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Complexes of Magnesium(II) and other Divalent Metal Ions with Adenosine 5′-Triphosphate and 2,2′-Dipyridylamine in Aqueous Solution

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Abstract

Binary and ternary systems involving adenosine 5′-triphosphate (ATP), 2,2′-dipyridylamine (DPA) and magnesium, calcium, strontium, manganese, cobalt, copper, and zinc(II) metal ions have been investigated in aqueous media by potentiometric titrations. The analysis of the titration curves shows the existence of $M(ATP)^{2-}$, $M(ATP)(H)^-$, and $M(ATP)_2(H)_2{}^{4-}$ species for alkaline-earth metal ions, while no ternary complex can be detected. For transition metal ions both binary and ternary species are found. Binary $M(ATP)_2(H)_2{}^{4-}$ complexes are present in solutions containing manganese and cobalt(II) metal ions but these species cannot be revealed in the case of copper and zinc(II). Ternary complexes as $M(ATP)(DPA)^{2-}$ and $M(ATP)(DPA)(H)^-$ are common to all transition metals. Binuclear and hydroxo complexes as $M_2(ATP)(OH)^-$ and $M(ATP)(OH)^{3-}$ are found only for copper and zinc(II). A hypothesis on the possible role of the species M–ATP in 1:2 ratio in the dephosphorylation mechanism is advanced on the basis of a comparison between the equilibrium data in the solution phase and the solid state structures of the magnesium, calcium, and manganese(II)–ATP–DPA systems.

Introduction

During the last decade, the interest in the interactions between metal ions and adenosine 5′-triphosphate (ATP) has always been growing, due to the considerable importance of the phosphoryl transfer enzymatic processes which all involve divalent cations such as magnesium and manganese(II) [1, 2]. The recent success in crystallizing ternary complexes containing a metal ion, ATP, and an aromatic base as 2,2′-bipyridyl [3, 4], 1,10-phenanthroline [5], or 2,2′-dipyridylamine (DPA) [6, 7] has facilitated the identification of interesting molecular structures, by single crystal X-ray diffraction technique. In the study of these ternary systems, for the first time, it has been possible to isolate and deeply characterize a complex containing magnesium(II) and ATP in the solid state. In fact, the study revealed that in the magnesium(II)–ATP–DPA ternary system, as well as in the corresponding systems of calcium, manganese, and cobalt(II), $M(ATP)_2(H)_2{}^{4-}$ units are present in the crystal lattice, while a $M(H_2O)_6{}^{2+}$ ion does not interact with any basic site of the purine moiety, neither with the DPA nitrogen atom [7]. These hydrated metal ions form strong hydrogen bonds with the γ-phosphate group. The information obtained about the donor ability of ATP towards divalent metal ions has been exploited in hypothesizing a possible hydrolysis mechanism of ATP, *i.e.* a pathway for the phosphoryl transfer at the end of the triphosphate chain [7].

However, it is evident that one must be very careful in drawing such conclusions on the basis of solid-state data alone. For these reasons, we considered that investigations on these and similar systems in the solution phase could be useful in shedding light on the very important role which metal ions, such as magnesium and manganese(II), play in biological systems.

Our study was extended to other biologically significant divalent metal ions as calcium, copper, and zinc(II), as well as to strontium and cobalt(II), in order to obtain deeper information on the relationship between the complex stability constants and the metal ion nature.

An aromatic base such as DPA was chosen with the intention of imitating the aromatic aminoacid moieties present in enzymes.

For all the metal ions, measurements were performed in $NaClO_4$ aqueous solutions. Also, for copper and zinc(II) systems, KNO_3 aqueous media have been investigated.

Experimental

Materials

Metal(II) perchlorates or nitrates were reagent grade, purchased from Fluka AG, Buchs, Switzerland. Zinc and strontium(II) perchlorates were

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prepared as reported in refs. 8 and 9, respectively. $NaClO_4$, KNO_3, and DPA were from Merck AG, Darmstadt, F.R.G. and adenesine 5′-triphosphoric acid disodium salt was obtained from Sigma, St. Louis, Mo., U.S.A. (inorganic phosphate less than 0.04%).

Stock solutions of the proper metal salt were standardized by conventional instrumental and non-instrumental analyses. The DPA commercial product was twice crystallized from ethanol. Na_2H_2ATP was standardized by acid-base titration. Twice distilled water was used for preparing the solutions.

Apparatus

In the potentiometric titrations, the titrant was added by a Metrohm Dosimat equipped with the proper syringe, and the pH values of the solutions were read on a Metrohm E 603 digital pH-meter.

