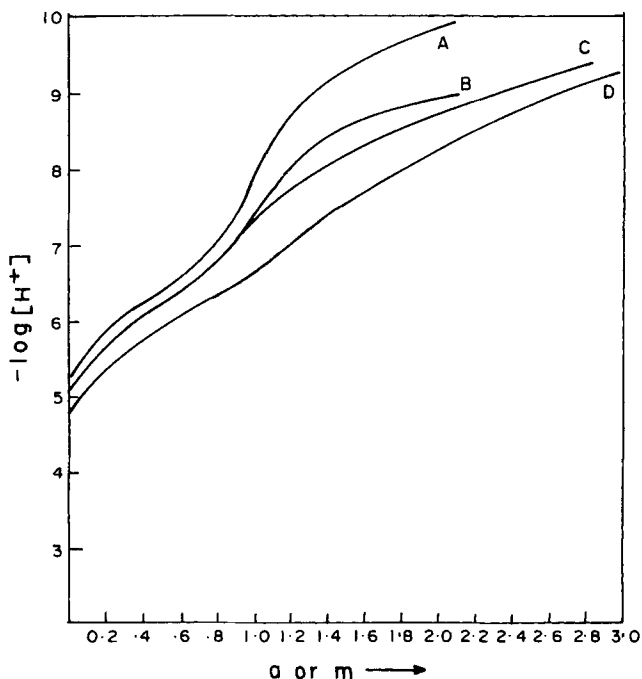


Figure 1. Potentiometric titration curves at 35°C and $I=0.10$ M (KNO_3) A = Free 5'-GMP ligand, B = Co(II)-GMP (1:1), C = Co(II)-GMP-Ala (1:1:1), D = Ni(II)-GMP-Ala (1:1:1) and m = moles of base added per mole of metal ion.

MAL excluding hydroxo and polynuclear species, using computer program BEST¹⁷. The error limits in these constants were minimised (Sigma fit = 0.001 to 0.0001). BEST was also used to generate the complete species distribution curves at various pH values.

4. Results and discussion

The free ligand titration curves of 5'-GMP (figure 1A) and 5'-IMP showed an inflection at $a = 1$ (where, a = moles of base added per mole 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 ($-\text{NH}_2$) group. All these constants are listed in table 1.

4.1 $M(\text{II})$ -GMP (1:1) systems

In the course of titration of Cu(II) and Zn(II) with GMP in an equimolar ratio, a precipitate invariably appeared before inflection could be reached. However, their protonated stability constants (K_{MHL}^M) were determined taking experimental points much below the precipitation region, $a = 0-0.7$, using (1).

The titration curve of Co(II) showed an inflection at $a = 1$ (figure 1B) followed by buffer region. Accordingly, 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

Table 1. Ionisation constants* and corresponding binary stability constants** of the ligands
 [Temp = 35°C; I = 0.10 M (KNO₃)]

| Metal ion | M-GMP | | M-IMP | | | | M-nLeu (pK _a = 9.59) K _{MMA} ^M |
|-----------|--|--|---|---|---|---|---|
| | (pK _a = 6.36, pK _{2a} = 9.36) K _{MHL} ^M | (pK _a = 6.32, pK _{2a} = 8.93) K _{MHL} ^M | (pK _a = 9.42) K _{MMA} ^M | (pK _a = 9.50) K _{MMA} ^M | (pK _a = 9.57) K _{MMA} ^M | (pK _a = 9.59) K _{MMA} ^M | |
| Co(II) | 2.65 | 3.97 | 2.51 | 3.85 | 4.42 | 4.08 | 4.22 |
| Ni(II) | 3.09 | 4.19 | — | — | 5.34 | 5.12 | 5.36 |
| Cu(II) | 3.80 | — | 3.68 | — | 7.93 | 8.07 | 8.36 |
| Zn(II) | 2.97 | — | 2.83 | — | 5.00 | 4.62 | 4.88 |

*Constants are accurate to ± 0.02 pK units.

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

K_{MHL}^M and K_{ML}^{MHL} were determined using (1) and (2) respectively. Similar trends were observed for Ni(II) system also. The constants thus calculated are presented in table 1.

