Metal Ion Catalysed Adenosine-5'-triphosphate Hydrolysis: Part II—Zn(II) & Mn(II) Catalysed Hydrolysis

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The kinetics of the Zn(II) and Mn(II) catalysed hydrolysis of adenosine-5'-triphosphate in aqueous solutions have been studied at 46°, 56° and 64°, over a broad pH range and $\mu = 0.1$ (KNO$_3$). pH-rate profiles have been analysed and the overall rate constants have been resolved into specific rate constants relating to the various metal-ATP chelate species in solution. Activation parameters, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta F^{\ddagger}$ for the specific rate constants of the various chelate species are reported. Possible mechanism for the hydrolysis of adenosine-5'-triphosphate in the presence of Zn(II) and Mn(II) ions are discussed.

IN previous papers[1,2] the kinetics of uncatalysed and Cu(II)-catalysed hydrolyses of adenosine-5'-triphosphate (ATP) were reported. In the present paper a detailed physicochemical study of the effect of Zn(II) and Mn(II) ions on the rate of ATP hydrolysis is reported. Previous investigations by Lowenstein[3] were only semi-quantitative in nature and various buffer known to complex with metal ions[4] were used to maintain a constant pH. In this study specific rate constants of the protonated and normal chelate species of ATP with Zn(II) and Mn(II) ions were evaluated over a broad pH range at 46°, 56° and 64°, and the corresponding activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta F^{\ddagger}$ determined.

Materials and Methods

A chromatographically pure crystalline sample of adenosine-5'-triphosphate dipotassium salt (Mann Research Laboratories, USA) was used. Zn(II) and Mn(II) solutions were prepared from analytical grade metal salts and were standardized with ethylenediamine tetraacetic acid by the procedure outlined by Schwarzenbach[5].

The experimental set-up and procedure used for studying the catalytic effect of Zn(II) and Mn(II) ions on the rate of ATP hydrolysis are essentially the same as those described for the uncatalysed and Cu(II) catalysed ATP hydrolysis[1,2]. Kinetic runs were carried out with equimolar amounts of metal ion and ATP ($\sim 2.0 \times 10^{-3}M$). First order rate constants were evaluated in the initial stages of the hydrolysis reactions, from plots of log $a/(a-x)$ against t (where a = initial concentration of ATP and x = amount reacted in time t). Acid or alkali were used to maintain a constant pH throughout the kinetic runs. Buffers were not used, since the basic component of most buffers are known to complex with metal ions[4]. All calculations were carried out with an IBM 370/155 computer.

Results

First order rate constants evaluated from kinetic runs with a 1:1 mole ratio of metal-ATP, at 46°, 56° and 64°, over a broad pH range are listed in Tables 1 and 2. These constants have been corrected for the spontaneous hydrolysis of the uncomplexed ATP at a particular pH. The data in Table 1 show

TABLE 1 — EXPERIMENTAL AND CALCULATED RATE CONSTANTS (min^{-1} × 10^4) FOR THE Zn(II) CATALYSED ATP HYDROLYSIS

[$\mu = 0.1$ (KNO$_3$)]

pH	46°		56°		64°	
	Expl	Calc.	Expl	Calc.	Expl	Calc.
3·5	0·65	0·66	1·72	1·82	3·84	3·98
4·0	1·15	1·12	2·87	3·00	6·13	6·26
4·5	1·65	1·61	4·36	4·23	9·27	8·90
5·0	2·06	2·11	5·64	5·53	12·00	11·66
5·5	2·61	2·55	6·90	6·73	14·94	14·23
6·0	2·31	2·86	6·58	7·56	14·20	16·07
6·5	2·05	3·03	5·00	8·05	10·50	17·11
7·0	1·70	3·11	4·07	8·26	9·57	17·59
7·5	1·25	3·13	3·76	8·34	7·40	17·75

TABLE 2 — EXPERIMENTAL AND CALCULATED RATE CONSTANTS (min^{-1} × 10^4) FOR THE Mn(II) CATALYSED ATP HYDROLYSIS

[$\mu = 0.1$ (KNO$_3$)]

pH	46°		56°		64°	
	Expl	Calc.	Expl	Calc.	Expl	Calc.
3·5	0·14	0·15	0·93	0·94	1·90	2·02
4·0	0·59	0·61	1·56	1·58	3·11	3·29
4·5	0·88	0·84	2·18	2·14	4·57	4·41
5·0	1·08	1·04	2·70	2·65	5·59	5·48
5·5	1·26	1·21	3·19	3·12	6·58	6·50

