

Equilibrium studies on ternary Cu(II) complexes containing adenosine-5'-triphosphate

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Abstract. Interaction of adenosine-5'-triphosphate (ATP) with a series of binary Cu(II) complexes (ML) (where L = *o*-phenanthroline (Phen), 5-nitrophenanthroline (NPhen), 5-methyl phenanthroline (MPhen), 2,9-dimethylphenanthroline (DPhen), 2,9-dimethyl-4,7-diphenylphenanthroline (DPhPhen), 2,2'-bipyridyl (Bipy), bis(imidazol-2-yl) methane (BIM), oxalic acid (Ox), glycine (Gly), alanine (Ala), valine (Val), phenylalanine (Phe), tryptophan (Trp), methionine (Met), histidine (His) or aspartic acid (Asp) to form ternary complexes (MLA) was investigated by a pH-metric technique and the formation constants were evaluated at 35.0°C and $\mu = 0.2$ M (KNO₃). The influence exerted by ligand L on the binding of ATP to Cu(II) was quantitatively assessed and ATP was found to bind more strongly when L = bidentate nitrogen donors, relatively less strongly when L = amino acids and least strongly when L = a bidentate oxygen donor. With respect to the nitrogen donor ligands the stability of the ternary complexes decreases in the order NPhen > Phen > Bipy > MPhen > DPhen > BIM \approx DPhPhen, whereas in ternary complexes containing amino acids the stability decreases in the order: Phe \approx Trp > Ala > Gly \approx Val \approx Met > His > Asp. The trends in the stability of the various complexes are discussed in terms of the nature of the metal ion and the two ligands in its coordination sphere.

Keywords. Equilibrium studies; Cu(II) complexes; adenosine-5'-phosphate.

1. Introduction

In view of the fact that enzyme-metal ion-adenosine-5'-triphosphate (ATP) complexes act as intermediates in biochemical reactions (Bickel-Sandkotter 1985; Pearson and Cusack 1985; Kalbitzer 1986) we reported in an earlier paper (Rao and Srinivas Mohan 1989) *in vitro* model system studies on the formation and stability of ternary complexes involving Co(II), Ni(II), Zn(II), Ca(II) and Mg(II) with ATP as one ligand and a series of amino acids or pyrimidines as the second ligand. A survey of literature shows that solution studies on ternary Cu(II) complexes (MLA) containing ATP (A) have been investigated to a limited extent. Cu(II) complexes with A = ATP and L = Ala, Leu or Trp (Sigel *et al* 1983); Bipy (Chaudhuri and Sigel 1977) and Phen (Mitchell and Sigel 1978) were investigated in aqueous media at 25.0°C and $\mu = 0.1$ M (NaClO₄). The formation and stability of Cu(II) complexes containing ATP and L = Bipy, Phen, 2-(2'-pyridyl) benzimidazole, 2-(2'-pyridyl) imidazoline (Patel *et al* 1983); NPhen and dipyritylamine (Emanuel and Bhattacharya 1985) were studied in dioxane-water medium at 30°C and $\mu = 0.2$ M (NaClO₄). Ternary complexes

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containing ATP with L = pyridine-2,6-dicarboxylate or iminodiacetate have also been studied (Rafiq and Bhattacharya 1990). In order to obtain more broad based information we have carried out a detailed quantitative study on the binding of ATP to Cu(II) in the presence of a series of ligands (L) which possess different donor sites (N-N, N-O⁻ or ⁻O-O⁻), substituents, surface area of aromatic rings, magnitude of negative charge and varying σ and π bonding capabilities in aqueous media at 35.0°C and $\mu = 0.2$ M (KNO₃). The series of ligands (L) used were *o*-phenanthroline (Phen), 5-nitrophenanthroline (NPhen), 5-methylphenanthroline (MPhen), 2,9-dimethylphenanthroline (DPhen), 2,9-dimethyl-4,7-diphenylphenanthroline (DPhPhen), 2,2'-bipyridyl (Bipy), bis(imidazol-2-yl) methane (BIM), oxalic acid (Ox), glycine (Gly), alanine (Ala), valine (Val), phenylalanine (Phe), tryptophan (Trp), methionine (Met), histidine (His) and aspartic acid (Asp). The influence exerted by ligand L on the binding of ATP with Cu(II) was quantitatively compared under identical experimental conditions.