Methods

The acidity constants of the H_2ATP^{2-} and $HDPA^+$ were determined by titration of carefully de-aerated solutions containing known amounts of ATP or DPA, of $HClO_4$ or HNO_3, and of $NaClO_4$ or KNO_3 in order to obtain an ionic strength equal to 0.1, employing 0.01 M NaOH in $NaClO_4$ or KNO_3 as titrant. The titrations were carried out at 25 ± 0.1 °C.

The M–ATP and M–DPA binary systems, as well as the M–ATP–DPA ternary ones, were studied by acid-base titrations carried out under experimental conditions similar to those followed in evaluating the above cited acidity constants. The solutions contained known amounts (about 0.002 M) of $M(ClO_4)_2$ or $M(NO_3)_2$, H_2ATP^{2-} and/or DPA in molar ratios ranging from 3:1 to 1:3. Some titrations were performed after adding suitable quantities of $HClO_4$ or HNO_3. $NaClO_4$ or KNO_3 were also added to the solution up to an ionic strength equal to 0.1.

The evaluation of the acidity constants was performed by the least-squares computer programs ESAB [10], which minimizes the error-square sum in the titre, or BETAREF which, as well as the new program SUPERQUAD [11], minimizes an error-square sum based on measured electrode potentials. Both were implemented on a PDP 11/23 PLUS (Digital Equipment Corporation) minicomputer. Calculations for evaluating formation constants and distribution diagrams for binary and ternary systems were carried out by means of BETAREF which, also, allowed us a statistical analysis of the results obtained. $\beta^{M}_{M(DPA)}$ and $\beta^{M}_{M(DPA)_2}$ values resulted always in excellent agreement with those already reported [12].

Results and Discussion

Equations (1)–(13) define the overall stability constants for the binary (M–ATP) and ternary (M–ATP–DPA) complexes studied:

$$M^{2+} + ATP^{4-} \rightleftharpoons M(ATP)^{2-} \qquad \beta^{M}_{M(ATP)} = [M(ATP)]/[M][ATP] \qquad (1)$$

$$M^{2+} + ATP^{4-} + H^+ \rightleftharpoons M(ATP)(H)^- \qquad \beta^{M}_{M(ATP)(H)} = [M(ATP)(H)]/[M][ATP][H] \qquad (2)$$

$$M^{2+} + ATP^{4-} + 2H^+ \rightleftharpoons M(ATP)(H)_2 \qquad \beta^{M}_{M(ATP)(H)_2} = [M(ATP)(H)_2]/[M][ATP][H]^2 \qquad (3)$$

$$M^{2+} + 2ATP^{4-} \rightleftharpoons M(ATP)_2{}^{6-} \qquad \beta^{M}_{M(ATP)_2} = [M(ATP)_2]/[M][ATP]^2 \qquad (4)$$

$$M^{2+} + 2ATP^{4-} + 2H^+ \rightleftharpoons M(ATP)_2(H)_2{}^{4-} \qquad \beta^{M}_{M(ATP)_2(H)_2} = [M(ATP)_2(H)_2]/[M][ATP]^2[H]^2 \qquad (5)$$

$$2M^{2+} + ATP^{4-} \rightleftharpoons M_2(ATP) \qquad \beta^{M}_{M_2(ATP)} = [M_2(ATP)]/[M]^2[ATP] \qquad (6)$$

$$2M^{2+} + ATP^{4-} + H^+ \rightleftharpoons M_2(ATP)(H)^+ \qquad \beta^{M}_{M_2(ATP)(H)} = [M_2(ATP)(H)]/[M]^2[ATP][H] \qquad (7)$$

$$M^{2+} + ATP^{4-} + OH^- \rightleftharpoons M(ATP)(OH)^{3-} \qquad \beta^{M}_{M(ATP)(OH)} = [M(ATP)(OH)]/[M][ATP][OH] \qquad (8)$$

$$2M^{2+} + ATP^{4-} + OH^- \rightleftharpoons M_2(ATP)(OH)^- \qquad \beta^{M}_{M_2(ATP)(OH)} = [M_2(ATP)(OH)]/[M]^2[ATP][OH] \qquad (9)$$

$$M^{2+} + ATP^{4-} + DPA \rightleftharpoons M(ATP)(DPA)^{2-} \qquad \beta^{M}_{M(ATP)(DPA)} = [M(ATP)(DPA]/[M][ATP][DPA] \qquad (10)$$

$$M^{2+} + ATP^{4-} + DPA + H^+ \rightleftharpoons M(ATP)(DPA)(H)^- \qquad \beta^{M}_{M(ATP)(DPA)(H)} = [M(ATP)(DPA)(H)]/[M][ATP][DPA][H] \qquad (11)$$

