Thermodynamic Quantities Associated with the Interaction of Adenosine Triphosphate with Metal Ions[1,2]

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Abstract: Potentiometric equilibrium measurements have been made at 0.4, 12, 25, and 40° for the combination of protonated and normal chelate species of adenosine triphosphate (ATP) with nine bivalent metal ions, at a 1:1 molar ratio of ligand to metal ion. Equilibrium constants are reported in the same temperature range for the formation of the monohydroxo mononuclear complex, the dihydroxo mononuclear complex, and the μ-dihydroxo binuclear complex of Cu(II) and ATP. The enthalpy and entropy changes for the formation of protonated and normal metal chelate species and for the hydrolysis and olation reactions of the Cu(II)–ATP chelate were calculated from temperature coefficient data. The enthalpy terms for the formation of the protonated and normal chelate species of ATP are relatively small. The enthalpy changes in the formation of protonated and normal chelate species of the first transition series metals, and Mg(II), are very nearly the same. For Ba(II), Sr(II), and Ca(II), $\Delta H°$ is much less for the protonated chelates than for the normal chelate compounds. The relatively small values of $\Delta H°$, coupled with large positive values of $\Delta S°$, indicate entropy to be the principal driving force for the formation of ATP–metal chelate species in solution. A regular trend is observed in the entropy of formation, $\Delta S°$, which varies inversely as the ionic radius of the cation.

Because of the recent interest in the role of biologically important metals and ligands, it has been considered important to investigate the stability constants and thermodynamics of the alkaline earth and transition metal chelates of adenosine triphosphate. A previous investigation by Naninga[3] was restricted to an ion-exchange study of calcium and magnesium complexes of ATP at pH 8.8 (μ = 0.15). George, *et al.*,[4] computed thermodynamic data for magnesium complexes of ATP and ADP. Their calculations were based on an empirical extrapolation of the data of Burton and Krebs[5] compiled in the pH range 7.7–8.8 to zero ionic strength. In the investigations of Naninga[3] and of George, *et al.*,[4] only the normal (or unprotonated) complexes of ATP were considered. Recently George, *et al.*,[6] have reported thermodynamic data of the normal (unprotonated) Mg(II) complexes of ATP and ADP, and on the ionization of the protonated complexes of these ligands, by an empirical extrapolation based on activity coefficient ratios from secondary phosphate ionization data.

In this study stability constants of monoprotonated and normal chelate species of Mg(II), Ba(II), Sr(II), Ca(II), Co(II), Mn(II), Zn(II), Ni(II), and Cu(II) were measured at 0.4, 12, 25, and 40°, and the corresponding thermodynamic quantities $\Delta H°$, $\Delta F°$, and $\Delta S°$ were calculated. The thermodynamic constants with adenosine diphosphate and adenosine 3- and 5-phosphates are now in progress and will be the subject matter of future publications.

Experimental Methods

The experimental method employed consisted of the potentiometric titration of ATP, in the absence and in the presence of the metal ion being investigated, as described previously.[7] The ionic strength was maintained constant by using 0.1 M KNO_3 as the supporting electrolyte and relatively low concentrations of ligand and metal ion. The titrations were carried out using a 1:1 ratio of concentrations of ligand to metal ion. All measurements were carried out at 0.4, 12, 25, and 40 ± 0.02°. The pK values of acetic acid at 0.4, 12, 25, and 40° used in the calibration of the electrode system were obtained from Harned and Owens.[8] The pH regions below 3.5 and above 10.5 were calibrated by measurements in HCl and KOH solutions, respectively.

Thermodynamic Constants. For this investigation, as well as for a large number of similar investigations in the literature, the standard state is defined as 0.100 M KNO_3 solution, so that the activity of any reacting ionic or nonionic species X becomes

$$\lim_{[X]\to 0} \frac{a_X}{[X]} = 1$$

in the pure ionic medium.[9] On this basis all of the equilibrium constants determined as described above are true thermodynamic constants, as are the values of $\Delta H°$ and $\Delta S°$ calculated for the temperature coefficients of these equilibrium constants. These constants are of value not only in themselves, but because they are comparable to the equilibrium constants in the literature determined under the same conditions for other metal ions and other ligands. These equilibrium constants differ somewhat in magnitude from constants obtained for a standard state of zero concentration of electrolyte; however, experience has shown that values of $\Delta H°$ are about the same for the two conventions. Hence the main difference will occur in the entropy changes of the ionic species.

