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Stability of Drugs in Aqueous Solutions. III.[1,2] Kinetic Studies on Anaerobic Hydrolysis of Adenosine-5′-triphosphate

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The nature of the decomposition products of adenosine-5′-triphosphate (ATP) and the kinetics of decomposition in aqueous solution were investigated at 60, 70 and 80°C, ionic strength 0.5, pH 3.2—10.1 by high pressure liquid chromatography. Adenosine-5′-diphosphate (ADP) and adenoside-5′-monophosphate (AMP) were identified as the decomposition products of ATP. The time courses of the ATP and ADP concentrations were measured and the apparent 1st-order rate constants of the decomposition of ATP to ADP (k_1), ADP to AMP (k_2) and ATP to AMP (k_3) were calculated. The order of decomposition rates was $k_1>k_2>k_3$ in the pH range of 3.2—10.1.

The observed decomposition rates of ATP and ADP were influenced significantly by spontaneous or water-catalyzed reaction. The specific rate constants of the ionic species of ATP and ADP were calculated, and it was found that decomposition rate of ATP including its decomposition products could be expressed by the summation of the products of the specific rate constants and mole fractions of the ionic species.

The Arrhenius plots of these specific rate constants were linear and the apparent activation energies were all in the range of 28.8—31.8kcal/mol.

The primary salt effects on the decomposition of ATP were almost negligible.

Therefore the overall decomposition rate of ATP including its decomposition products could be expressed as a function of the specific rate constants at arbitrary pH, temperature and ionic strength in several buffer species.

Keywords——ATP; decomposition; kinetics; aqueous solution; high pressure liquid chromatography

Adenosine-5′-triphosphate (ATP) is one of the most important chemicals involved in energy transfer in living systems. ATP injection is clinically applied for muscular neural, cerebromuscular and circulatory diseases. The hydrolysis of ATP has been studied previously at selected temperatures and pH values both in distilled water and in the presence of several inorganic ions which might act as catalysts,[3,4] but in most of these studies, the residual concentration of ATP was indirectly determined by measuring the concentration of inorganic orthophosphate.[3]

Further, only the decomposition of ATP has been treated in previous reports,[3,4] not the overall decomposition kinetics including the decomposition products.

In this paper, the concentrations of ATP and its decomposition products such as adenosine-5′-diphosphate (ADP) and adenosine-5′-monophosphate (AMP) were determined by using high pressure liquid chromatography (HPLC) in order to investigate the overall kinetics of the decomposition of ATP including the behavior of the decomposition products.

Experimental

Materials——Disodium salts of adenosine-5′-triphosphate (ATP, Daiichi Seiyaku Co. Ltd., >99%), the sodium salt of adenosine-5′-diphosphate (Sigma Chemical Company, 97%, from equine muscle), the sodium salt of adenosine-5′-monophosphoric acid (Sigma Chemical Company, 99%, from yeast) and adenosine (Daiichi Seiyaku Co. Ltd., >99%) were used without further purification. All other chemicals used were of the highest grade commercially available. The water used was distilled water for injection (J.P.).

Kinetic Procedures——An accurately weighed quantity of ATP was dissolved in an appropriate buffer solution bubbled through with nitrogen gas. The initial concentration of ATP in the buffer solutions was

8×10^{-4} M. Each buffer solution contained 0.02 M phosphoric acid, 0.02 M acetic acid, 0.02 M boric acid and appropriate amounts of sodium hydroxide. The solutions were adjusted to the ionic strength of 0.5 with sodium chloride. The ATP solution was put into 1 ml ampoules, the air in the ampoules was replaced with nitrogen gas, and the ampoules were sealed. Then they were allowed to stand in a constant temperature water bath (accuracy ±0.1°C). The ampoules were removed from the water baths at appropriate intervals and the concentrations of ATP and its decomposition products were determined. The pH values of ATP solutions were measured at the experimental temperatures with a pH meter (Hitachi–Horiba, Model M-7) standardized with standard buffer solutions of pH 4, 7 and 9 at the same temperatures.

Analytical Procedure——HPLC was used to determine the concentrations of ATP, ADP and AMP. A high pressure liquid chromatograph (Kyowa Seimitsu Co. Ltd., Model K88D) with a variable wavelength ultra violet (UV) spectrophotomerter (JASCO, Model UVIDEC-100-II) and an integrator (Hewlett Packard, Model 3380A) were employed. For chromatographic separation, a 500 mm × 3 mm i.d. glass column packed with strong anion exchanger, Zipax® SAX (Dupont Instruments), was used. The mobile phase was 0.3 M monobasic potassium phosphate and its flow rate was 2 ml/min. The column temperature was ambient. The UV absorbance at 258 nm, $\lambda_{\max}$ of ATP, was measured. The concentration of each species was calculated from the area of each peak.