$$M^{2+} + ATP^{4-} + DPA + 2H^+ \rightleftharpoons M(ATP)(DPA)(H)_2 \qquad \beta^{M}_{M(ATP)(DPA)(H)_2} = [M(ATP)(DPA)(H)_2]/[M][ATP][DPA][H]^2 \qquad (12)$$

$$M^{2+} + 2ATP^{4-} + DPA + H^+ \rightleftharpoons M(ATP)_2(DPA)(H)^{5-} \qquad \beta^{M}_{M(ATP)_2(DPA)(H)} = [M(ATP)_2(DPA)(H)]/[M][ATP]^2[DPA][H] \qquad (13)$$

TABLE I. Logarithms of the Stability Constants of the Binary Complexes M–ATP and Ternary Complexes M–ATP–DPA as Obtained by Potentiometric Titrations

m n q r[a]	log $\beta(\sigma)$								
	Mg[b]	Ca[b]	Sr[b]	Mn[b]	Co[b]	Cu[b]	Cu[c]	Zn[b]	Zn[c]
1 0 1 0	4.365(4)	4.032(5)	3.664(6)	5.32(4)	5.056(4)	6.507(3)	6.455(3)	5.81(5)	5.44(1)
1 0 1 1	8.57(5)	8.94(2)	8.99(1)	9.83(6)	9.23(2)	10.236(7)	10.055(7)	10.25(6)	9.75(2)
1 0 1 2				13.49(7)				13.86(7)	13.09(4)
2 0 1 0							9.22(3)	8.27(7)	7.52(4)
2 0 1 2	18.33(3)	17.56(11)	17.5(1)	18.81(6)	18.53(4)				
1 0 2 0				7.43(8)				8.26(8)	7.22(6)
1 0 2 1								11.98(12)	
1 0 1 −1						−1.21(2)	−1.55(2)	−2.56(12)	
1 0 2 −1							3.54(2)	1.56(6)	1.28(5)
1 1 1 0				8.46(3)	10.15(3)	14.313(5)	14.129(5)	9.62(1)	9.27(2)
1 1 1 1				15.53(4)	15.52(4)	18.476(8)	18.157(7)	15.23(4)	
1 1 1 2				20.27(4)	19.80(8)			20.16(2)	19.09(3)
2 1 1 1					19.77(10)				

[a]The indexes refer to the reaction mATP + nDPA + qM + rH $\rightleftarrows$ $M_q(ATP)_m(DPA)_n(H)_r$; when r is negative the ligand is OH^-. [b]$I = 0.1$; $NaClO_4$. [c]$I = 0.1$; KNO_3.

TABLE II. Acidity Constants of the Binary and Ternary Complexes

m n q r	pK_a[a]								
	Mg[b]	Ca[b]	Sr[b]	Mn[b]	Co[b]	Cu[b]	Cu[c]	Zn[b]	Zn[c]
1 0 1 2				3.66				3.61	3.34
1 0 1 1	4.205	4.91	5.33	4.51	4.17	3.73	3.60	4.44	4.31
1 1 1 2				4.74	4.28			4.93	
1 1 1 1				7.07	5.37	4.16	4.03	5.61	

[a]For the complexes $M_q(ATP)_m(DPA)_n(H)_r$. [b]$I = 0.1$; $NaClO_4$. [c]$I = 0.1$; KNO_3.

Table I shows the β values for the binary and ternary complexes which we have found to exist in the media studied. On the basis of this data, we have computed the acidity constants of both binary and ternary complexes (see Table II), as well as the difference in the stability between the corresponding binary and ternary complexes, computed as [13]:

$$\Delta \log K = \log K^{M(DPA)}_{M(ATP)(DPA)} - \log K^{M}_{M(ATP)}$$

This difference corresponds to log K of the following equilibrium [14]:

$$M(ATP)^{2-} + M(DPA)^{2+} \rightleftarrows M(ATP)(DPA)^{2-} + M^{2+} \qquad (14)$$

The resulting data are reported in Table III.