Reagents. The ATP used in this investigation was obtained from the Nutritional Biochemicals Corporation as the disodium salt. Appropriate samples were weighed individually before each titration to obtain the concentration desired, as a stock solution could not be prepared and kept for long periods of time. During the course of the experimental work the ATP was frequently checked for purity (and for hydrolysis) by potentiometric titration. The results were always consistent with the titrations of the purified materials, so that no decomposition appeared to take place. The metal salt solutions were standardized by titration with the disodium

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(9) At a recent meeting of the Commission on Equilibrium Data of the IUPAC, it was decided to define all equilibrium constants determined under carefully controlled conditions in this manner, to correct the misconception that has developed that constants determined at finite supporting electrolyte concentrations are "apparent" constants, rather than true equilibrium constants.

salt of EDTA as described by Schwarzenbach.[10a] Carbonate-free KOH was prepared by the method of Schwarzenbach and Biedermann[10b] and was standardized by titration with potassium acid phthalate.

Calculations. The acid dissociation constants K_a and K_{2a} for the disodium salt of ATP (H_2L^{2-}) were calculated by a direct algebraic method. The equilibria involved in the formation of a 1:1 complex of ATP and a divalent metal ion may be expressed as

$$M^{2+} + HL^{3-} \rightleftharpoons MHL^- \quad K_1 = \frac{[MHL^-]}{[M^{2+}][HL^{3-}]} \tag{1}$$

$$M^{2+} + L^{4-} \rightleftharpoons ML^{2-} \quad K_2 = \frac{[ML^{2-}]}{[M^{2+}][L^{4-}]} \tag{2}$$

Related equilibria may be defined as

$$M^{2+} + H_2L^{2-} \rightleftharpoons MHL^- + H^+ \quad K_1' = \frac{[MHL^-][H^+]}{[M^{2+}][H_2L^{2-}]} \tag{3}$$

$$MHL^- \rightleftharpoons ML^{2-} + H^+ \quad K_2' = \frac{[ML^{2-}][H^+]}{[MHL^-]} \tag{4}$$

The constants K_1 and K_2 were calculated from the potentiometric data with the help of the procedure previously described.[7]

Solution Equilibria for the Cu(II)-ATP Complex. As reported in a previous paper,[7] titration of the Cu(II)-ATP system beyond 2 equiv of base produces concentration-dependent buffer regions characteristic of polymerization through hydroxo bridges. The data were treated mathematically on the basis that four species are present: a normal 1:1 chelate, a monohydroxo complex $Cu(OH)L^{3-}$, a dihydroxo species $Cu(OH)_2L^{4-}$, and a dimer $(Cu(OH)L)_2^{6-}$. The corresponding solution equilibria may be expressed in terms of the equations

$$CuL^{2-} \rightleftharpoons Cu(OH)L^{3-} + H^+ \quad K_{Cu(OH)L} = \frac{[Cu(OH)L^{3-}][H^+]}{[CuL^{2-}]} \tag{5}$$

$$CuL^{2-} \rightleftharpoons Cu(OH)_2L^{4-} + 2H^+ \quad K_{Cu(OH)_2L} = \frac{[Cu(OH)_2L^{4-}][H^+]^2}{[CuL^{2-}]} \tag{6}$$

$$2CuL^{2-} \rightleftharpoons (Cu(OH)L)_2^{6-} + 2H^+ \quad K_{(Cu(OH)L)_2} = \frac{[(Cu(OH)L)_2^{6-}][H^+]^2}{[CuL^{2-}]^2} \tag{7}$$

$$2Cu(OH)L^{3-} \rightleftharpoons (Cu(OH)L)_2^{6-} \quad K_d = \frac{[(Cu(OH)L)_2^{6-}]}{[Cu(OH)L^{3-}]^2}$$