4.2 $M(II)$ -IMP (1:1) systems

The tendency of the titration curves of Co(II), Cu(II) and Zn(II) with IMP are similar to those observed for the corresponding $M(II)$ -GMP system. However, in the case of Ni(II) system, no inflection was observed. Accordingly, formation of normal complex was considered in the buffer region between $a = 0-2$, which gave constant values. The constant K_{ML}^M was determined with the help of (3). The stability constants are listed in table 1.

4.3 $M(II)$ -AA (1:1) systems

The binary constants K_{MA}^M of Ala, Aba, nVal and nLeu were determined using (4) and are compiled in table 1.

4.4 $M(II)$ -GMP-Ala (1:1:1) systems

In Cu(II) and Zn(II) ternary systems, a precipitate invariably appeared before $m = 2$ (where, m = moles of base added per mole of metal ion). However, calculations were performed taking experimental points well ahead of the precipitation region. The formation of a mono-protonated ternary complex ($K_{M(HL)A}^M$) is assumed in the buffer region between $m = 0-2$. The constants were determined with the help of (5).

The mixed ligand titration curve of Co(II)/Ni(II)-GMP-Ala in an equimolar ratio showed an inflection at $m = 1$ (figure 1C and D) followed by a buffer region. In comparison with binary (1:1) metal-nucleotide and metal-alanine titration curves, it was observed that the mixed ligand titration curve in the buffer region between $m = 0-1$ coincided exactly with that of the metal-nucleotide curve. This confirms the formation of protonated binary (1:1) (K_{MHL}^M) complex in the said buffer region. Accordingly, it was assumed that the formation of ternary complex takes place only in the buffer region between $m = 1-3$. The normal stability constant K_{MHA}^{MHL} was determined using (6).

The other amino acid ternary systems behaved in a manner exactly similar to that observed for the corresponding $M(II)$ -GMP-Ala systems. The stability constant values are included in table 2.

4.5 $M(II)$ -IMP-Ala (1:1:1) systems

The trends of potentiometric titration curves of $M(II)$ -IMP-Ala systems were similar to that of the corresponding $M(II)$ -GMP-Ala systems except for Ni(II) system 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_{MLA}^M was determined using (7). Similar trends were observed with the other amino acids studied. These constants are presented in table 2.

The experimental conditions and results were presented as per the IUPAC recommendations¹⁸.

Although the protonation and binary stability constants of amino acids^{13, 19-24} and nucleotides²⁵⁻²⁹ were reported, they were remeasured to minimise the errors which might result in the evaluation of various parameters reported in this work. In GMP and IMP, N(7) also acts as a potential metal binding site in addition to phosphate oxygen

Table 2. Stability constants* of ternary complexes (1:1:1) of M(II) with nucleotides and amino acids
 [Temp = 35°C, I = 0.10 M (KNO₃)]

| Metal ion (II) | M-GMP-Ala | | M-GMP-Aba | | M-GMP-nVal | | M-GMP-nLeu | | M-IMP-Ala ^a | | M-IMP-Aba ^b | | M-IMP-nVal ^c | | M-IMP-nLeu ^d | |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------------|-----------------|------------------------|-----------------|-------------------------|-----------------|-------------------------|-----------------|
| | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} | $K_{M(HL)_A}^M$ | K_{MLA}^{MHL} |
| Co | — | 8.50 | — | 8.34 | — | 8.47 | — | 8.57 | — | 8.36 | — | 8.21 | — | 8.33 | — | 8.43 |
| Ni | — | 9.62 | — | 9.61 | — | 9.77 | — | 9.95 | — | — | — | — | — | — | — | — |
| Cu | 11.88 | — | 12.22 | — | 12.38 | — | 12.60 | — | 11.74 | — | 12.07 | — | 12.23 | — | 12.44 | — |
| Zn | 8.16 | — | 8.07 | — | 8.22 | — | 8.42 | — | 8.01 | — | 7.89 | — | 8.04 | — | 8.24 | — |

For Ni(II)-IMP-AA complexes: [a = K_{MLA}^M = 9.83; b = K_{MLA}^M = 9.81; c = K_{MLA}^M = 9.97; d = K_{MLA}^M = 10.15]

* Constants are accurate to $\pm 0.03 \log K$ units.

and N(1) ³⁰. However, N(7) was not considered in this work since titration curves of binary and ternary systems overlapped with that of free ligand curve in the region of N(7) involvement. Similar observations were also made earlier with other systems ²⁸. The interaction of metal ions with nucleotides is highly pH 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 pH increases ^{31,32}. The metal ions under investigation show high selectivity in complexation with nucleotides which is evident from the type of complexes the Co(II), Ni(II), Cu(II) and Zn(II) have formed. It is very well established that amino acids act as bidentate ligands involving carboxylate (COO⁻) and amino (NH₂) groups. Similar type of ligation was assumed in the present investigation.