2. Materials and methods

Bis(imidazol-2-yl) methane was synthesized by a reported procedure (Joseph *et al* 1977). The other ligands used were obtained from Sigma and Aldrich Chemical Co. USA. A stock solution of Cu(II) was prepared from analytical grade copper(II) nitrate and standardized by titrating with disodium EDTA (Flashka 1964). Carbonate-free sodium hydroxide was prepared and standardized by titrating with potassium hydrogen phthalate (Schwarzenbach and Biederman 1948). Further details of the experimental procedure are given in the earlier paper (Rao and Srinivas Mohan 1989). The formation constants for the various binary and ternary constants were computed from the potentiometric data using the computer programme SCOGS (Sayce 1968) by taking into consideration the relevant protonation, binary and ternary formation constants.

3. Results and discussion

Potentiometric titration curves for binary systems containing Cu(II) and the various diprotonated bidentate ligands (L) or ATP in a 1:1 molar ratio exhibit an inflexion at $a = 2$ (a = moles of base added per mole of ligand) while the titration curves for the 1:1:1 ternary systems containing Cu(II), diprotonated ligand (L) and ATP (A) show a steep inflexion at $m = 4$ (m = moles of base added per mole of metal ion). For ternary systems containing the triprotonated ligands His or Asp an inflexion was observed at $m = 5$. Formation constants for the relevant systems are listed in table 1. The relative stability of the ternary complexes (MLA) and the relevant binary complex (MA) were quantitatively compared in terms of the parameter $\Delta \log K$ given by (1) and are listed in table 1,

$$\Delta \log K = \log K_{MLA}^{ML} - \log K_{MA}^M \quad (1)$$

The above parameter represents the difference in the stability for the addition of ATP to the various binary 1:1 [Cu-L] complexes relative to the aquo cupric ion. In order to obtain precise $\Delta \log K$ values the formation constants for the binary (ML, MA)

Table 1. Formation constants^a of ternary Cu(II) complexes (MLA) containing various ligands (L), ATP (A) and corresponding $\Delta\log K$ and $\Delta\Delta\log K$ values. $t = 35.0^\circ\text{C}; \mu = 0.2 \text{ M (KNO}_3\text{)}$

Ligand (L)	$\log K_{MLA}^{ML}$	$\Delta\log K^b$	$\Delta\Delta\log K^c$
5-Nitrophenanthroline	6.65	+ 0.69	+ 0.12
<i>o</i> -Phenanthroline	6.53	+ 0.57	
5-Methylphenanthroline	6.21	+ 0.25	- 0.32
2,9-Dimethylphenanthroline	5.66	- 0.30	- 0.87
2,9-Dimethyl-4,7-diphenyl phenanthroline	5.33	- 0.43	- 1.00
2,2'-Bipyridyl	6.38	+ 0.42	
Bis(imidazol-2-yl)methane	5.56	- 0.40	- 0.82
Glycine	6.96	- 1.04	- 0.11
Alanine	7.01	- 0.93	
Valine	7.04	- 1.04	- 0.11
Phenylalanine	6.96	- 0.68	+ 0.25
Tryptophan	7.20	- 0.70	+ 0.23
Methionine	6.61	- 1.09	- 0.16
Histidine	8.54	- 1.22	- 0.29
Aspartic acid	6.93	- 1.45	- 0.52
Oxalic acid	4.45	- 1.51	

^aTernary constants accurate to ± 0.1 . Binary constants for Cu(II)-ATP are $\log K_{MHA}^M = 2.78$ and $\log K_{MA}^M = 5.96$. Formation constants for binary ML complexes from our earlier work under identical experimental conditions (Prasad and Srinivas Mohan 1987; Prasad *et al* 1987; Prasad *et al* 1988).

