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Physicochemical studies on ternary complexes containing adenosine-5′-triphosphate, divalent metal ions and selected biomolecules

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Abstract. Ternary complexes of Co(II), Ni(II), Zn(II), Cd(II), Mg(II) and Ca(II) with adenosine-5′-triphosphate (ATP) as the primary ligand and glycine, alanine, valine, norvaline, leucine, serine, methionine, threonine, aspartic acid, uracil and thymine as secondary ligands have been studied potentiometrically and the formation constants are reported at 35·0°C and $\mu = 0{\cdot}2$ (KNO_3). The differences between the stability constants of the ternary complexes and the corresponding binary complexes are expressed quantitatively in terms of the parameter $\Delta \log K$. Ternary complexes of all metal ions containing phenylalanine, tryptophan, uracil or thymine have $\Delta \log K$ values more positive than expected on statistical grounds, while the $\Delta \log K$ values for ternary complexes of all metal ions with glycine, alanine, valine, norvaline, leucine, serine, threonine and methionine are more negative than expected. This trend is more noticeable in ternary complexes containing aspartic acid. Probable explanations for these are provided.

Keywords. Ternary complexes; formation constants; adenosine-5′-triphosphate; amino acids; pyrimidines.

1. Introduction

Study of ternary, metal complexes containing adenosine-5′-triphosphate (ATP) is of much biological relevance since enzymatic reactions involving ATP are metal ion-dependent and are postulated to proceed through the formation of ternary enzyme–metal ion–ATP complexes (Person and Cusack 1985; Bickel-Sandkotter 1985; Kalbitzer 1986). Information regarding ternary ATP–metal ion–amino acid complexes is scanty. Sigel and coworkers have investigated the stability and structure of a few ternary systems with ATP as the primary ligand, Cu(II), Mn(II) or Zn(II) as the metal ion and the amino acids alanine, leucine or tryptophan as the secondary ligands (Sigel and Neumann 1976; Sigel *et al* 1983). We have therefore carried out a comprehensive study of the ternary complexes of Co(II), Ni(II), Zn(II), Cd(II), Mg(II) and Ca(II) with ATP as the primary ligand and various amino acids and pyrimidines such as glycine, alanine, valine, norvaline, leucine, serine, threonine, methionine, aspartic acid, uracil and thymine as secondary ligands. The formation constants of these complexes have been determined potentiometrically in aqueous solutions at 35·0°C and $\mu = 0{\cdot}2$ (KNO_3). The relative stabilities of the various binary and ternary complexes are compared and discussed in terms of the nature of the primary and secondary ligands.

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2. Materials and methods

DL-amino acids glycine (Gly), alanine (Ala), Valine (Val), norvaline (Nval), serine (Ser), threonine (Thr), methionine (Met), phenylalanine (Phe), tryptophan (Trp) and aspartic acid (Asp) were obtained from the Sigma Chemical Co., USA. Uracil, thymine and adenosine-5′-triphosphate disodium salt were also from Sigma. The purity of these ligands was checked by potentiometric titration with standard sodium hydroxide.

Stock solutions of Co(II), Ni(II), Zn(II), Cd(II), Mg(II) and Ca(II) were prepared from reagent grade nitrate salts and standardized by complexometric titration with EDTA (Flashka 1964). Carbonate-free sodium hydroxide was prepared and standardized by titrating with potassium hydrogen phthalate (Schwarzenbach and Biederman 1948).

Acid dissociation constants of the free ligands and the formation constants of the binary metal complexes were determined by potentiometric titrations of the ligands with standard carbonate-free sodium hydroxide in the absence and presence of the metal ions respectively. For binary systems a 1:3 molar ratio of metal–amino acid was employed. For ternary systems a 1:1:1 molar ratio of ATP–metal ion–secondary ligand was used. The concentration of the metal ion in binary and ternary systems was $1{\cdot}0 \times 10^{-3}$ M. For each experiment fresh, solid ATP was weighed and titrations were completed in a short time to minimize any dephosphorylation taking place. In order to minimize metal ion-promoted intermolecular self-stacking interactions between ATP molecules, low concentrations of ($\approx 1{\cdot}0 \times 10^{-3}$ M) ATP were used. Under these conditions the ATP molecules are predominantly in the monomeric form (Scheller 1981). The potentiometric titrations were carried out in a double-walled titration cell maintained at 35·0°C. The ionic strength was maintained effectively constant at 0·2 by appropriate addition of reagent grade KNO_3. Further details of the experimental procedure for determination of formation constants are given in earlier papers (Srinivas Mohan 1981; Prasad and Srinivas Mohan 1987). The stability constants for the binary metal complexes were calculated from the potentiometric data using a Rossotti and Rossotti (1955) modification of the Bjerrum's method. Comparison of the binary and ternary potentiometric titration curves indicates that in the ternary systems, the 1:1 M (II)–ATP complex is formed in the lower buffer region and the addition of the secondary ligand takes place in the upper buffer region. Ternary complex formation was therefore considered to take place in a stepwise fashion as represented by the equilibria (1) and (2) (L = ATP, A = secondary ligand).