In order to rationalise the stabilisation of these systems in solution a quantity Δlog K has been evaluated. (The Δlog K is the difference between the stabilities of binary and ternary systems). The Δlog K values of nucleotide systems are more positive when compared to their corresponding nucleoside systems ¹³. This may be due to the metal ion interaction with phosphate in the former which exerts an influence on the stability of nucleotide complexes ³³. In the present nucleotide-aliphatic amino acid ternary complexes, aromatic-aliphatic type of inter ligand-ligand interactions are possible. Among the amino acids, alanine was considered as a base for comparisons since it has limited tendency for hydrophobic interactions ^{34,35}. The Δlog K values of metal-nucleotide-amino acid systems (table 3) increase in the order: Ala < Aba < nVal < nLeu. This can be attributed to the extent of intramolecular hydrophobic interactions of ternary complexes on the length of the aliphatic side chain of the amino acids. In aqueous media the intramolecular hydrophobic ligand-ligand interactions in mixed ligand complexes emerge from the lipophilicity of one part of the ligand (amino acid) and hydrophilicity of the remainder of the complex (metal-nucleotide). This unique organizing force ⁶ is the result of repulsion between the solvent and amino acid side chain. The dependence of intramolecular interaction on solvent was also investigated and the effect of organic solvents to aqueous solution was established ³⁶ by observing the changes in the water activity. The less positive or nearer to zero Δlog K values of alanine system may be due to the presence of the methyl side chain which is too short to allow any interaction with the aromatic rings of nucleotides. However, with other amino acid systems, the increase in chain length, viz. ethyl, propyl and butyl groups result in more positive Δlog K values, indicating better hydrophobic interactions. The difference in the Δlog K values of GMP and IMP systems may be due to the presence of an additional exocyclic amino group in GMP, which may exert an influence on the hydrophobic interactions.

The extent of side chain influence of amino acids on the hydrophobic interactions are further quantified in terms of ΔΔlog K, which is,

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

In addition, these complexes exist in solution in two different forms i. e. 'closed or stacked' and 'open' in equilibrium with each other, which can be expressed in terms of a dimensionless constant K_I , which is given as,