Determination of Dissociation Constants——The apparent pKa values of ATP and ADP were determined potentiometrically[5] at the ionic strength of 0.5 (NaCl) at 60, 70 and 80°C.

The Rate Determination——On the basis of Chart 1, the differential equation for the concentrations of ATP, ADP and AMP can be expressed as eqs. (1), (2) and (3), because ATP and ADP were reported to be decomposed according to apparent 1st-order kinetics;[3c,e]; the decomposition of AMP is neglected.

$$\begin{tikzcd}
\text{ATP} \arrow[r, "k_1"] \arrow[rr, bend right, "k_3"'] & \text{ADP} \arrow[r, "k_2"] & \text{AMP}
\end{tikzcd}$$

Chart 1. Possible Decomposition Pathway of ATP

$$\mathrm{d}[X_1]/\mathrm{d}t = -(k_1+k_3)[X_1] \tag{1}$$

$$\mathrm{d}[X_2]/\mathrm{d}t = k_1[X_1] - k_2[X_2] \tag{2}$$

$$\mathrm{d}[X_3]/\mathrm{d}t = k_2[X_2] + k_3[X_1] \tag{3}$$

where $[X_1]$ is the concentration of ATP, $[X_2]$ is the concentration of ADP and $[X_3]$ is the concentration of AMP.

After integration of eqs. (1), (2) and (3) between $[X_1]=[X_1]_0$, $[X_2]=0$, and $[X_3]=0$ at time $t=0$ and $[X_1]$, $[X_2]$ and $[X_3]$ at some later time t using Laplacian transformation, $[X_1]$, $[X_2]$ and $[X_3]$ can be expressed as eqs. (4), (5) and (6).

$$[X_1] = [X_1]_0 \exp\{-(k_1+k_3)t\} \tag{4}$$

$$[X_2] = [X_1]_0 \frac{k_1}{k_2-k_1-k_3}[\exp\{-(k_1+k_3)t\} - \exp(-k_2t)] \tag{5}$$

$$[X_3] = [X_1]_0 \left[1 - \frac{k_2-k_3}{k_2-k_1-k_3}\exp\{-(k_1+k_3)t\} + \frac{k_1}{k_2-k_1-k_3}\exp(-k_2t)\right] \tag{6}$$

By differentiating eq. (5), it can be shown that the concentration of ADP, $[X_2]$, has a maximum at time $t=t_a$.

$$[X_2]_{\max} = \frac{k_1[X_1]_0}{k_1+k_3}\left(\frac{k_1+k_3}{k_2}\right)^{\frac{k_2}{k_2-k_1-k_3}} \tag{7}$$

$$t_a = \frac{1}{k_1+k_3-k_2}\ln\left(\frac{k_1+k_3}{k_2}\right) \tag{8}$$

Using eqs. (5) and (7), $[X_2]$ can be expressed as follows.

$$[X_2] = \frac{[X_2]_{\max} k_2 \left(\frac{k_1+k_3}{k_2}\right)^{\frac{k_1+k_3}{k_1+k_3-k_2}}}{k_2-(k_1+k_3)}[\exp\{-(k_1+k_3)t\} - \exp(-k_2t)] \tag{9}$$

When $k_1+k_3=P$ and $[X_2]_{\max}=Q$,

$$[X_2] = \frac{Qk_2(P/k_2)^{\frac{P}{P-k_2}}}{k_2-P}\{\exp(-Pt)-\exp(k_2t)\} \tag{10}$$

where the value of P can be obtained from a semi-logarithmic plot of the concentration of ATP against time and the value of Q can be obtained from the maximum concentration of ADP by using multinomial regression. The value of Q can be obtained accurately because the number of data near the maximum of the ADP concentration is usually large.

The k_2 value can be obtained by minimizing the sum of the squares of the difference between all the ADP concentration data at each time and the value of $[X_2]$ from eq. (10) by means of a computer.

When the k_2 value is obtained, k_1 and k_3 can be obtained from the following equiatons.

$$k_1 = Qk_2(P/k_2)^{\frac{P}{P-k_2}} \tag{11}$$

$$k_3 = P - k_1 \tag{12}$$

This method is more useful than the method of residuals when the k_1+k_3 value is slightly larger than the k_2 value, because all the data can be used in this method.

Data Analysis——When the P and Q values are obtained, the theoretical value of $[X_2]_i$ can be obtained at time t_i from eq. (10) as a function of k_2. Further, the experimental value of $[X_2]_i$ is obtained at time t_i. When the theoretical value is replaced by $y_i(k_2)$ and the experimental value is replaced by x_i, the sum of the squares of variations is given by eq. (13).

$$S = \sum_i^n \{x_i-y_i(k_2)\}^2 \tag{13}$$

The k_2 value which minimizes the S value of eq. (13) is obtained by changing the k_2 value in small increments from 0.0001 to 1.0000 by computer.