that in the presence of Zn(II) ions, the rate of ATP hydrolysis increases with pH until a maximum value is reached at about pH 5·75, and then decreases with an increase in pH. Precipitation was found to occur above pH 8. In the presence of Mn(II) ions, the rate of ATP hydrolysis also increases with increasing pH (Table 2). However, precipitation in this case takes place at pH 6. The concentrations of the metal-ATP chelate species were calculated with the help of the thermodynamic data of Khan and Martell[6]. From the concentrations of the metal-ATP species, the corresponding mole fractions were calculated. The values obtained at 56° are given in Table 3. The overall experimentally evaluated rate constants for the pH region up to 5·5 have been resolved (by solution of simultaneous equations) into the specific rate constants relating to the catalytically active protonated and normal complexes of ATP with Zn(II) and Mn(II). These values along with the specific rate constants of the diprotonated, monoprotonated and fully dissociated forms of ATP are listed in Table 4. Correlation between the measured rate constants and the calculated rate constants (obtained from the specific rate constants of protonated and normal metal-ATP complex species listed in Table 4) is good as shown in Tables 1, and 2 and Figs. 1 and 2. The activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta F^{\ddagger}$ for the hydrolysis of the metal-ATP species have been evaluated and are listed in Table 4.

Discussion

Zn(II) and Mn(II) interact moderately with ATP^{4-} to form two types of metal complex species, namely a protonated complex and a normal complex. The mole fractions of these metal species in solution, as a function of pH at 56°, are given in Table 3.
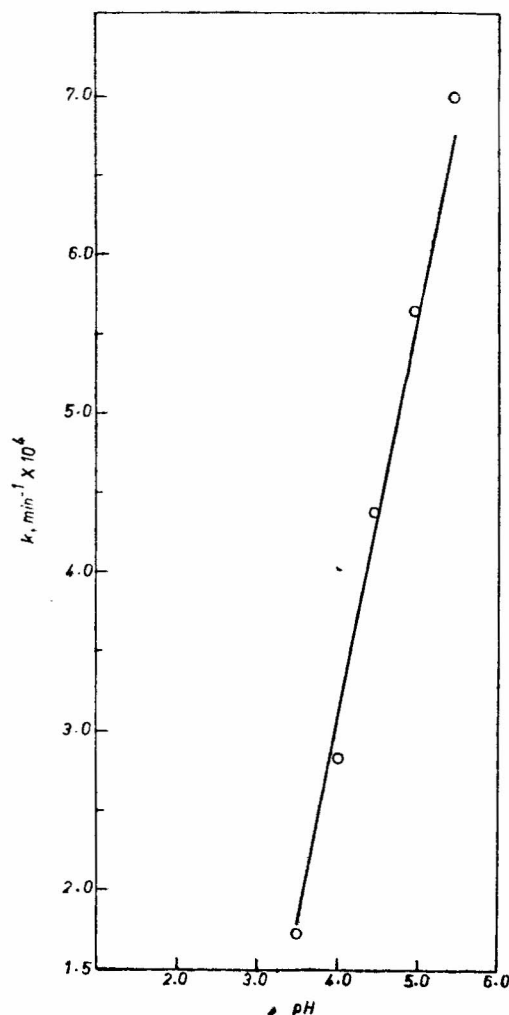

Fig. 1 — pH-rate profile for the Zn(II) catalysed ATP hydrolysis [t = 56°, μ = 0·1 (KNO₃). Solid line — calculated rates, ○ — measured rates. Similar pH-rate profiles were obtained at 46° and 64°]

TABLE 3 — MOLE FRACTIONS OF THE Zn(II)-ATP AND Mn(II)-ATP CHELATE SPECIES IN SOLUTION AT 56°

[μ = 0·1 (MNO₃)]

pH	Zn(II)-ATPH⁻	Zn(II)-ATP²⁻	Mn(II)-ATPH⁻	Mn(II)-ATP²⁻
3·0	0·078	0·004	0·045	0·003
3·5	0·152	0·022	0·093	0·020
4·0	0·202	0·092	0·131	0·091
4·5	0·176	0·254	0·115	0·253
5·0	0·104	0·476	0·068	0·470
5·5	0·046	0·671	0·030	0·659
6·0	0·017	0·794	0·011	0·781
6·5	0·006	0·859	0·004	0·847
7·0	0·002	0·886	0·007	0·876
7·5		0·897		0·886
8·0		0·900		0·890

TABLE 4 — SPECIFIC RATE CONSTANTS (min⁻¹ × 10⁴) AND ACTIVATION PARAMETERS* FOR THE UNCATALYSED AND METAL CATALYSED ATP HYDROLYSIS

[μ = 0·1 (KNO₃)]