The amount of various chelate species present under varying conditions of pH and total concentration may be calculated from the above equilibria with the relationships outlined in a previous paper.[7]

Values of enthalpy of complex formation, $\Delta H°$, were obtained by plotting log K against $1/T$. A linear relationship was observed in all the plots over the temperature range studied. $\Delta H°$ is obtained from these plots by multiplying the slopes by $2.303R$, according to the Van't Hoff isochore. Values of $\Delta F°$ and $\Delta S°$ for the various reactions involved were calculated with the usual thermodynamic relationships.

Results

ATP. The acid dissociation constants of ATP at 0.4, 12, and 40° were determined from the corresponding titration curves. The titration curves are similar to that at 25° described earlier,[7] with two sharp inflections separated by a buffer region. The dissociation constants obtained at several temperatures are shown in Table I. The thermodynamic constants for the ionization of ATP are given in Table II.

Mg(II), Ba(II), Sr(II), Ca(II), Co(II), Mn(II), Zn(II), and Ni(II) Complexes. Titration of equimolar amounts of ATP and the metal ions mentioned above gave an inflection at $a = 2$, corresponding to the formation of a 1:1 chelate. The titration curves for these metal ions at 0.4, 12, and 40° are similar to those reported[7] at 25°.

Table I. Acid Dissociation Constants[a] of ATP[b]

Temp, °C	pK_2	pK_{2a}
0.4	4.29	6.56
12.0	4.14	6.54
25.0	4.06	6.53
40.0	3.87	6.52

[a] Standard deviation ±0.01 pK unit. [b] $\mu = 0.1\ M\ (KNO_3)$.

Table II. Thermodynamic Constants for Ionization Reactions of ATP[a]

Reaction	$\Delta H°$, kcal/mole	$\Delta F°$ (25°), kcal/mole	$\Delta S°$ (25°), cal/deg mole
$H_2L^{2-} \rightleftharpoons HL^{3-} + H^+$	$+4.1 \pm 0.1$	$+5.53 \pm 0.01$	-4.5 ± 0.5
$HL^{3-} \rightleftharpoons L^{4-} + H^+$	$+0.5 \pm 0.1$	$+8.90 \pm 0.01$	-27.8 ± 0.5

[a] $\mu = 0.1\ M\ (KNO_3)$.

The stability constants at the temperatures investigated are given in Table III. The stabilities of the normal complexes ML increase in the order Ba < Sr < Ca < Mg < Co < Mn < Zn < Ni < Cu. The same order is also followed by the acido complexes of ATP at 25°. At 0.4, 12, and 40°, however, the order is not strictly followed for some metal ions, as may be seen from Table III. Thermodynamic constants pertaining to the interaction of the above-mentioned metal ions with ATP are given in Table IV.

Cu(II)-ATP. The 1:1 titration curves of Cu(II)-ATP show a steep inflection at $a = 2$, corresponding to the formation of a 1:1 chelate compound, followed by an additional concentration-dependent buffer region of the type characteristic of polynuclear metal complex formation. The curves at 0.4, 12, and 40° are similar to those discussed in an earlier publication.[7] A summary of the equilibrium constants obtained at the four temperatures is given in Table V.

Thermodynamic constants for the hydrolysis and olation reaction of Cu(II)-ATP are presented in Table VI.

Discussion

Since Mg(II) seems to combine with ATP (H_4L) to form a complex MgH_2L, which has not been detected potentiometrically, it has been suggested that potentiometric measurements give only a "lower bound" for complex formation.[11] Such a complex, if formed, would, however, be much less stable than those ($MgHL^-$ and MgL^{2-}) measured in the present investigation. Under the conditions employed in the present work, MgH_2L would be completely dissociated, so that its possible existence at high concentration of reactants in no way affects the validity of the constants reported in Tables II, III, and IV.

