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Thermodynamics of Mg and Ca Complexation with AMP, ADP, ATP

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Abstract

The thermodynamic parameters (ΔG, ΔH, ΔS) of complexation have been measured by potentiometric and calorimetric titration for formation of ML and MHL (M = Mg^{2+}, Ca^{2+}; L = AMP^{2-}, ADP^{3-}, ATP^{4-}). The parameters are interpreted to support a model of inner sphere complexation of the metal cations to the phosphate groups with no evidence of metal–ring interaction in the ML complexes. In the MHL complexes, the protonation (of a ring nitrogen) seemingly leads to 'backfolding' interaction between the metal and the ring system in addition to the interaction between the metal and the phosphate groups.

Introduction

The adenosine phosphates are present in all forms of life in 2–10 millimolar concentrations. The enzymatic hydrolysis reactions of adenosine-5'-triphosphate, ATP, require either Mg(II) or Ca(II) as cofactors. There is substantial evidence that the substrate is a metal–nucleotide complex and not the free nucleotide but the actual role of the metal ion and the structure of the complex are still not clear. The spectroscopic inertness of these two cations has hindered development of a satisfactory understanding of the complexes formed by these alkaline cations and the adenosine phosphates. We have studied the complexation of Mg(II) and Ca(II) with ATP as well as the analogous diphosphate, ADP, and the monophosphate, AMP, by potentiometric and calorimetric titration to obtain thermodynamic data which could aid in developing a satisfactory model of the interactions.

The structure of the ATP complexes with divalent cations has been interpreted as involving interaction with the phosphate groups in α,β,γ-tridentation [1, 2], β,γ-bidentation [3, 4], β-monodentation [5] and a mixture of α,β-, α,γ- and β,γ-bidentation [6] (Fig. 1). There is also controversy about whether the metal ions interact with the basic sites of the heterocyclic rings. It has been proposed from the UV spectral data [7] that the alkaline earth metal ions possibly interact with the purine and pyrimidine rings. By contrast, Britzinger [8] and Khalil and Brown [9] from IR studies concluded that Ca^{2+}, Mg^{2+} and the bivalent 3d transition metal ions bind exclusively to the phosphate chain. Weser *et al.* interpreted UV studies as showing that Co^{2+} and Ni^{2+} ions are preferentially coordinated to the phosphate chain [10]. However, a thorough NMR study by Swift *et al.* [11] led to the conclusion that Ni^{2+} is bound to the adenine ring in ATP, but Co^{2+} and Mn^{2+} show no evidence for such interaction [11]. Granot and Fiat [12] based on NMR shifts, proposed that divalent Mg, Ca, Ni, and Co bind ATP predominantly through the phosphate chain but in the protonated complexes some metal–ring interaction occurs.

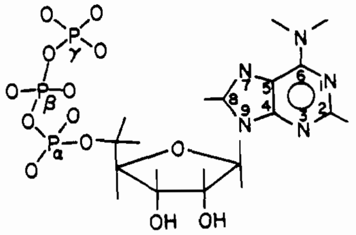

Fig. 1. The structure and nomenclature of adenosine 5'-triphosphate (ATP$_4^{4-}$) 6-aminopurine 9-ribose 5'-triphosphate.

This thermodynamic study of the binary (ML) and protonated (MHL) complexation provides additional data for clarifying the role of various donors in the adenosine phosphates.

Experimental

Solutions

All chemicals were reagent grade. H_2AMP, $NaH_2ADP \cdot 3H_2O$ and $Na_2H_2ATP \cdot H_2O$ were obtained from Aldrich Chemical Co. in 99% purity and were stored cold until used. Solutions of about 0.02 M were prepared from $Mg(ClO_4)_2$ and $Ca(ClO_4)_2$ and standardized by titration with 0.02 M EDTA solution. The ionic strength was adjusted to 0.10 M with $NaClO_4$. The NaOH stock solution was prepared from solid NaOH to be 0.10 M and analyzed by