Results and Discussion

HPLC Studies

A typical chromatogram is shown in Fig. 1. The concentrations of AMP, ADP and ATP were 4.03×10^{-5} M and the concentration of adenosine was 2.02×10^{-5} M. The sensitivity for ATP is increased by a factor of four in this chromatogram. The retention times of adenosine, ATP, ADP and ATP are 1.06, 1.17, 1.69 and 4.46 minutes, respectively, so it takes about six minutes to determine the concentrations of all the compounds. The concentration of each species was calculated from the peak areas and plots of the areas against the concentration were linear for each species. In the actual kinetic runs, the peak of adenosine was scarcely observed, so AMP, ADP and ATP were completely separated.

Dissociation Constant

The apparent pK_a values of ATP and ADP at the ionic strength of 0.5, at 60, 70 and 80°C are shown in Table I. The three ionic forms of ATP in the pH range 3.2—10.1 are shown in Chrt 2.[3f] The pK_a values of ATP were different from the values (pK_{a1}=3.87, pK_{a2}=6.52, 40°C, ionic strength 0.1) reported by Kahn *et al.*,[6] because the pK_a values of ATP are greatly dependent on ionic strength. On the other hand, the pK_a values of ATP and ADP scarcely changed with temperature between 60°C and 80°C.

The Time Courses of ATP

Fig. 2 illustrates typical time courses of ATP concentration during hydrolysis in buffer, ionic strength 0.5 (NaCl), at 70°C, at various pH values. In the range of pH 3.2—10.1, semi-logarithmic plots of the ratios of the remaining concentration of ATP at various pH values are linear against reaction time, as shown in Fig. 2. This indicates that the decomposition of ATP is an apparent first-order reaction in this range of pH. The pseudo-1st-order rate constants were calculated by least-squares analysis of the data. These rate constants correspond to k_1+k_3 in Chart 1. The values of k_1+k_3 are shown in Table II. In the ranges of pH 3.2—5.1

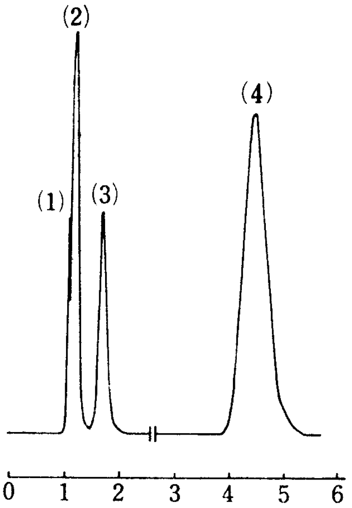

Fig. 1. HPLC Chromatogram of (1) Adenosine, (2) AMP, (3) ADP, and (4) ATP

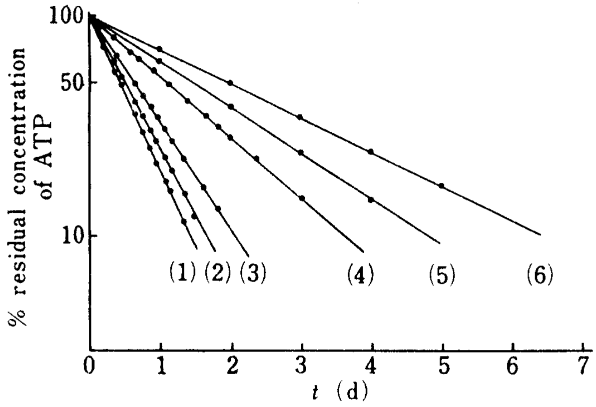

Fig. 2. Observed Pseudo-1st-Order Kinetic Degradation of ATP in Buffer Solutions at Different pH Values at 70°C ($\mu=0.5$)

(1) pH 4.10, (2) pH 5.52, (3) pH 6.00, (4) pH 6.60, (5) pH 7.04, (6) pH 9.40.