Brintzinger[12] has recently reported similar shifts in the phosphate absorption bands on combination with divalent metal ions and hydrogen ions. The changes observed, which were decreases in absorption intensities of the bands at 1015 cm^{-1} (symmetrical $-PO_3^{2-}$ stretching) and 1120 cm^{-1} (symmetrical $>PO_2^-$ and asymmetrical $-PO_3^-$ stretching) indicated that the influence

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Table III. Equilibrium Constants[a] for the Interaction of ATP with Various Metal Ions[b]

Metal ion	0.4°		12°		25°		40°	
	Log K_1	Log K_2	Log K_1	Log K_2	Log K_1	Log K_2	Log K_1	Log K_2
Ba(II)	2.02	3.58	1.92	3.42	1.85	3.29	1.75	3.12
Sr(II)	2.17	3.80	2.11	3.66	2.05	3.54	2.00	3.45
Ca(II)	2.34	4.10	2.21	3.99	2.13	3.97	2.13	3.94
Mg(II)	1.95	3.97	2.16	4.10	2.24	4.22	2.29	4.28
Co(II)	2.45	4.80	2.39	4.69	2.32	4.66	2.24	4.55
Mn(II)	2.55	4.97	2.48	4.82	2.39	4.78	2.30	4.63
Zn(II)	2.81	5.00	2.73	4.88	2.67	4.85	2.58	4.71
Ni(II)	2.88	5.18	2.80	5.05	2.72	5.02	2.59	4.90
Cu(II)	3.32	6.42	3.20	6.20	3.12	6.13	3.01	5.97

[a] The constants are accurate to ±0.02 log K unit. [b] $\mu = 0.1\ M(KNO_3)$.

Table IV. Values of $\Delta H°$, $\Delta F°$, and $\Delta S°$ for the Interaction of ATP with Various Metal Ions[a]

Metal ion	$\Delta H°$ (K_1), kcal/mole	$\Delta H°$ (K_2), kcal/mole	$\Delta F°$ (K_1),[b] kcal/mole	$\Delta F°$ (K_2),[b] kcal/mole	$\Delta S°$ (K_1),[b] cal/deg mole	$\Delta S°$ (K_2),[b] cal/deg mole
Ba(II)	−2.1 ± 0.2	−3.9 ± 0.2	−2.52 ± 0.02	−4.59 ± 0.02	+2 ± 1	+2 ± 1
Sr(II)	−1.6 ± 0.3	−3.0 ± 0.3	−2.80 ± 0.02	−4.83 ± 0.02	+4 ± 1	+6 ± 1
Ca(II)	−0.3 ± 0.2	−0.9 ± 0.3	−2.90 ± 0.02	−5.40 ± 0.02	+9 ± 1	+12 ± 1
Mg(II)	+3.4 ± 0.1	+2.6 ± 0.1	−3.10 ± 0.02	−5.80 ± 0.02	+21.5 ± 0.5	+27.5 ± 0.5
Co(II)	−2.1 ± 0.3	−2.2 ± 0.3	−3.20 ± 0.02	−6.40 ± 0.02	+4 ± 1	+14 ± 1
Mn(II)	−2.3 ± 0.3	−3.0 ± 0.3	−3.30 ± 0.02	−6.50 ± 0.02	+3 ± 1	+12 ± 1
Zn(II)	−2.4 ± 0.3	−2.7 ± 0.3	−3.60 ± 0.02	−6.60 ± 0.02	+4 ± 1	+13 ± 1
Ni(II)	−2.4 ± 0.2	−2.5 ± 0.2	−3.70 ± 0.02	−6.90 ± 0.02	+4 ± 1	+15 ± 1
Cu(II)	−3.0 ± 0.2	−4.3 ± 0.2	−4.30 ± 0.02	−8.40 ± 0.02	+4 ± 1	+14 ± 1

[a] $\mu = 0.1\ M(KNO_3)$. [b] At 25°.