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titration with potassium acid phthalate. Stock $HClO_4$ solution of 0.1 M was prepared by dilution of concentrated perchloric acid and titrated with the standardized NaOH solution. These stock solutions were diluted as required in the experiments with adjustment to an ionic strength of 0.10 M with $NaClO_4$. Tetraethylammonium perchlorate, TEAP, from Eastman Kodak Co. was used to adjust the ionic strength to 0.10 M in the NaATP complexation measurements. Approximately 5 mM solutions of AMP, ADP and ATP were prepared by dissolving the required amount of the solid in deionized water. The ionic strength of the solutions was adjusted to 0.1 M using $NaClO_4$. The AMP solutions showed no change in titration behavior but after about four days the ADP and ATP solutions had slight changes, probably due to hydrolysis. Therefore, fresh solutions were prepared for each set of experiments. These solutions were kept in the dark by wrapping the container with aluminum foil and storing in a dark cabinet.

Procedures

For the potentiometric titrations, the pH readings were obtained with a Radiometer research pH meter model PHM 84. An Ag–AgCl reference electrode and a glass electrode (Corning pH combination electrode model H4330-5 with saturated KCl filling solution) was used. The titrations were conducted under an atmosphere of N_2 in a jacketed titration vessel in which the temperature was maintained by circulating constant temperature (±0.1 °C) water in the jacket. Magnetic stirring was used.

Two NBS standard solutions, 0.05 M potassium hydrogen phthalate (pH 4.008) and 0.025 M disodium hydrogen phosphate +0.025 M sodium dihydrogen phosphate (pH 6.865) were used to calibrate and standardize the pH meter at different temperatures. The temperature dependence of the pH of these two solutions is known from 0 to 80 °C.

The adenosine phosphates were obtained in the dibasic acid forms. Approximately 5 mM solutions of these ligands were titrated with 0.01 M NaOH. The sodium–ATP complexation study was done at 25 °C in TEAP solution. Three different solutions at 0.02, 0.04 and 0.06 M $NaClO_4$ concentrations with 5 mM concentrations of ATP were titrated with 0.01 M NaOH solution. Correction was made for the addition of Na^+ ions by the titrant. The acid stability constants of ATP and AMP were also determined in the TEAP medium by titration of approximately 5 mM solutions of the acid ligands with 0.01 M NaOH solution.

The Mg and Ca complexation constants with ATP, ADP and AMP were determined at 25 °C. Twenty milliliters of solution containing approximately 3 mM concentration of the metal ion along with approximately 5 mM concentration of the adenosine phosphate were titrated with 0.02 M solution of NaOH.

The calorimeter system has been described elsewhere [13, 14]. The solution calorimeter was operated in an adiabatic mode at an initial water bath temperature of 25.000 ± 0.001 °C. Typically, 50 ml of a solution containing approximately 3 mM concentration of the metal ion (Ca^{2+} or Mg^{2+}) and approximately 5 mM concentration of the ligand (ATP, ADP or AMP, partially neutralized) was titrated with a 0.02 M solution of $HClO_4$ The heats of protonation of the ligands were determined by a similar titration of a partially neutralized ligand solution in the absence of metal ions. The heat of dilution of the titrant was determined by titrating a 50 ml solution of 0.1 M $NaClO_4$ (pH = 4.5) with the $HClO_4$ solution. Since the observed heats were 0.005 J per addition (0.5 ml), no correction was applied to the observed heat changes in the case of complexation titrations.

Data Analysis

In the case of the potentiometric titrations, the total hydrogen concentration was varied over a wide range. The total hydrogen concentration, C_H, was calculated by:

$$C_H = [H] + \beta_{011}[H][L] + 2\beta_{021}[H]^2[L] + \beta_{111}[M][H][L] \tag{1}$$

where β_{011} and β_{021} are the overall protonation constants for formation of HL and H_2L, [H] is the measured 'free' hydrogen ion concentration, [L] is the free, non-protonated ligand concentration, [M] is the free metal ion concentration and β_{111} is defined by:

$$\beta_{111} = \frac{[MHL]}{[M][H][L]} \tag{2}$$

Similar mass balance equations for total ligand concentration, C_L, and total metal concentration, C_M were:

$$C_L = [L] + \beta_{101}[M][L] + \beta_{111}[M][H][L] + \beta_{011}[H][L] + \beta_{021}[H]^2[L] \tag{3}$$

and

$$C_M = [M] + \beta_{101}[M][L] + \beta_{111}[M][H][L] \tag{4}$$

where β_{101} is defined by:

$$\beta_{101} = \frac{[ML]}{[M][L]} \tag{5}$$

These equations involve four unknowns [ML], [L], β_{101} and β_{111}. To solve for β_{101} and β_{111}, initial guesses for β_{101} and β_{111} were introduced into these equations and the values were systematically varied until the best agreement with the experimental data was obtained. The agreement between the experimental and calculated values of C_H was taken as a measure of the fit of the values for β_{101} and β_{111}. The actual fitting of the data was done by computer using the non-linear least-squares program PSCENT [15]. This program chooses the best set of constants β_{101} and β_{111} that minimizes the error square sum S given by:

$$S = \Sigma_i [C_{Hi}(\text{calc.}) - C_{Hi}(\text{exp.})]^2$$

where the summation is taken over all the experimental points.

Since it is possible that the higher complexes, ML_2, $M(HL)_2$ or $M(L)(HL)$ may also be formed along with the ML and MHL complexes, calculations were performed assuming the presence of one or more of these species. These calculations however, either gave unreasonable values for the computed constants or the standard errors were very high. Hence, it was assumed that only ML and MHL complexes were formed in the solution. Accordingly, calculations were made to determine β_{101} and β_{111} from the experimental data.

The enthalpy data were analyzed by following the general procedure described in refs. 14 and 16. The computer calculations were modified to consider the formation of protonated complex species.

Results

The protonation constants, log β_{011} and log β_{021} for formation of the species HL and H_2L as well as the associated enthalpy and entropy values are given in Table I. The data are the average of triplicate potentiometric and calorimetric titrations for each ligand with the errors based on the differences.

TABLE I. Protonation Thermodynamics for AMP, ADP and ATP $I = 0.10$ M ($NaClO_4$); $T = 298$ K

	AMP	ADP	ATP
log β_{011}	6.29 ± 0.02	6.41 ± 0.02	6.51 ± 0.02
ΔH_{011}[a]	3.34 ± 0.10	2.35 ± 0.10	5.08 ± 0.01
ΔS_{011}[b]	132 ± 1	131 ± 1	142 ± 1
log β_{021}	10.22 ± 0.04	10.36 ± 0.06	10.54 ± 0.04
ΔH_{021}[a]	−15.20 ± 0.40	−16.40 ± 0.40	−13.34 ± 0.40
ΔS_{021}[b]	144 ± 2	143 ± 2	157 ± 2

[a] kJ mol^{-1}. [b] J mol^{-1} K^{-1}.

The values reported in the literature vary over a wide range depending upon the experimental conditions like temperature, ionic strength, supporting electrolyte, etc. Our values for ATP and ADP are in good agreement with those of Kahn and Martell [17] who used similar conditions. For AMP, our values are in good agreement with those of Frey *et al.* [18].

The complexation studies with Mg^{2+} and Ca^{2+} were performed in solutions of 0.1 M ionic strength adjusted using $NaClO_4$. However, Na^+ ion is known to interact with ATP which would decrease the free ATP concentration. To evaluate this effect, the Na + ATP complexation constant was determined using inert tetraethylammonium perchlorate (TEAP) to maintain the ionic strength at 0.10 M in titrations at 0.02, 0.04 and 0.06 M concentrations of $NaClO_4$. The stability constant, β_{NaATP}, for the $NaATP^{3-}$ complex was determined to be 19.5 ± 0.6. This value is slightly higher than 14.0 reported by Smith and Alberty [19]. The $KATP^{3-}$ complex stability constant has been reported [17] to be 14.3 which would suggest a value larger than 14 for the sodium complexation.

The acid stability constants for ATP and AMP were also determined in TEAP medium. The constants β_{011} and β_{021} obtained under similar conditions in the $NaClO_4$ medium and in the TEAP medium are compared in Table II.