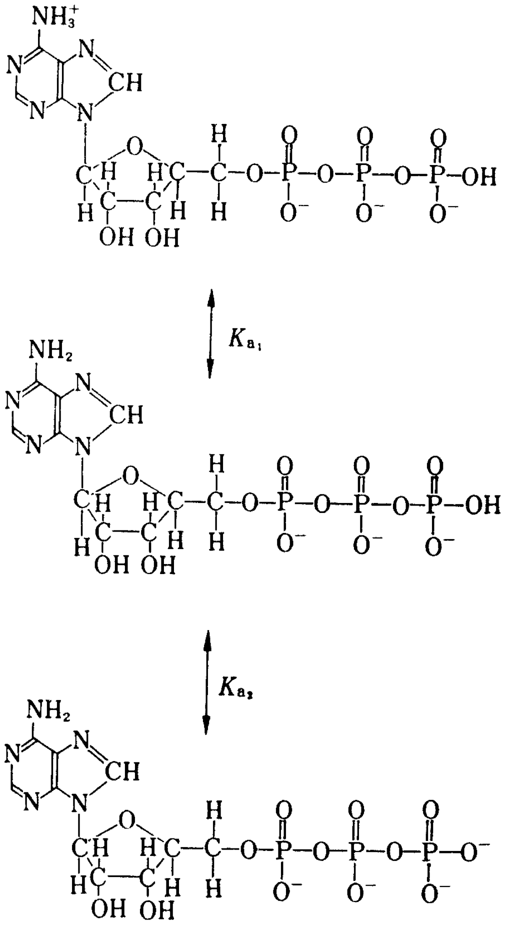

Chart 2. Dissociation Steps for ATP

TABLE I. Apparent pK_a Values of ATP and ADP at the Ionic Strength of 0.5 (NaCl)

Temp. (°C)	ATP		ADP	
	pK_{a_1}	pK_{a_2}	pK_{a_1}	pK_{a_2}
60	3.51	6.17	3.57	6.15
70	3.53	6.26	3.57	6.20
80	3.54	6.27	3.52	6.21

and 7.8—10.1, the behavior of ATP was almost the same, so the plots in these pH ranges are represented by those at 4.10 and 9.40 in Fig. 2.

The Time Courses of ADP

Fig. 3 shows typical time courses of ADP during the decomposition of ATP in buffer, ionic strength 0.5 (NaCl), at 70°C at various pH values. In the ranges of pH 3.2—5.1 and

8.7—10.6, the behavior of ADP during the decomposition of ATP was almost the same, so the plots in these pH ranges are represented by those at pH 4.10 and 9.40.

Observed Rate Constants of ATP and ADP

The observed rate constants (k_1, k_2 and k_3) were determined from eqs. (10), (11) and (12) by means of a computer by using the decomposition rates of ATP, the maximum concentrations of ADP determined by multinomial regressions and the time courses of ADP concentration. The observed rate constants k_1, k_2, and k_3 at each pH are shown in Tables II and III.

The experimental values of ATP, ADP and AMP concentrations coincided well with the values calculated from eqs. (4), (5) and (6). The experimental and calculated values for each

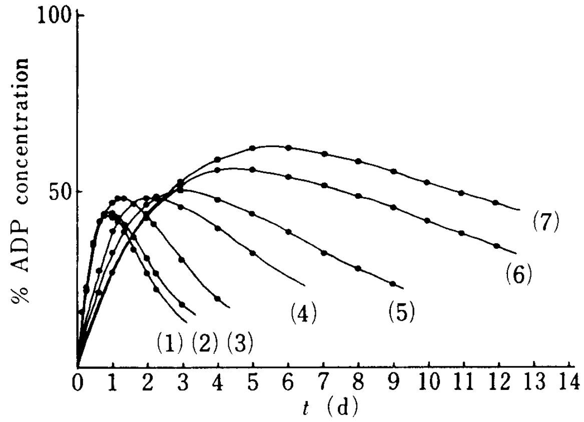

Fig. 3. Time Courses of ADP during the Degradation of ATP in Buffer Solutions at Different pH Values at 70°C ($\mu=0.5$)

(1) pH 4.10, (2) pH 5.52, (3) pH 6.00, (4) pH 6.60, (5) pH 7.04, (6) pH 7.83, (7) pH 9.40.

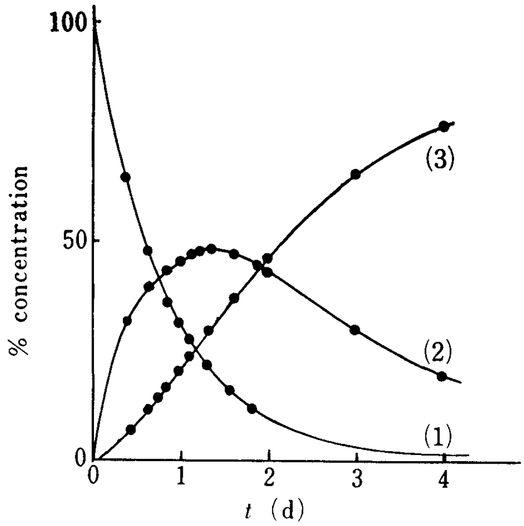

Fig. 4. Time Courses of ATP, ADP and AMP at pH 6.00 (70°C, $\mu=0.5$)

Solid lines: calculated profiles, Dots: observed values.
(1) ATP, (2) ADP, (3) AMP.