$$M + L \rightleftharpoons ML, \tag{1}$$

$$ML + A \rightleftharpoons MLA. \tag{2}$$

The stability constant for the ternary complexes (3) was calculated in the upper buffer region using suitable material balance equations described in an earlier paper (Prasad *et al* 1987)

$$K_{MLA}^{ML} = \frac{[MLA]}{[ML][A]} \tag{3}$$

3. Results and discussion

Potentiometric titration curves of binary systems containing a 1:3 molar ratio of metal ion and the amino acids Gly, Ala, Val, Nval, Leu, Phe, Tyr, Ser, Thr, Met (in the

diprotonated form) exhibit a steep inflexion at $m = 3$ followed by a buffer region till $m = 6$ (m = moles of base added per mole of metal ion). The formation constants for the normal *mono* (MA) and *bis* (MA_2) binary complexes were calculated in the upper buffer region taking into consideration the pK_2 of the amino acids. In binary systems involving aspartic acid (triprotonated form) the titration curve for the 1:2 metal–ligand ratio exhibits an inflexion at $m = 3$ followed by a buffer region upto $m = 6$. The formation constants for the MA and MA_2 complexes were calculated in the region of $m = 3$ to 6 using the pK_3 value of aspartic acid. In binary systems involving pyrimidines as ligands a 1:1 molar ratio of metal–ligand was employed and the stability constants for the binary complex MA was calculated by taking into consideration the pK_1 value of pyrimidines. Stability constants could be calculated for Co(II) and Ni(II)-pyrimidine systems only. Precipitation was found to take place in the case of Zn(II) and Cd(II). For Mg(II) and Ca(II) the pyrimidine titration curves are superimposable in the absence and presence of metal ions indicating the lack of complex formation. The formation constants for the various binary systems investigated are listed in table 1. Although some of these constants have been reported earlier by other investigators (Sillen and Martell 1971), we have redetermined these constants at 35·0°C and $\mu = 0{\cdot}2$ (KNO_3). This permits an accurate comparison of the binary and ternary constants which have been determined under identical experimental conditions (Martin *et al* 1973). Potentiometric titration curves for ternary systems, containing a 1:1:1 molar ratio of metal ion, ATP (diprotonated form) and the diprotonated amino acids listed above, show an inflexion at $m = 3$ followed by a buffer region upto $m = 4$. The stability constants for the ternary complex MLA, (3), were calculated in the buffer region of 3 to 4 taking into consideration the pK_2 value of the amino acids. In ternary systems involving triprotonated aspartic acid an inflexion was obtained at $m = 4$. The ternary constant was calculated in the buffer region $m = 4$ to 5 using the pK_3 value of aspartic acid. For uracil and thymine the titration curves show an inflexion at $m = 2$ and the stability constants for the ternary complex was calculated in the region of $m = 2$ to 3 using the pK_1 value of the pyrimidines. The formation constants for the various ternary complexes are listed in table 2. The difference in the stability of the binary and corresponding ternary complexes have been quantitatively expressed in terms of the parameter $\Delta \log K$ which is defined by the expression

$$\Delta \log K = \log K^{ML}_{MLA} - \log K^{M}_{MA}. \tag{4}$$

The $\Delta \log K$ values for the various ternary systems investigated are listed in table 2.