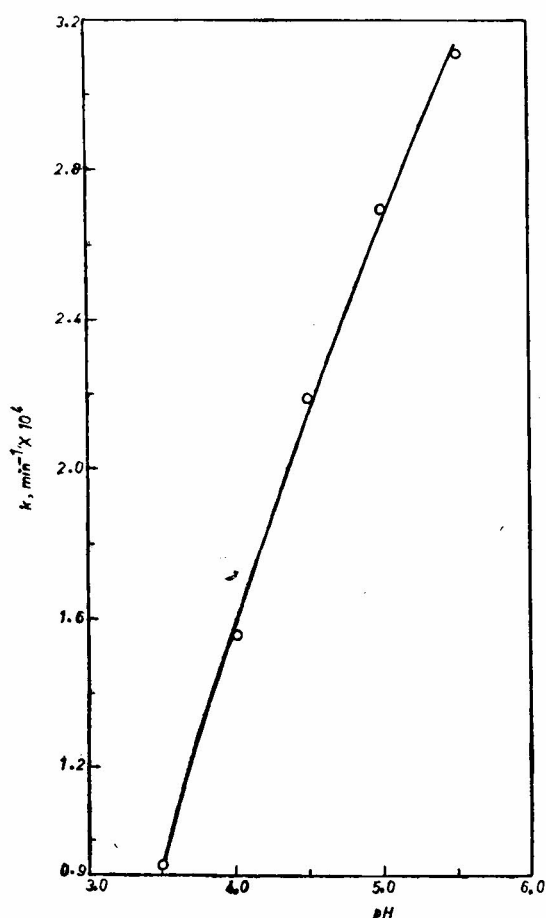| ATP species	46°	56°	64°	78°	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)	$\Delta F^{\ddagger}$ (kcal/mole)
H₂ATP²⁻		1·87±0·10	5·75±0·24	37·6±0·15	31·2	10·9	27·6
HATP³⁻		1·63±0·06	4·50±0·15	23·7±0·14	27·8	0·3	27·7
ATP⁴⁻		0·36±0·23	0·76±0·34	2·63±0·25	20·9	−24·0	28·7
Zn(II)-ATPH⁻	3·89±0·07	10·6±0·06	23·0±0·08		21·1	−16·4	26·5
Zn(II)-ATP²⁻	3·47±0·09	9·30±0·09	19·9±0·10		21·0	−16·9	26·6
Mn(II)-ATPH⁻	3·45±0·10	9·10±0·09	19·3±0·13		20·5	−18·5	26·6
Mn(II)-ATP²⁻	1·64±0·08	4·32±0·11	9·15±0·06		20·4	−20·3	27·1

*Reference temperature = 56·0°.

Fig. 2 — pH-rate profile for the Mn(II) catalysed ATP hydrolysis [t = 56°, μ = 0·1 (KNO$_3$). Solid line—calculated rates, O—measured rates. Similar pH-rate profiles were obtained at 46° and 64°]

The rate law for the hydrolysis of ATP in the presence of Zn(II) or Mn(II) ions is given by

$$\frac{-dL}{dt} = k_{obs.}[T_L] \qquad \qquad ...(1)$$

where

$k_{obs.} = k_1 M_1 + k_2 M_2$

$T_L = [\text{Me-ATPH}^-] + [\text{Me-ATP}^2]$

$\text{Me} = \text{Zn(II) or Mn(II)} \qquad \qquad ...(2)$

k_1 and k_2 are the specific rate constants and M_1 and M_2 are the corresponding mole fractions of the protonated and normal Me-ATP species, respectively. A comparison of the specific rate constants of Zn-ATPH$^-$ and Zn-ATP^{2-} species with those of the uncomplexed ATPH^{3-} and ATP^{4-} species (Table 4) shows that Zn(II) ions increase the rate of hydrolysis by a factor of 6·5 and 26·0 respectively at 56°. The corresponding increase of the rates of protonated and normal ATP complexes in the presence of Mn(II) ions, as compared to the uncomplexed ATPH$^-$ and ATP^{4-} species, is by a factor of 5·6 and 12·0 at 56° respectively. Zn(II) ions are thus more effective than the Mn(II) ions in catalysing the rate of ATP hydrolysis.

The magnitude of the entropies of activation, $\Delta S^{\ddagger}$ (Table 4) for the protonated and normal ATP