Table II. Correlation of Hydrolysis Rates with Fractions of Ionic Species of ATP at 70°C, $\mu=0.5$

Mole fractions of the ionic species of ATP (70°C, $\mu=0.5$)				k_1+k_3 (d^{-1})	k_1 (d^{-1})		k_3 (d^{-1})	
pH	H_2ATP^{2-}	$HATP^{3-}$	ATP^{4-}	Observed	Measured	Calculated	Measured	Calculated
3.20	0.681	0.319	—	1.708	1.572	1.570	0.136	0.136
4.10	0.211	0.784	0.005	1.586	1.463	1.461	0.123	0.122
4.59	0.079	0.902	0.019	1.535	1.422	1.417	0.113	0.117
5.11	0.024	0.911	0.065	1.461	1.359	1.355	0.102	0.111
5.52	0.009	0.839	0.152	1.371	1.270	1.257	0.101	0.101
6.00	0.002	0.644	0.354	1.144	1.063	1.036	0.081	0.080
6.60	—	0.134	0.686	0.633	0.603	0.676	0.030	0.045
7.04	—	0.142	0.858	0.479	0.458	0.489	0.021	0.027
7.83	—	0.026	0.974	0.355	0.344	0.363	0.011	0.015
8.70	—	0.004	0.996	0.336	0.323	0.339	0.013	0.013
9.40	—	0.001	0.999	0.353	0.342	0.336	0.011	0.012
10.06	—	—	1.000	0.353	0.340	0.335	0.013	0.012

The observed values of k_1+k_3 were determined from the pseudo-1st-order decomposition rate of ATP. The measured values of k_1 and k_3 were determined by means of eqs. (10), (11) and (12). The calculated values of k_1 and k_3 were determined from eqs. (13) and (15), where $k'_{1(H_2ATP^{2-})}=1.64\ d^{-1}$, $k'_{1(HATP^{3-})}=1.42\ d^{-1}$, $k'_{1(ATP^{4-})}=0.335\ d^{-1}$, $k'_{8(H_2ATP^{2-})}=0.145\ d^{-1}$, $k'_{3(HATP^{3-})}=0.117\ d^{-1}$ and $k'_{3(ATP^{4-})}=0.0121\ d^{-1}$.

Table III. Correlation of Hydrolysis Rates with Fractions of Ionic Species of ADP at 70°C, $\mu=0.5$

	Mole fractions of the ionic species of ADP (70°C, $\mu=0.5$)			k_2 (d^{-1})	
pH	H_2ADP^-	$HADP^{2-}$	ADP^{3-}	Measured	Calculated
3.20	0.700	0.300	—	0.956	0.956
4.10	0.226	0.768	0.006	0.894	0.907
4.59	0.085	0.893	0.022	0.881	0.879
5.11	0.026	0.901	0.073	0.840	0.834
5.52	0.009	0.820	0.171	0.763	0.753
6.00	0.002	0.612	0.386	0.521	0.578
6.60	—	0.285	0.715	0.300	0.311
7.04	—	0.126	0.874	0.207	0.183
7.83	—	0.023	0.977	0.113	0.099
8.70	—	0.003	0.997	0.083	0.083
9.40	—	0.001	0.999	0.079	0.081
10.06	—	—	1.000	0.080	0.080

The measured values of k_2 were determined from eq. (10). The calculated values of k_2 were determined by means of eq. (14), where $k'_{2(H_2ADP^-)}=0.984$ d^{-1}, $k'_{2(HADP^{2-})}=0.891$ d^{-1}, and $k'_{2(ADP^{3-})}=0.084$ d^{-1}.

species at pH 6.00 and 7.83 are shown in Figs. 4 and 5 as examples. As no decomposition products of ATP other than ADP and AMP were found in this experiment, ATP was concluded to be decomposed according to Chart 1.

pH Rate Profile at 70°C

The values of log k_1, log k_2 and log k_3 were plotted against pH to obtain the pH-rate profile (Fig. 6). Generally, specific acid-base reactions can be considered for the hydrolysis of ATP and ADP. The log $k'_{1,2,3}$-pH profile (Fig. 6) suggests that two apparent second-order rate

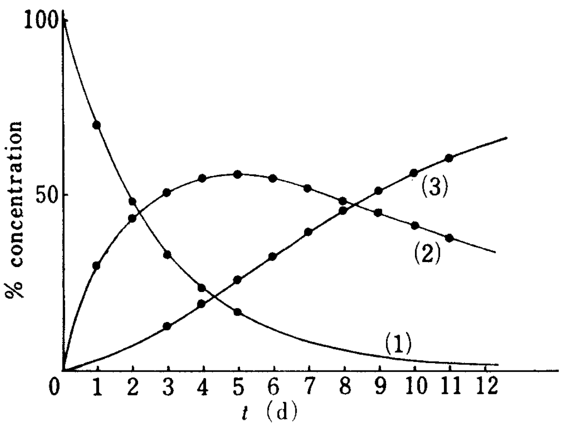

Fig. 5. Time Courses of ATP, ADP and AMP at pH 7.83 (70°C, $\mu=0.5$)

Solid lines: calculated profiles, Dots: observed values.
(1) ATP, (2) ADP, (3) AMP.