The ATP molecule is potentially a quadridentate ligand wherein the three phosphate oxygens and the N-7 of the purine ring may act as possible metal-binding sites. In binary metal complexes it has been shown by NMR and other techniques that ATP binds octahedral metal ions strongly through the β and γ phosphate oxygens. Further the N-7 of the purine ring may simultaneously bind the metal ion to give rise to a macrochelate (Martin and Mariam 1979). The extent to which macrochelates are formed depends on the nature of the metal ion (Sigel *et al* 1987). However, in ternary complexes containing ATP, a metal ion and a second ligand such as NH_3, OH^- or imidazole, the N-7 binding to the metal ion is displaced (Tribolet *et al* 1987). Therefore in ternary complexes we may consider that ATP binds metal ions mainly through the β and γ phosphate oxygens and is effectively acting as a bidentate ligand. The statistical factor for the interaction of an incoming bidentate amino acid to an ATP-bound octahedral metal ion is therefore 5/2; i.e. $\Delta \log K = -0{\cdot}4$. Correspondingly the

Table 1. Formation constants of binary metal complexes $t = 35{\cdot}0°C$; $\mu = 0{\cdot}2$ (KNO_3).

Ligand* (A)	Co(II)		Ni(II)		Zn(II)		Cd(II)		Mg(II)	Ca(II)
	$\log K^{M}_{MA}$	$\log K^{MA}_{MA_2}$	$\log K^{M}_{MA}$	$\log K^{MA}_{MA_2}$	$\log K^{M}_{MA}$	$\log K^{MA}_{MA_2}$	$\log K^{M}_{MA}$	$\log K^{MA}_{MA_2}$	$\log K^{M}_{MA}$	$\log K^{M}_{MA}$
Glycine	4·62	4·11	5·90	4·54	4·86	4·13	4·12	3·45		
Alanine	4·35	3·42	5·60	4·10	4·80	4·05	4·10	3·40	3·29	3·40
Valine	4·24	3·56	5·70	4·29	4·70	4·02	3·91	3·32		
Norvaline	4·27	3·75	5·80	4·42	4·70	3·87	3·85	3·19		
Leucine	4·27	3·66	5·47	4·43	4·69	3·99	4·01	3·29		
Serine	4·19	3·52	5·42	4·34	4·66	3·83	3·78	3·02		
Threonine	4·13	3·78	5·52	4·28	4·69	3·84	3·89	3·28		
Methionine	3·98	3·52	5·32	4·34	4·37	3·56	3·63	2·88		
Aspartic acid	5·78	4·72	7·17	5·99	5·23	4·09	4·31	3·29		
Phenylalanine	3·90	3·65	5·13	4·02	4·61	3·66	3·78	3·27	2·25	2·02
Tryptophan	4·10	3·91	5·25	4·45	4·59	4·05	3·66	3·42	2·28	2·06
Uracil	3·93		3·99							
Thymine	4·10		3·87							

* pK values for amino acids from Srinivas Mohan (1981) and Prasad and Srinivas Mohan (1987). Uracil $pK_1 = 9{\cdot}28$, thymine $pK_1 = 9{\cdot}59$ ($t = 35{\cdot}0°C$, $\mu = 0{\cdot}2$ (KNO_3).

Table 2. Formation constants for ternary complexes (MLA)[a] and $\Delta \log K$ values[b], $t = 35{\cdot}0°C$; $\mu = 0{\cdot}2$ (KNO_3).

Secondary ligands	Co(II)		Ni(II)		Zn(II)		Cd(II)		Mg(II)		Ca(II)	
	$\log K^{ML}_{MLA}$	$\Delta \log K$	$\log K^{ML}_{MLA}$	$\Delta \log K$	$\log K^{ML}_{MLA}$	$\Delta \log K$	$\log K^{ML}_{MLA}$	$\Delta \log K$	$\log K^{ML}_{MLA}$	$\Delta \log K$	$\log K^{ML}_{MLA}$	$\Delta \log K$
Glycine	3·91	−0·71	5·18	−0·72	4·13	−0·73	3·43	−0·69				
Alanine	3·74	−0·61	4·91	−0·69	3·99	−0·81	3·37	−0·73	2·53	−0·76	2·60	−0·80
Valine	3·57	−0·67	5·07	−0·63	3·95	−0·75	3·23	−0·68				
Norvaline	3·59	−0·68	5·20	−0·60	4·02	−0·68	3·21	−0·64				
Leucine	3·62	−0·65	4·78	−0·69	3·96	−0·73	3·32	−0·69				
Serine	3·36	−0·83	4·62	−0·80	3·85	−0·81	2·97	−0·81				
Threonine	3·39	−0·72	4·73	−0·79	3·93	−0·76	3·11	−0·78				
Methionine	3·21	−0·77	4·59	−0·73	3·59	−0·78	2·83	−0·80				
Aspartic acid	4·81	−0·97	6·18	−0·99	4·23	−1·00	3·32	−0·99				
Phenylalanine	3·71	−0·19	4·90	−0·23	4·37	−0·24	3·54	−0·24	2·16	−0·09	1·90	−0·12
Tryptophan	3·98	−0·12	5·08	−0·17	4·43	−0·16	3·50	−0·16	2·38	+0·10	2·14	+0·08
Uracil	4·35	+0·42	4·34	+0·35								
Thymine	4·47	+0·37	4·20	+0·33								