complexes with Zn(II) and Mn(II), suggests a bimolecular S_N2 mechanism[7]. The catalytic effect of Zn(II) and Mn(II) ions can be attributed to two factors, namely (i) the screening of the negative charge and (ii) the polarization of the P-O bonds. On complexation with the ATP molecule, the bivalent metal ion reduces the negative charge by two units. Due to this screening of the negative charge on the phosphorous oxygen atoms, the approach of a nucleophile to the site of attack is facilitated. Further, the complexation of the metal ion with the oxygen atoms of the phosphate chain will result in the withdrawal of electrons from the oxygen atoms to the sites of metal binding. This leads to polarization of P-O bond, the phosphorous atom becoming much more positive. Nucleophilic attack on the polarized phosphorous atom will thus be greatly facilitated. Cu(II) is found to be a more effective catalyst[2], as compared to the Zn(II) or Mn(II). This greater effectiveness of the Cu(II) ions can be traced to the higher stability of the Cu-ATP complexes as compared with the corresponding complexes of Zn(II) or Mn(II)[6]. Stronger interaction between the metal ion and the ATP molecule leads to a greater degree of polarization of the P-O bond leading to higher rate of hydrolysis. The data in Table 4 show that the monoprotonated ATP complexes with Zn(II) and Mn(II) are slighly more reactive than the corresponding normal complex species. This is expected on the basis of the lower negative charge on the monoprotonated species, and also the polarization of the phosphorous atom undergoing attack by both the metal ion and the proton on the γ-phosphorous atom. On the basis of the similarity in the stability constants of the Zn(II) and Mn(II) complexes with ATP[6], it is to be expected that these metal ions would be equally effective as catalysts in the hydrolysis of ATP. The reason for the lower activity of Mn(II) ions as compared to the Zn(II) ions is due to the difference in the metal-ATP structures. NMR studies[8,9] show that Zn(II) ions are coordinated with the β- and γ-phosphate oxygens of the ATP molecule, while Mn(II) ions are found to interact with all the three phosphate oxygens, namely α, β and γ. Since Zn(II) ions are bound only to the two terminal phosphate oxygens, its polarizing effect would be mainly concentrated on the β- and γ-phosphorous atoms. In the case of Mn(II), however, binding to all the three phosphate oxygens, would lead to polarization of all the three P-O bonds, resulting in an overall lowering of polarization of each individual phosphorous atom. In other words, the γ-phosphorous atom may be more positive in the Zn-ATP complexes than in the Mn-ATP complexes, leading to higher rate of hydrolysis in the former case than in the latter. The S_N2 hydrolysis of ATP in the presence of Zn(II) and Mn(II) ions, involving the nucleophilic attack by a polar water molecule, may be diagrammatically represented as in Figs. 3 and 4 respectively.

The data in Table 3 shows that the Zn-ATP chelate species increase in concentration from 67% at pH 5·50 to 90% at pH 8. The rate of ATP hydrolysis will also be expected to increase with pH in accordance with the relationship (2). However,
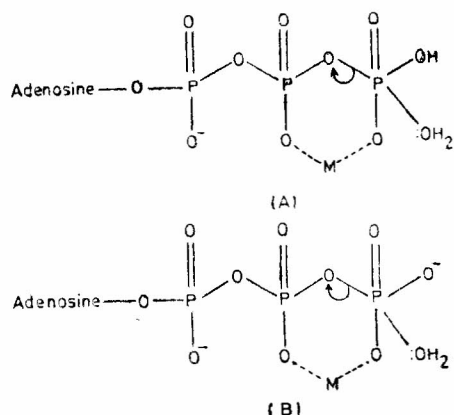
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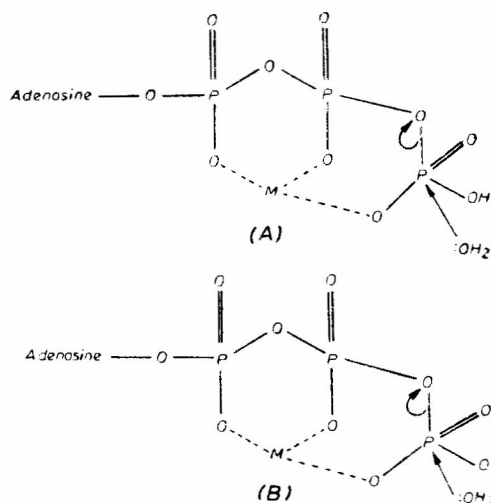Fig. 3 — Mechanism for the Zn(II) catalysed ATP hydrolysis [A — protonated complex; and B — normal complex]

Fig. 4 — Mechanism for the Mn(II) catalysed ATP hydrolysis [A — protonated complex; and B — normal complex]

above pH 5·75, the rate of ATP hydrolysis in the presence of Zn(II) ions is found to decrease with increasing pH (Table 1). This behaviour is similar to that obtained in the presence of Cu(II) ions[2]. In the Cu-ATP system, it has been shown by Khan and Martell[6] that hydroxo species are involved. It is therefore possible that in the Zn-ATP systems also, hydroxo species may be formed above pH 5·75. No such evidence was obtained by Khan and Martell[6] at 40°. It is, however, possible that at higher temperatures used in the present work, hydroxo species may be formed. It is interesting to note in this connection that formation of hydroxo species has been reported in the Zn-ATP chelate systems[10].

Acknowledgement

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