Table V. Hydrolysis and Olation of Cu(II)–ATP[a]

Temp, °C	Log K_{ML}	p$K_{M(OH)L}$	$pK_{M(OH)_2L}$	p$K_{(M(OH)L)_2}$	Log K_d
0.4	6.42 ± 0.02	7.05 ± 0.04	14.37 ± 0.07	11.00 ± 0.05	3.10 ± 0.04
12	6.20 ± 0.02	6.74 ± 0.04	13.92 ± 0.07	10.58 ± 0.05	2.80 ± 0.04
25	6.13 ± 0.02	6.47 ± 0.04	13.50 ± 0.07	10.35 ± 0.05	2.59 ± 0.04
40	5.97 ± 0.02	6.19 ± 0.04	13.10 ± 0.07	10.00 ± 0.05	2.38 ± 0.04

[a] $\mu = 0.1\ M(KNO_3)$.

Table VI. Thermodynamic Constants for Hydrolysis and Olation Reactions of Cu(II)–ATP

	Reaction	$\Delta H°$, kcal/mole	$\Delta F°$ (25°), kcal/mole	$\Delta S°$ (25°), cal/deg mole
1	$CuL^{2-} \rightleftharpoons Cu(OH)L^{3-} + H^+$	+8.0 ± 0.3	+8.80 ± 0.04	−3 ± 1
2	$CuL^{2-} \rightleftharpoons Cu(OH)_2L^{4-} + 2H^+$	+12.0 ± 1.0	+18.4 ± 0.1	−22 ± 3
3	$2Cu^{2-}L \rightleftharpoons (Cu(OHL)_2^{6-} + 2H^+$	9.9 ± 0.3	+14.1 ± 0.1	−14 ± 1
4	$2Cu(OH)L^{3-} \rightleftharpoons (Cu(OH)L)_2^{6-}$	−6.4 ± 0.5	−3.50 ± 0.04	−10 ± 2

of Zn(II) on infrared absorptions of ATP, orthophosphate, and methyl diphosphate is greater than those of Cu(II), Ni(II), and Co(II). By the use of infrared phosphate absorptions to detect proton displacement by metal ions, Khalil and Brown[11] found the apparent interaction with Zn(II) to be greater than that of Ni(II). While this seems to be a reversal of the normal stability order, the normal order (*i.e.*, Ni(II) > Zn(II) has been previously reported for ATP complexes of the transition metals and Zn(II).[7]

In the calculated thermodynamic constants given in Table IV, the enthalpies of formation are very small in general and are exothermic, with the only exception for Mg(II), in which case $\Delta H°$ is positive (endothermic). For the transition metal ions and Mg(II), the enthalpy changes in the formation of protonated and normal chelate species are roughly the same. The remaining alkaline earths, Ba(II), Sr(II), and Ca(II), however, show a variation in $\Delta H°$ values for the protonated and normal chelate species. The enthalpy for the normal species $\Delta H°$ (K_2) is more negative than the value $\Delta H°$ (K_1) for protonated species. A positive enthalpy value for Mg(II) is due to a very high heat of hydration of Mg(II) as compared to the other alkaline earths. There is a regular change in the values of $\Delta H°$ for both the normal and protonated complexes in the series Ca(II), Sr(II), and Ba(II). The value of $\Delta H°$ becomes more negative the larger the metal ion. The trend is in accord with the heats of hydration of the alkaline earths which steadily decrease through Ca(II), Sr(II), and Ba(II). The enthalpy changes in the interaction of ATP with alkaline earths are not very much different from the corresponding enthalpy changes for the hydration of the gaseous ions Mg(II), Ca(II), Sr(II), and Ba(II). Thus