$$K_I = [\text{M(GMP/IMP)(AA)}_{cl}] / [\text{M(GMP/IMP)(AA)}_{op}].$$

This can be calculated using the following equation,

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

Table 3. Extent of intramolecular hydrophobic interaction in ternary complexes

| Metal ion | Parameters | M:GMP:Ala | M:GMP:Aba | M:GMP:nVal | M:GMP:nLeu | M:IMP:Ala | M:IMP:Aba | M:IMP:nVal | M:IMP:nLeu |
|-----------|--------------------------|-----------|-----------|------------|------------|-----------|-----------|------------|------------|
| Co(II) | $\Delta \log K$ | 0.11 | 0.29 | 0.34 | 0.38 | 0.09 | 0.28 | 0.32 | 0.36 |
| | $\Delta \Delta \log K_M$ | | 0.18 | 0.23 | 0.27 | | 0.19 | 0.23 | 0.27 |
| | K_f | | 0.51 | 0.70 | 0.86 | | 0.55 | 0.70 | 0.86 |
| | % of (MLA) _{cl} | | 33.71 | 41.17 | 46.23 | | 35.48 | 41.17 | 46.23 |
| | $-\Delta G^\circ$ | | 1.06 | 1.35 | 1.59 | | 1.12 | 1.35 | 1.59 |
| Ni(II) | $\Delta \log K$ | 0.09 | 0.30 | 0.35 | 0.40 | 0.07 | 0.27 | 0.32 | 0.37 |
| | $\Delta \Delta \log K_M$ | | 0.21 | 0.26 | 0.31 | | 0.20 | 0.25 | 0.30 |
| | K_f | | 0.62 | 0.82 | 1.04 | | 0.58 | 0.77 | 0.99 |
| | % of (MLA) _{cl} | | 38.27 | 45.05 | 50.98 | | 36.71 | 43.50 | 49.74 |
| | $-\Delta G^\circ$ | | 1.23 | 1.53 | 1.83 | | 1.18 | 1.47 | 1.77 |
| Cu(II) | $\Delta \log K$ | 0.15 | 0.35 | 0.39 | 0.44 | 0.13 | 0.32 | 0.36 | 0.40 |
| | $\Delta \Delta \log K_M$ | | 0.20 | 0.24 | 0.29 | | 0.19 | 0.23 | 0.27 |
| | K_f | | 0.58 | 0.73 | 0.95 | | 0.55 | 0.70 | 0.86 |
| | % of (MLA) _{cl} | | 36.71 | 42.19 | 48.71 | | 35.48 | 41.17 | 46.23 |
| | $-\Delta G^\circ$ | | 1.18 | 1.41 | 1.71 | | 1.12 | 1.35 | 1.59 |
| Zn(II) | $\Delta \log K$ | 0.19 | 0.48 | 0.53 | 0.57 | 0.18 | 0.44 | 0.49 | 0.53 |
| | $\Delta \Delta \log K_M$ | | 0.29 | 0.34 | 0.38 | | 0.26 | 0.31 | 0.35 |
| | K_f | | 0.95 | 1.18 | 1.40 | | 0.82 | 1.04 | 1.23 |
| | % of (MLA) _{cl} | | 48.71 | 54.12 | 58.33 | | 45.05 | 50.98 | 55.15 |
| | $-\Delta G^\circ$ | | 1.70 | 2.00 | 2.24 | | 1.53 | 1.83 | 2.06 |

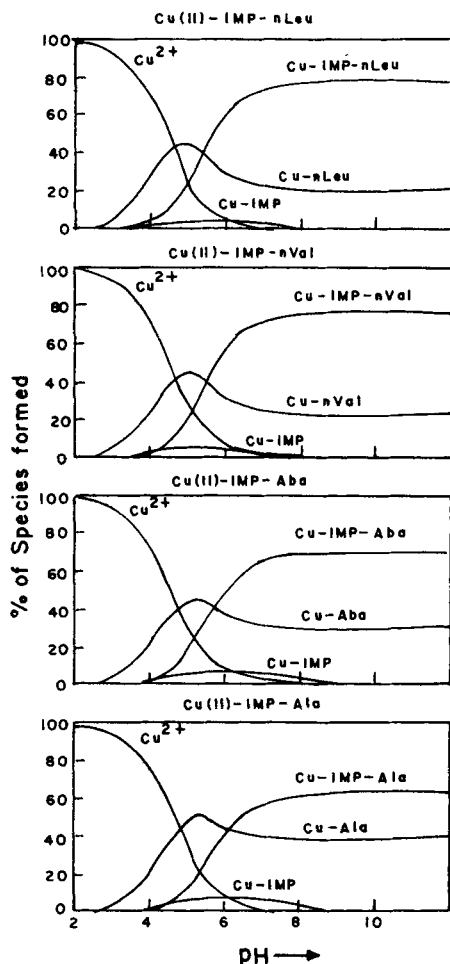


Figure 2. Species distribution curves of Cu(II)-5'-IMP-amino acids (1:1:1) ternary systems.

The K_f values lead to the calculation of percentage of the closed species,

$$\% \text{ of } (\text{MLA})_{cl} = \left(\frac{K_f}{1 + K_f} \right) 100.$$

The free energy (ΔG°) change in kJ/mole was also calculated from $\Delta \Delta \log K$,

$$\Delta G^\circ = -RT \Delta \Delta \log K.$$

A detailed discussion of these parameters can be found elsewhere³⁷⁻⁴⁰.

These parameters were computed and listed in table 3. Though the values are only rough estimates, they unambiguously reveal a regular trend which correlates well with the experimental results. The more positive K_f values for nVal and nLeu systems account for more closed species which suggest that the closed form enhances the stability of the ternary complexes in solution. This is further reflected in species distribution curves of the systems shown in figure 2, where formation of the complex

Cu-IMP-nLeu reaches maximum (~ 77%) at biological pH 7.5, followed by those of corresponding ternary complexes of nVal (~ 73%), Aba (~ 68%) and Ala (~ 58%).

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