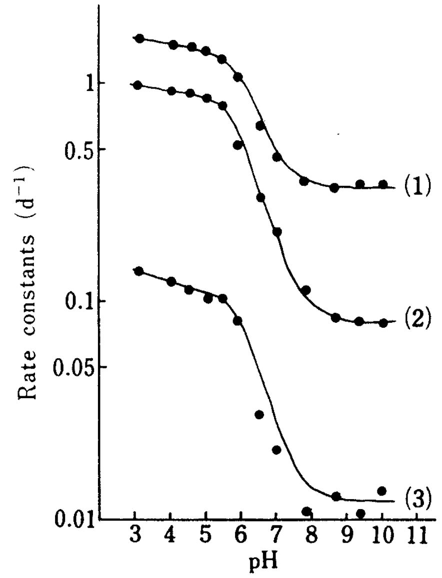

Fig. 6. pH-Rate Profiles for the Degradation of ATP and ADP in Buffer Solutions at 70°C ($\mu=0.5$)

Solid lines: theoretical profiles, Dots: observed values.
(1) ATP → ADP(k_1), (2) ADP → AMP(k_2),
(3) ATP → AMP(k_3).

constants, one for the interaction of H^+ ion and the other for the interaction of OH^- ion, have no significance and are not related to the acid- or base-catalyzed reaction. That is to say that spontaneous or water-catalyzed decomposition takes place, and the overall reaction rate represents a summation of the following separate reactions.

$$\mathrm{H_2ATP^{2-}} \xrightarrow{\mathrm{H_2O}} \text{products} \qquad \text{reaction 1}$$

$$\mathrm{HATP^{3-}} \xrightarrow{\mathrm{H_2O}} \text{products} \qquad \text{reaction 2}$$

$$\mathrm{ATP^{4-}} \xrightarrow{\mathrm{H_2O}} \text{products} \qquad \text{reaction 3}$$

$$\mathrm{H_2ADP^{-}} \xrightarrow{\mathrm{H_2O}} \text{products} \qquad \text{reaction 4}$$

$$\mathrm{HADP^{2-}} \xrightarrow{\mathrm{H_2O}} \text{products} \qquad \text{reaction 5}$$

$$\mathrm{ADP^{3-}} \xrightarrow{\mathrm{H_2O}} \text{products} \qquad \text{reaction 6}$$

The rate constants $k_{1,\mathrm{obs}}$, $k_{2,\mathrm{obs}}$ and $k_{3,\mathrm{obs}}$, therefore, represent the sum of these reactions.

$$k_{1,\,\mathrm{obs}} = k'_{1(\mathrm{H_2ATP^{2-}})} f_{(\mathrm{H_2ATP^{2-}})} + k'_{1(\mathrm{HATP^{3-}})} f_{(\mathrm{HATP^{3-}})} + k'_{1(\mathrm{ATP^{4-}})} f_{(\mathrm{ATP^{4-}})} \tag{14}$$

$$k_{2,\,\mathrm{obs}} = k'_{2(\mathrm{H_2ADP^{-}})} f_{(\mathrm{H_2ADP^{-}})} + k'_{2(\mathrm{HADP^{2-}})} f_{(\mathrm{HADP^{2-}})} + k'_{2(\mathrm{ADP^{3-}})} f_{(\mathrm{ADP^{3-}})} \tag{15}$$

$$k_{3,\,\mathrm{obs}} = k'_{3(\mathrm{H_2ATP^{2-}})} f_{(\mathrm{H_2ATP^{2-}})} + k'_{3(\mathrm{HATP^{3-}})} f_{(\mathrm{HATP^{3-}})} + k'_{3(\mathrm{ATP^{4-}})} f_{(\mathrm{ATP^{4-}})} \tag{16}$$

where k_1', k_2' and k_3' are each spontaneous or water-catalyzed reaction rate constants and f's are the mole fractions of each ionic species.

The specific rate constants of k'_1, k'_2 and k'_3 were obtained by solving the simultaneous equations (14), (15) and (16) over the entire pH range of 3.2—10.1. The specific rate constants are independent of pH over the pH range studied and the values at 70°C are shown in Tables II and III. The calculated and observed experimental rates are also shown in Tables II and III and Fig. 5. They are in good agreement with each other.

Effect of Temperature

The effect of temperature on k_1,' k'_2 and k'_3 was determined by measuring the decomposition rates at 60 and 80°C with the same samples as had been used at 70°C. The behavior of ATP and ADP at 60 and 80°C was almost the same as at 70°C. As shown in Figs. 7, 8 and 9, Arrhenius plots of log k'_{123} *vs.* $1/T$ were linear. The apparent activation parameters obtained from the Arrhenius plots are given in Table IV.