TABLE II. Comparison of Protonation Constants in $NaClO_4$ and TEAP

Medium	AMP		ATP	
	log β_{011}	log β_{021}	log β_{011}	log β_{021}
$NaClO_4$	6.29	10.22	6.51	10.54
TEAP	6.46	10.32	6.93	10.93

These values in Table II are consistent with the fact that Δ (where $\Delta = \log \beta(\text{TEAP}) - \log \beta(NaClO_4)$) is expected to be $\log(1 + \beta_{NaL}(Na))$ which is 0.47 for ATP (based on $\beta_{NaL} = 19.5$). The constants for AMP are similar, suggesting a smaller stability constant (perhaps about 3 or 4) for the $NaAMP^-$ complex.

Typical sets of potentiometric (for Ca + ADP) and calorimetric (for Mg + ATP) titration data are presented in Tables III and IV, respectively. Table V lists the results of the calculations of the thermodynamic parameters for formation of MgL and CaL (eqn. (2)) and for MgHL and CaHL (eqn. (3)). The errors in the β_{101} and β_{111} values are estimated to be 1–2% (1σ) and are reported with the ΔG values. The estimated errors for ΔS_{101} and ΔS_{111} are $\geqslant 1$ J mol^{-1} K^{-1}.

Discussion

The protonation data in Table I agree fairly well with the corresponding values in ref. 20b for log β_{011},

TABLE III. Potentiometric Titration Data for Calcium–ADP System[a]

Titer volume (ml)	pH	[H] (× 10^5 M)	[L] (× 10^5 M)	[M] (× 10^3 M)
0.70	4.177	8.015	1.360	2.873
0.80	4.213	7.378	1.511	2.850
1.00	4.292	6.151	1.894	2.802
1.20	4.376	5.069	2.390	2.754
1.40	4.464	4.139	3.024	2.707
1.60	4.559	3.326	3.878	2.657
1.70	4.659	2.642	4.988	2.606
1.80	4.765	2.070	6.462	2.552
1.90	4.823	1.811	7.420	2.522
2.00	4.881	1.585	8.499	2.492
2.10	4.940	1.383	9.734	2.461
2.20	4.999	1.208	11.12	2.428
2.30	5.059	1.052	12.71	2.393

[a] Initial conditions. Titrand solution: volume = 20.00 ml; total [H] = 7.384 × 10^{-3} M; total [L] = 4.986 × 10^{-3} M; total [M] = 3.330 × 10^{-3} M; temperature = 25 °C. Titrant: NaOH; total [OH] = 2.020 × 10^{-2} M. Ionic strength = 0.1 M ($NaClO_4$). Computed constants: β_{101} = 8.14 (0.09) × 10^2; β_{111} = 9.98 (0.15) × 10^7.

log β_{021}, and ΔS_{011}, but less satisfactorily for ΔH_{011}, ΔH_{021}, and ΔS_{021}. Our values are consistent with the accepted model [21] in which the first proton associates with a phosphate group and the second with a ring nitrogen (N-1). The values of log β_{011}, ΔH_{011}, and ΔS_{011} are consistent with their analogs for monoprotonation of polyphosphates such as $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$ [20a]. Similarly, the 021 values are consistent with ring nitrogen protonation [20b].

TABLE IV. Calorimetric Titration Data for Magnesium–ATP System[a]

Titer volume (ml)	Q (mJ)	[H] (× 10^5 M)	[L] (× 10^5 M)	[M] (× 10^3 M)
0.50	−72.5	0.246	28.70	0.604
1.00	−74.2	0.314	23.44	0.684
1.50	−80.9	0.396	19.23	0.766
2.00	−85.4	0.494	15.84	0.847
2.50	−85.2	0.610	13.10	0.926
3.00	−105.6	0.747	10.86	1.002
3.50	−102.3	0.908	9.031	1.076
4.00	−109.9	1.096	7.518	1.145
4.50	−126.5	1.314	6.266	1.210
5.00	−133.2	1.568	5.224	1.270
5.50	−130.2	1.859	4.357	1.325
6.00	−148.8	2.195	3.635	1.376
6.50	−149.6	2.578	3.032	1.423
7.00	−158.3	3.013	2.529	1.466
7.50	−159.2	3.506	2.109	1.504
8.00	−166.0	4.063	1.758	1.540
8.50	−171.3	4.691	1.464	1.573