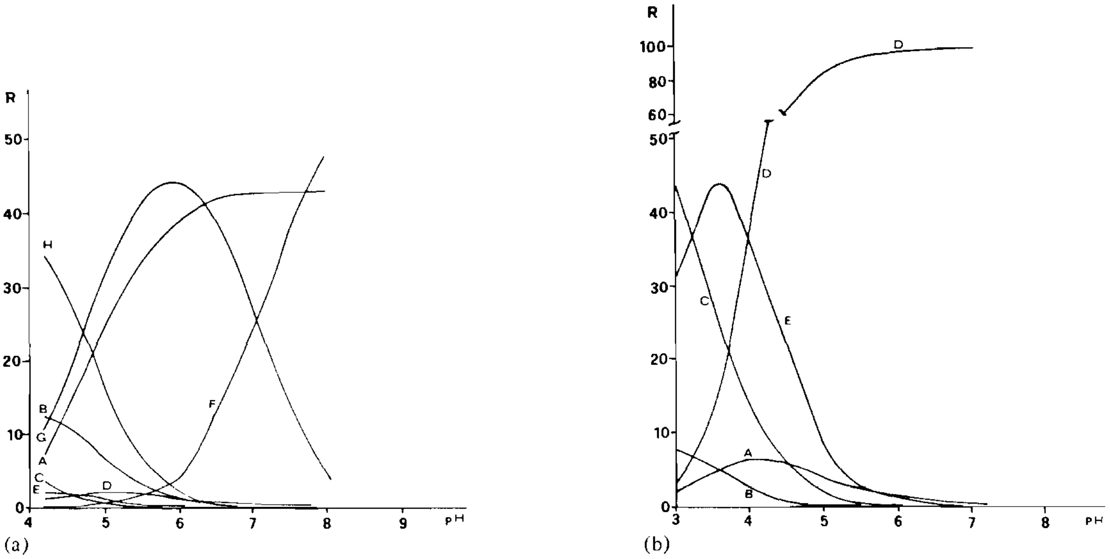
TABLE III. Comparison of the Stability Constants of Corresponding Ternary and Binary Complexes

Metal ion	Δ log K
Mn^{2+}	+0.70[a]
Co^{2+}	+0.31[a]
Cu^{2+}	−0.25[a]
Cu^{2+}	−0.37[b]
Zn^{2+}	+0.06[a]
Zn^{2+}	+0.08[b]

[a]$I = 0.1$; $NaClO_4$. [b]$I = 0.1$; KNO_3.

Alkaline-earth Metal Ion Complexes

Attempts to calculate stability constants for the M–DPA system in the case of alkaline-earth metal ions failed, as well as elaborating data obtained by titration of solutions with a M:DPA molar ratio as high as 20:1. This can be ascribed to the small value of the stability constants of these complexes, which appears reasonable, considering the corresponding values for similar complexes containing 1,10-phenanthroline [13]. Moreover, owing to the high K_b value of DPA, a large fraction of the base results to be protonated in the pH range investigated (3–10).



(a)

(b)

Fig. 1. Distribution diagrams for the M–ATP–DPA ternary systems showing the formation percentages (R) relative to total concentration of the metal, as a function of pH; (a) Mn–ATP–DPA; A = $Mn(ATP)^{2-}$; B = $Mn(ATP)(H)^{-}$; C = $Mn(ATP)(H)_2$; D = $Mn_2(ATP)$; E = $Mn(ATP)_2(H)_2{}^{4-}$; F = $Mn(ATP)(DPA)^{2-}$; G = $Mn(ATP)(DPA)(H)^{-}$; H = $Mn(ATP)(DPA)(H)_2$. (b) Cu-ATP-DPA; A = $Cu(ATP)^{2-}$; B = $Cu(ATP)(H)^{-}$; C = $Cu(DPA)^{2+}$; D = $Cu(ATP)(DPA)^{2-}$; E = $Cu(ATP)(DPA)(H)^{-}$. In both the systems the analytical concentrations of M and DPA were 0.002 M, while ATP was 0.004 mM.

The analysis of the titration curves of the M–ATP–DPA ternary systems for magnesium, calcium, and strontium(II) allows the existence of ternary species to be excluded and the stability constants of the M–ATP binary complexes in the ternary sytems result are equal to those determined by titrations of the binary mixtures [13]. This data allowed us to ascertain the existence of the species $M(ATP)_2(H)_2{}^{4-}$, in agreement with the X-ray data for the solid state [7]. As it was previously suggested, these anionic complexes could exist in enzymatic systems where stacking interactions could link $M(ATP)_2(H)_2{}^{4-}$ or $M(ATP)_2{}^{6-}$ complex species to some aromatic centres present in the protein, close to the active site. The strong π interactions extensively present in the crystal lattices involving DPA and the adenine system, could not be revealed in the solution phase, also because rather low concentrations (*ca.* 0.002 M) were used in our titrations.

As regards the log β values relevant to the binary complexes, as expected on the basis of the relative charge density on the metal centre, magnesium(II) cation forms the strongest complexes, and for all of the three complex species determined the stability constants decrease in the order magnesium > calcium > strontium(II).