Primary Salt Effect

The values of $k'_1+k'_3$ at 56°C and ionic strength 0.5 were obtained by extrapolation of Arrhenius plots (Figs. 7 and 9), while Kahn *et al.*[3e] obtained the rate constants of the decom-

Table IV. Apparent Activation Parameters for the Ionic Species of ATP and ADP, $\mu=0.5$ (NaCl), Ref. temp. =70°C

Ionic species	Ea (kcal/mol)	log A	$\Delta H^{\neq}$ (kcal/mol)	$\Delta S^{\neq}$ (cal/mol deg)	$\Delta G^{\neq}$ (kcal/mol)
$k'_{1(\mathrm{H_2ATP^{2-}})}$	29.8	14.22	29.1	4.26	27.6
$k'_{1(\mathrm{HATP^{3-}})}$	29.8	14.13	29.1	3.84	27.8
$k'_{1(\mathrm{ATP^{4-}})}$	30.0	13.50	29.3	0.96	29.0
$k'_{2(\mathrm{H_2ADP^{-}})}$	29.6	14.00	28.9	3.25	27.8
$k'_{2(\mathrm{HADP^{2-}})}$	29.6	13.89	28.9	2.75	28.0
$k'_{2(\mathrm{ADP^{3-}})}$	30.7	13.38	30.0	0.14	29.9
$k'_{3(\mathrm{H_2ATP^{2-}})}$	29.6	13.58	28.9	1.33	28.4
$k'_{3(\mathrm{HATP^{3-}})}$	29.6	13.53	28.9	1.10	28.5
$k'_{3(\mathrm{ATP^{4-}})}$	31.8	13.32	31.1	0.14	31.1

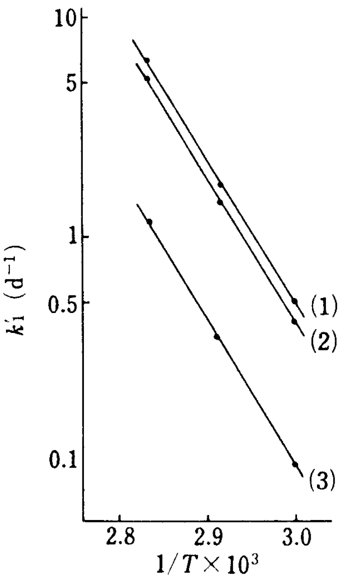

Fig. 7. Arrhenius Plots for the Specific Rate Constants (k_1') of the Decomposition of ATP to ADP ($\mu=0.5$)

(1) $k'_{1(H_2ATP^{2-})}$, (2)$k'_{1(HATP^{3-})}$, (3) $k'_{1(ATP^{4-})}$.

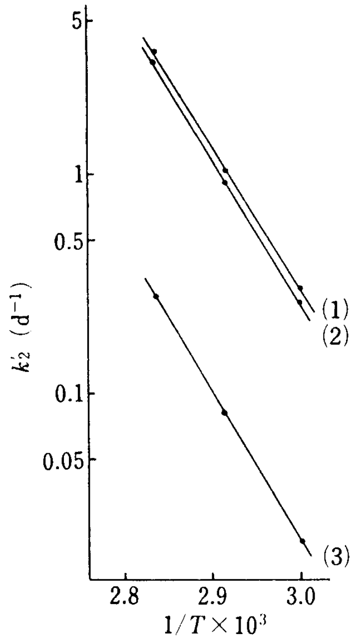

Fig. 8. Arrhenius Plots for the Specific Rate Constants (k_2') of the Decomposition of ADP to AMP ($\mu=0.5$)

(1) $k'_{2(H_2ADP^{-})}$, (2) $k'_{2(HADP^{2-})}$, (3) $k'_{2(ADP^{3-})}$.

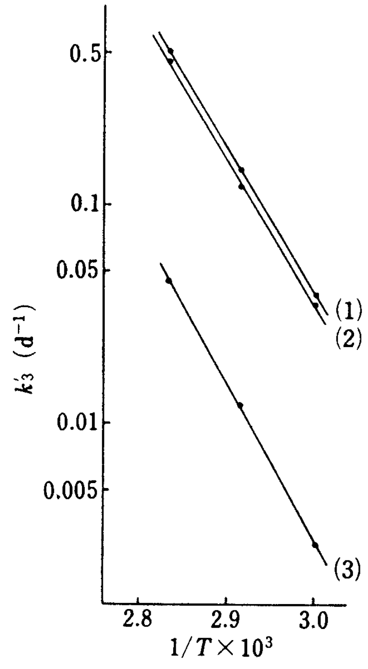

Fig. 9. Arrhenius Plots for the Specific Rate Constants (k_3') of the Decomposition of ATP to AMP ($\mu=0.5$)

(1) $k'_{3(H_2ATP^{2-})}$, (2) $k'_{3(HATP^{3-})}$, (3) $k'_{3(ATP^{4-})}$.