[a] Initial conditions. Solutions in the cup: volume = 53.00 ml; total [H] = 2.430 × 10^{-3} M; total [L] = 4.700 × 10^{-3} M; total [M] = 2.641 × 10^{-3} M; temperature = 25 °C. Titrant: $HClO_4$; total [H] = 2.060 × 10^{-2} M. Ionic strength = 0.1 M ($NaClO_4$). Computed constants: ΔH_{101} = 18.08 (±0.10) kJ mol^{-1}; ΔH_{111} = 14.65 (±0.20) kJ mol^{-1}).

The stability constants, β_{101}, for the binary complexes of Mg^{2+} and Ca^{2+} with AMP, ADP and ATP have been reported in a number of publications. The values in Table V differ somewhat from those in ref. 17 (the 'selected' values of ref. 20b), but the differences are consistent with that expected from the use of KNO_3 in ref. 17 and $NaClO_4$ in this

TABLE V. Thermodynamic Parameters for Formation of Mg^{2+} and Ca^{2+} Complexes I = 0.10 M ($NaClO_4$); T = 298 K

Complex	log β_{101}	ΔG_{101}[a]	ΔH_{101}[a]	ΔS_{101}[b]
A. M + L = ML				
MgAMP	2.10	11.99 ± 0.03	5.71 ± 0.10	59
MgADP	3.28	18.72 ± 0.03	17.53 ± 0.10	121
MgATP	4.03	23.01 ± 0.04	18.08 ± 0.10	138
CaAMP	2.03	11.61 ± 0.03	4.23 ± 0.10	53
CaADP	2.90	16.58 ± 0.02	13.80 ± 0.10	101
CaATP	3.70	21.13 ± 0.04	13.45 ± 0.10	116
Complex	log β_{111}	ΔG_{111}[a]	ΔH_{111}[a]	ΔS_{111}[b]
B. M + H + L = MHL				
MgHADP	8.31	47.45 ± 0.06	10.01 ± 0.20	126
MgHATP	8.63	49.28 ± 0.06	14.65 ± 0.20	116
CaHADP	7.00	39.97 ± 0.06	8.62 ± 0.20	105
CaHATP	8.55	48.82 ± 0.06	13.04 ± 0.20	120

[a] kJ mol^{-1}. [b] J mol^{-1} K^{-1}.

work for the ionic strength adjustment. However, there is rather substantial disagreement with the ΔH_{101} and ΔS_{101} values of Table V and those in the literature [20b, 22]. The literature data generally are based on measurements of β_{101} at various temperatures, often over rather limited ranges. The values from this work are more consistent and, since they were obtained directly by calorimetry, are more likely to be reliable.

These thermodynamic parameters can be analyzed for information on the mode of bonding in the binary complexes. For example, the log β_{101} values can be compared with those from polyphosphate bonding where the number of phosphonyl groups and the anion charge are the same. Since the sum of the acid constants is higher for the phosphate ligand relative to that of the adenosine phosphate analog, if only M–O–P bonding is involved in the complexation, the log β_{101} for the phosphate analog should be somewhat larger. Table VI shows this is the case except for the AMP complexation. This could mean that there is some interaction of the metal and ring donor atom in the AMP complexes or that the HPO_4^{2-} data are not reliable.

TABLE VI. Comparison of log β_{101} for Complexation of Mg^{2+} and Ca^{2+} with AMP, ADP and ATP and with Polyphosphates

Ligand	log β_{101} (Mg)	log β_{101} (Ca)	Reference
HPO_4^{2-}	1.7	1.5	20a
AMP^{2-}	2.1	2.0	p.w.[a]
$HP_2O_7^{3-}$	3.9	3.4	23
ADP^{3-}	3.3	2.9	p.w.
$HP_3O_{10}^{4-}$	4.2	4.1	23
ATP^{4-}	4.0	3.7	p.w.

[a] p.w., present work.