^b $\Delta\log K$ defined by (1). $\Delta\log K$ values reported earlier: For Phen-Cu-ATP and Bipy-Cu-ATP + 0.56 and + 0.33 respectively in aqueous media at 25.0°C and $\mu = 0.1 \text{ M NaNO}_3$ (Sigel 1986) and - 0.29 and - 0.24 respectively in 50% v/v dioxane-water medium at 30.0°C and $\mu = 0.2 \text{ M (NaClO}_4\text{)}$ (Patel *et al* 1983). For NPhen-Cu-ATP + 0.31 in 50% v/v dioxane-water medium at 30.0°C and $\mu = 0.2 \text{ M}$ (Emannuel and Bhattacharya). For Ala-Cu-ATP, Leu-Cu-ATP and Trp-Cu-ATP - 1.50, - 1.40 and - 1.31 respectively in aqueous medium at 25.0°C and $\mu = 0.2 \text{ M (NaClO}_4\text{)}$ (Sigel *et al* 1983).

^c $\Delta\Delta\log K$ defined by (2).

and ternary complexes (MLA) were determined under identical experimental conditions. The $\Delta\log K$ values obtained for some relevant ternary systems by other investigators under different experimental conditions are also listed in table 1. These constants have been redetermined by us at the experimental conditions used in the present work to precisely evaluate the influence of the nature of ligand L on the binding of Cu(II) with ATP.

3.1 Ternary Cu(II) complexes containing N-N donor ligands and ATP

Although in binary metal complexes ATP behaves as a tridentate ligand and binds to metal ions using the β and γ phosphate oxygens and N-7 of the purine ring (Martin and Mariam 1979), in ternary complexes the N-7 binding is displaced and ATP

becomes bidentate and coordinates through the β and γ Phosphate oxygens (Tribolet *et al* 1987). The statistically expected $\Delta\log K$ value for binding of two different bidentate ligands to square planar Cu(II) is 1/4 i.e. $\Delta\log K = -0.6$. The $\Delta\log K$ values for Phen and its derivatives (table 1) are more positive than expected on purely statistical grounds indicating the preference of ATP to bind the binary ML complex relative to the aquo cupric ion. The extra stabilization of Phen–Cu–ATP and Bipy–Cu–ATP complexes has been attributed to metal $d^x \rightarrow$ ligand p^x backbonding which causes the metal ion to become more electropositive and thereby interact strongly with the phosphate oxygens of ATP and also to intramolecular metal ion mediated stacking interactions between the aromatic moieties of the primary amine and the adenine ring of ATP (Mitchell and Sigel 1978; Chaudhuri and Sigel 1977). Stacking interactions have been confirmed by UV–Vis and NMR spectroscopy by these investigators. In the present work the influence of the substituted Phen ligands on the stability of the ternary complexes has been quantitatively assessed in terms of the parameter $\Delta\Delta\log K$ given by

$$\Delta\Delta\log K = \Delta\log K_1 - \Delta\log K_2. \quad (2)$$

$\Delta\log K_1$ corresponds to ternary systems containing substituted Phen ligands and $\Delta\log K_2$ corresponds to the parent compound (Phen). The positive $\Delta\Delta\log K$ values for ternary systems containing NPhen (table 1) can be attributed to the stronger binding of the phosphate oxygens of ATP with the relatively more positive metal centre resulting from the electron withdrawing effect of the nitro group. Such a stabilizing effect has been previously observed for a similar system in dioxane–water medium (Emanuel and Bhattacharya 1985). However, the magnitude of the effect is more pronounced in the aqueous media used in the present work. The negative $\Delta\Delta\log K$ values for MPhen containing complexes reflect the lower stability resulting from the electron donating effects of the methyl group. The still more negative $\Delta\Delta\log K$ values for ternary systems containing DPhen and DPhPhen may be due to the electron donating tendency and the steric hindrance between the incoming ATP molecule and the two methyl groups located adjacent to the ligating nitrogen donor atoms of the two ligands. The more negative $\Delta\Delta\log K$ values for ternary complexes containing BIM ($\Delta\log K_1$) as compared to the Bipy ($\Delta\log K_2$) systems reflects the destabilization arising from (a) the lesser capacity of the imidazole rings in BIM to participate in backbonding relative to the pyridyl rings of Bipy (Sundberg and Martin 1974) and (b) to smaller surface area available for stacking on the imidazole rings in comparison to the pyridyl rings. A schematic representation of the BIM–Cu(II)–ATP complex is shown in figure 1.