Transition Metal Ion Complexes

Figure 1 shows the distribution curves for the manganese and copper(II) ternary systems.

In the ternary systems of manganese and cobalt(II), the most significant binary species are $M(ATP)^{2-}$ and $M(ATP)(H)^{-}$ in the pH range 3–9. However, the $M(ATP)_2(H)_2{}^{4-}$ species could be detected again, although they form in very small amounts. On the contrary, in the case of copper and zinc(II) metal ions, this last binary species could not be identified at all. In this context, it is interesting to note that while it is possible to grow crystals containing the $Mn(ATP)_2(H)_2{}^{4-}$ and $Co(ATP)_2(H)_2{}^{4-}$ units from almost neutral aqueous solution [15], many trials to get single crystals of the corresponding copper and zinc(II) species failed.

Figure 1a shows that, for the system containing manganese(II), $M(ATP)(DPA)^{2-}$, $M(ATP)(DPA)(H)^{-}$ and $M(ATP)(DPA)(H)_2$ are the prevalent ternary species. Similar results are obtained with cobalt and zinc(II) cations. The corresponding β values for the ternary complexes of these metal ions were rather close to each other.

In the copper(II) ternary system, the prevalent species resulted in $Cu(ATP)(DPA)^{2-}$, $Cu(ATP)(DPA)(H)^{-}$, and $Cu(DPA)^{2+}$, together with smaller amounts of $Cu(ATP)^{2-}$, $Cu(ATP)(H)^{-}$, $Cu(ATP)_2{}^{6-}$, $Cu(ATP)(OH)^{3-}$, and $Cu_2(ATP)(OH)^{-}$. It is significant that the copper(II)-containing binary and ternary complexes are the most stable in our series.

Acidity Constants

The acidity constant values of the complexes $M(ATP)(H)^{-}$, $M(ATP)(DPA)(H)^{-}$, and $M(ATP)(DPA)(H)_2$, calculated as previously indicated and reported in Table II, show that, with regard to the alkaline-earth metal complexes, the species $Mg(ATP)(H)^{-}$ exhibits the highest acidity character, followed by the corresponding calcium and strontium(II) complexes.

Since the pK_a value of the γ-phosphate is equal to 6.63 and that of N(1) is equal to 4.09 in absence of metal ion, it seems plausible to identify the γ-phosphate as the first protonation site in the strontium and calcium(II) complexes, but no conclusive statement can be formulated in the case of the magnesium compound. In fact, the pK_a value for this compound could be attributed to a γ-phosphate strongly interacting with the metal ion but, taking into account the standard deviations, the protonation of the N(1) atom of the adenine ring cannot be completely disregarded.

The pK_a values for the complex Mn(ATP)(DPA)-$(H)_2$ show that $HDPA^+$ has not undergone any appreciable increase in acidity (pK_a of free protonated DPA = 7.14), while a remarkable change is evidentiated in the basicity of the terminal phosphate group. We recall that in the solid state protonation of N(1) has been ascertained for the manganese(II) complex [15].

Less conclusive statements can be drawn for the other ternary complexes, even if a strong decrease in the proton basicity of DPA should be evident in any case, suggesting the DPA is directly implied in strong bonding formation with the metal.

Low pK_a values are found for all the binary complexes involving transition metal ions. These values do not allow us to ascertain the nature of the protonation site.

The Δ log K values, listed in Table III, indicate that an increased stability of ternary complexes with respect to the binary ones, occurs for the manganese, cobalt, and zinc(II) systems, but no stabilizing effect can be observed for the copper(II) complexes [14]. This means that the equilibrium (14) is shifted towards the right for manganese, cobalt, and zinc-(II).

To conclude, there is evidence that suggests the importance of the species with metal–ATP in 1:2 ratio in the case of the alkaline-earth and manganese(II) metal ions in water solution. These molecules could be considered as interacting with the aromatic moieties and, at the same time, with a second metal ion such as $M(H_2O)_6{}^{2+}$ which could be coresponsible for the terminal phosphate activation towards hydrolysis. However, species with metal–ATP in 1:1 ratio are predominant in the aqueous phase and similar units might just be the proper substrate in the enzymatic reaction.

On the basis of our data, it is obviously impossible to state the actual configuration of the binary and ternary complexes in enzymatic systems but, at least, for magnesium, calcium, and manganese(II) (the cations which play the most significant role) we can hypothesize that the metal ion could interact only with the ATP triphosphate chain, even if a bridging role of the metal ion, itself, between the nucleoside 5′-triphosphate and the protein macromolecule, cannot be disregarded.

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