The ΔS_{101} data can also be considered for insight into the metal–ligand bonding. ΔS_{101} for MADP is roughly twice that for MAMP for both Mg^{2+} and Ca^{2+}. This is strong support for a single M–OP bond in MAMP and two such bonds in MADP. The ΔS_{101} for MATP is slightly larger than for MADP, indicating that a third M–O–P bond may be formed but the additional bond is weaker than the other two (the ΔS_{101} is positive because the dehydration associated with complexation is the dominant entropy factor). The variation in ΔS_{101} for the metal–adenosine phosphate complexation parallels that of the phosphate complexation for the same potential number of P–O groups (*i.e.*, MPO_4^- *versus* MAMP, MP_2O_7 *versus* MADP and MP_3O_{10} *versus* MATP). This supports a model of only M–O–P bonding in the 1:1 complexes. Tran-Dinh *et al.* have interpreted ^{31}P NMR shift studies of nucleotides as support for binding of Mg^{2+} solely through the phosphate in the triphosphates and solely through the phosphate of the diphosphates [24]. However, Martin and Marian [25] reviewed the available data and concluded that the alkaline earth cations bind in non-protonated complexes to both phoshates of ADP and at least to the β and γ phosphates of ATP with some mixture of inner and outer sphere interaction with the α phosphates. Our data are more consistent with inner sphere complexation to all three phosphates in ATP with one bond, probably that with the α phosphate, being longer and, hence, weaker. The ΔH_{101} values in Table V add further support to metal phosphate binding with no evidence of metal-ring interaction in any of the binary complexes as the latter interaction should be primarily exoergic.

The formation of MHL complexes is evaluated more clearly by considering the protonation process:

$$ML + H = MHL \qquad (6)$$

$$K_{11} = [MHL]/[ML][H] \qquad (7)$$

Log K_{11} is obtained by subtracting log β_{101} from log β_{111}; the ΔH_{11} and ΔS_{11} values are obtained similarly. Table VIIA lists these values for eqn. (6). Presumably, this protonation is associated with addition to a ring nitrogen and can be compared to addition of the second proton to the free ligand (Table VIIB) which, as noted earlier, binds to a ring nitrogen. The agreement of log K_{11} and pK_2 support the model of ring protonation in MHL. However, the fact that ΔS_{11} is less positive than ΔS_{021} while ΔH_{11} is more exothermic than ΔH_{021} indicates additional interaction beyond simple protonation. NMR shifts of the protonated complexes indicate that protonation of the ring at N(1) leads to 'back-folding' due to the attraction between the positively charged

TABLE VII. Thermodynamic Parameters for Protonation $I = 0.10$ M ($NaClO_4$); $T = 298$ K

Complex	log K_{11}	$-\Delta G_{11}$[a]	ΔH_{11}[a]	ΔS_{11}[b]
A. ML + H = MHL				
MgHADP	5.03	28.73	−7.52	5
MgHATP	4.60	26.27	−3.43	−22
CaHADP	4.10	23.39	−5.18	4
CaHATP	4.85	27.69	−0.41	4
Complex	pK_2	$-\Delta G_{021}$[a]	ΔH_{021}[a]	ΔS_{021}[b]
B. HL + H = H_2L				
H_2ADP	3.95	22.55	−18.75	12
H_2ATP	4.03	23.01	−18.42	15

[a] kJ mol^{-1}. [b] J mol^{-1} K^{-1}.

ring and the residual negative charge of the phosphate chain. This back-folding allows interaction between the metal ion and ring donors – probably the N(7) site. This model is consistent with the differences in the values in Table VII, and explains the somewhat enhanced basicity as reflected in the $\log K_{11}$ *versus* pK_2 values.

Conclusion

The thermodynamic parameters of complexation are consistent with the models developed for Mg^{2+} and Ca^{2+} interaction with AMP, ADP and ATP from NMR, UV, IR, etc. data. In the binary complexes, the metal is bound to the phosphate chain. Protonation results in folding of the ring system over the phosphate chain and allows metal–ring interaction. An unexplained aspect of this study is the failure to observe formation of MHAMP complexes.

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