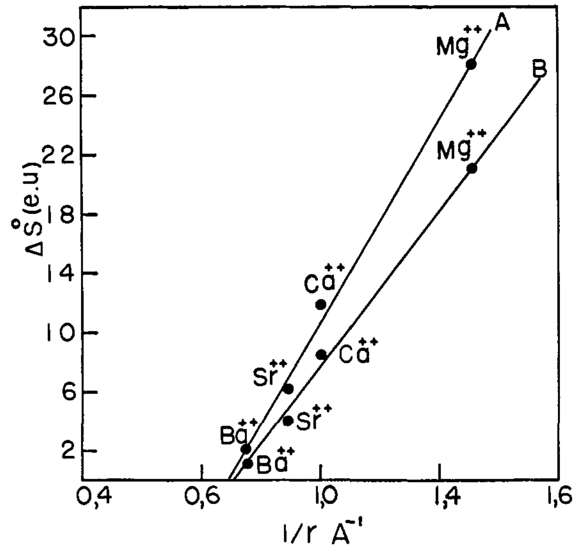

Figure 1. Plot of ΔS° against $1/\gamma$ for the alkaline earth metal ions where (A) ΔS° (K_2) = entropy change on the formation of the normal (1:1) ATP-metal chelate species in cal/deg mole; (B) ΔS° (K_1) = entropy change on the formation of the protonated (1:1) ATP-metal chelate species in cal/deg mole; and γ = ionic radius of the cation in Å.

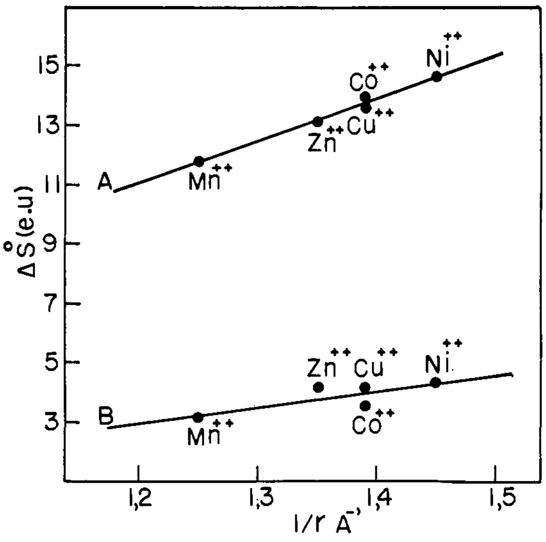

Figure 2. Plot of ΔS° against $1/r$ for the transition metal ions where (A) ΔS° (K_2) = entropy change on the formation of the normal (1:1) ATP-metal chelate species in cal/deg mole; (B) ΔS° (K_1) = entropy change on the formation of the protonated (1:1) ATP-metal chelate species in cal/deg mole; and γ = ionic radius of the cation in Å.

the coordinate bonds in both the normal and protonated species of the alkaline earth-ATP complexes seem to be as covalent as the metal oxygen bond in the hydrated ions.

The trend in the entropies of formation for the divalent metal chelates of ATP is much more regular than the enthalpies. The relatively small values of ΔH°, coupled with large positive values of ΔS°, offer qualitative evidence for the role of entropy as the primary factor favoring formation of these metal chelates in solution. The entropies of both the protonated and normal chelate species of the divalent metal ions studied are proportional to the reciprocal of their ionic radii. The alkaline earth and transition metal ions fall into two groups as shown in Figures 1 and 2. Martell[13] has suggested a linear dependence between ΔS° and $1/r$ for divalent metal ions. Similar dependence for ΔS° on the reciprocal of the ionic radii was observed by Staveley, *et al.*,[14] in their study of divalent and trivalent metal ion complexes of EDTA.

It may, however, be pointed out that there is a difference in the order of stabilities of divalent metal ions in EDTA and ATP complexes. In EDTA complexes, Irving–William order is strictly followed, whereas in ATP chelates the order is reversed with respect to Co(II) and Mn(II), Ca(II) and Mg(II). ATP in fact seems to be one of the few ligands in which the stability order in the alkaline earth series follows the reciprocal of their ionic radii.

In the thermodynamic constants for hydrolysis and olation of the Cu(II)–ATP system listed in Table VI, the constants corresponding to the dimerization reaction (4) merit consideration. The negative entropy for this reaction may be compared to ΔS°, for the dimerization of Fe(III)-EDTA and Fe(III)-CDTA reported by Gustafson and Martell.[15] The unfavorable entropies for dimerization in these completely chelated ligands may be due to the absence of water molecules in the coordination sphere of the complex. This effect may contribute in making the entropies of dimerization more negative. The driving force for dimerization in such systems seems to be a favorable enthalpy term which more than compensates the unfavorable entropy change.

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