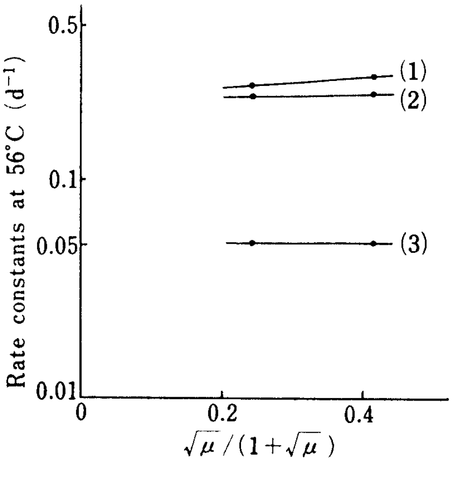

Fig. 10. Primary Salt Effects on the Hydrolysis of ATP

(1) $k'_{1(H_2ATP^{2-})}+k'_{3(H_2ATP^{2-})}$: slope=0.23.
(2) $k'_{1(HATP^{3-})}+k'_{3(HATP^{3-})}$: slope=0.07.
(3) $k'_{1(ATP^{4-})}+k'_{3(ATP^{4-})}$: slope=0.00.

position of ATP at the ionic strength $\mu=0.1$ without using buffers. The kinetic constants for the hydrolysis of ATP obtained indicectly by Kahn *et al.*[3e] can be adopted for rough discussions about the decomposition mechanism of ATP. Within a restricted range of ionic strength(μ), plots of log k *vs.* $\sqrt{\mu}$ or $\sqrt{\mu}/(1+\sqrt{\mu})$ should yield slopes theoretically equal to $2AZ_AZ_B$, where A is a constant for a given solvent at a given temperature and Z_A and Z_B are the charges on reaction species A and B, respectively.[7] These plots would be expected to be linear only within the limits of the Debye–Hückel expressions; Carstensen[8] reviewed kinetic salt effects in the pharmaceutical literature and concluded that plots of k *vs.* $\sqrt{\mu}/(1+\sqrt{\mu})$ may be linear at ionic strength below 1.0.

When the values of log $(k'_1+k'_3)$ were plotted against $\sqrt{\mu}/(1+\sqrt{\mu})$, the slopes were almost zero (Fig. 10) and no kinetic salt effect was observed.

This result requires that at least one reactant is noncharged according to the Debye–Hückel theory, and this is compatible with the reactions 1—6.

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References and Notes

1) Part II: H. Seki, T. Kagami, T. Hayashi and N. Okusa, *Chem. Pharm. Bull.*, **29**, 3680 (1981).
2) A Part of this paper was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, 1979 and the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, 1980.
3) *a)* S.L. Friess, *J. Am. Chem. Soc.*, **75**, 323 (1953); *b)* P.E. Amsler and H. Sigel, *J. Biochem.* (Tokyo), **63**, 569 (1976); *c)* H. R. Hulet, *Nature* (London), **225**, 1248 (1970); *d)* M. Tetas and J.M. Lowenstein, *Biochemistry*, **2**, 350 (1963); *e)* M.M.T. Kahn and M.S. Mohan, *J. Inorg. Nucl. Chem.*, **36**, 707 (1974); *f) Idem, Ind. J. Chem.*, **14A**, 945 (1976); *g) Idem, Ibid.*, **14A**, 951 (1976); *h)* J.T.H. Goossen and J.G. Kloosterboer, *Photochem. Photobiol.*, **27**, 703 (1978); *i)* D. H. Buisson and H. Sigel, *Biochim. Biophys. Acta*, **343**, 45 (1974); *j)* F. Reiff and H. Kirchhoff, *Arzneim. Forsch.*, **6**, 598 (1956).
4) *a)* D.L. Miller and F.H. Westheimer, *J. Am. Chem. Soc.*, **88**, 1507 (1966); *b)* A.M. Couture and L. Ouellet, *Can. J. Chem.*, **35**, 1248 (1957); *c)* A. Hock and G. Huber, *Biochem. Z.*, **328**, 44 (1956).
5) Shima, *J. Biochem (Tokyo).*, **29**, 1, 121, 147 (1939).
6) M.M.T. Kahn and A.E. Martel, *J. Am. Chem. Soc.*, **88**, 668 (1966).
7) A. Frost and R. Pearson, "Kinetics and Mechanisms," 2nd. ed., Wiley, New York, N.Y., 1971, chap. 7.
8) J.T. Carstensen, *J. Pharm. Sci.*, **59**, 1140 (1970).