[a] L = ATP; A = secondary ligand; [b] $\Delta \log K$ defined by (4).

statistical factor for the binding of a monodentate secondary ligand (such as the pyrimidines) will be 4/6 i.e. $\Delta \log K = -0{\cdot}2$.

Critical analysis of the $\Delta \log K$ values listed in table 2 reveal a number of interesting features. For bidentate amino acids such as Gly, Ala, Val, Nval, Leu, Ser, Thr and Met the $\Delta \log K$ values for the various metal ions studied are in the range $-0{\cdot}6$ to $-0{\cdot}88$. These values are more negative than the statistically expected value of $-0{\cdot}4$. The lower affinity of these mononegative amino acids for the 1:1 metal–ATP complex as compared to an aquo metal ion can be attributed to the electrostatic repulsion between the negative charges on the carboxylate oxygen of the amino acid and the phosphate oxygens of ATP. In the case of various ternary complexes containing aspartic acid the $\Delta \log K$ values are more negative ($-1{\cdot}0$) and may result from the greater electrostatic repulsion between the dinegative aspartate anion and the $(M\text{–}ATP)^{2-}$ complex. In ternary complexes of all metal ions involving the amino acids Phe and Trp, the $\Delta \log K$ values are substantially more positive than the statistically expected value. This enhanced stability can be attributed to intramolecular metal-bridged stacking interactions between the aromatic side chains of these amino acids and the purine moiety of ATP. Models of these ternary complexes show that when ATP is bound to the metal through the phosphate oxygens the length and flexibility of the ATP molecule permit the adenine ring to stack readily with the aromatic side chain of the metal-bound amino acids. A tentative structure for the ATP–metal ion–Phe complex is shown in figure 1. Evidence for such stacking interactions has been obtained by the use of NMR in ternary ATP–M–tryptophan complexes (Sigel and Neumann 1976), where M = Cu(II) or Zn(II).

The $\Delta \log K$ values for Ni(II) and Co(II) ternary complexes containing ATP and pyrimidines are much more positive ($\approx +0{\cdot}3$) than the statistically expected value of $-0{\cdot}2$. Uracil and thymine are monodentate ligands and bind the metal ions through the deprotonated N-3 of the pyrimidine ring. The enhanced stability for these ternary complexes can be due to intramolecular stacking of the adenine ring of ATP with the pyrimidine ring. Further, models of these ternary complexes show that additional stabilization may result from hydrogen bonding between the amino hydrogens of the adenine ring of ATP and the carbonyl oxygen on C-4 of the pyrimidine ring. The

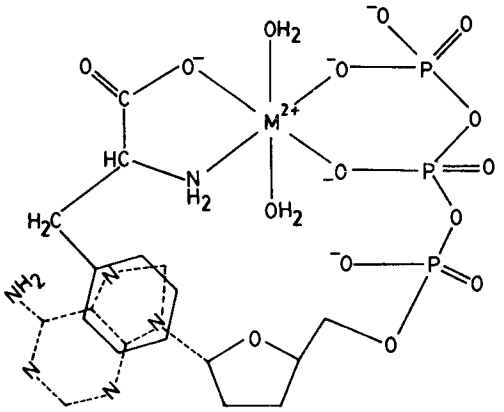

Figure 1. Tentative structure for the ATP–M–Phe ternary complex showing intramolecular stacking interaction.

present investigation shows that in ternary metal complexes an ATP molecule bound to the metal through phosphate oxygens is readily capable of intramolecular stacking with aromatic moieties present on other biomolecules attached to the metal ion, thereby leading to a considerable increase in the stability of ternary complexes. These stacking and other non-covalant interactions play a crucial role in biological selectivity and specificity (Frieden 1975).

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