3.2 Ternary Cu(II) complexes involving $N-O^- / ^-O-O^-$ donor ligands and ATP

The $\Delta\log K$ values for all ternary complexes containing amino acids are more negative than expected on statistical grounds and may result from the electrostatic repulsion between the negative charge on the carboxylate and phosphate oxygens of the two ligands. The influence of the nature of the amino acid side chain on the stability has been quantified in terms of the parameter $\Delta\Delta\log K$, (2). For these systems $\Delta\log K_1$ corresponds to ternary complexes containing Gly, Val, Phe, Trp, Met, His or Asp and $\Delta\log K_2$ corresponds to ternary systems containing Ala. The data for this parameter (table 1) show that ternary complexes containing Phe and Trp are

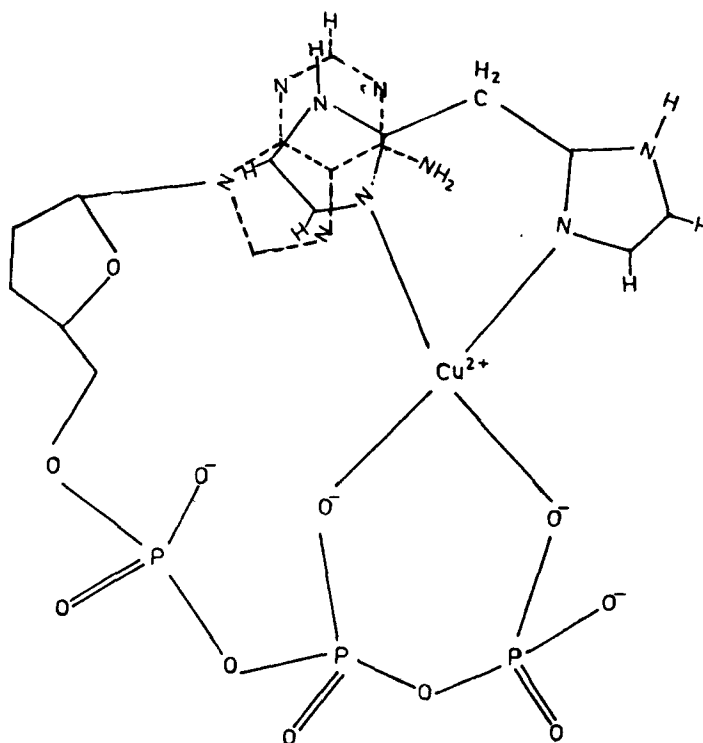


Figure 1. Tentative structure of bis(imidazol-2-yl) methane-Cu(II)-adenosine-5'-triphosphate showing intramolecular stacking interaction.

considerably stabilized whereas complexes containing His or Asp are destabilized considerably. The enhanced stability for Trp-Cu-ATP complexes observed earlier under different experimental conditions (Sigel and Mitchell 1978) was attributed to metal ion mediated intramolecular stacking interactions between the indole moiety of Trp and the adenine ring of ATP. Similar stacking interactions between the phenyl ring of Phe and adenine moiety of ATP leads to the extra stabilization observed for this system. The diminished stability of ternary complexes containing His or Asp may result from the decrease in denticity from tridentate in binary (Martin 1979) to bidentate in ternary complexes wherein electrostatic repulsion between the phosphate oxygens of ATP and the α -carboxylate of His and β -carboxylate of Asp may prevent these groups from binding to the weak axial site of Cu(II). Since His is ambidentate an alternate possible structure involves binding to metal in the glycine mode with the side chain imidazole moiety remaining unbound. Ternary complexes containing the bidentate dinegative oxygen donor ligand Ox are greatly destabilized due to repulsive interactions between the bound oxygens of the two ligands. Calculation of the concentration of various species as a function of pH using the computer programme BEST (Martell and Motekaitis 1988) shows that at pH 7.0 the percentage of the ternary Cu(II) complex containing BIM and ATP is 97 whereas the corresponding value for systems containing Ox and ATP is only 80. In conclusion it may be stated that the various factors which influence the formation and stability of the *in vitro*

model ternary complexes may also play a pivotal role in regulating the ability of ATP to bind metal ions in the presence of other coordinating biomolecules in *in vivo* systems.

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