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The Effect of Metal Ions on Labile Phosphates. I. The Hydrolysis of Acetyl Phosphate Dianion*

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ABSTRACT: The hydrolysis of acetyl phosphate dianion is catalyzed by the divalent ions of Zn, Co, Mn, Ni, Ca, and Mg in aqueous solutions of pH 5.8–8.8. The rate of hydrolysis is dependent upon the specific metal catalyst employed, catalyst concentration, pH, temperature, and the particular buffer if one is employed. The rate of reaction has been determined in three aqueous media by an indirect spectrophotometric procedure and by titration of the acid generated in the absence of buffer. The observed rate constants for the magnesium- and calcium-catalyzed systems are interpreted in terms of three simultaneous reactions: an uncatalyzed reaction, attack on an intermediate metal complex by water, and attack on the intermediate metal complex by hydroxide ion. Formation constants for the metal complexes of acetyl phosphate dianion with calcium and magnesium ions have been estimated to be 7.7 and 5.7, respectively.

The catalytic (nonenzymatic) reaction of acetyl phosphate dianion,[1] AcP^{2-}, with water has been examined by several investigators (Lipmann and Tuttle, 1944; Koshland, 1952; Kurz and Gutsche, 1960; Di Sabato and Jencks, 1961a). The objective of the present work was a more detailed and extensive investigation of the role of metal ions in this reaction. The pH and the temperature dependence of the catalytic and the noncatalytic reactions have been established in part for a variety of catalysts in several different aqueous media. A comparison of the catalytic activity of six metal ions in the same system has been achieved. The principal chemical reaction under investigation is

$$CH_3COOPO_3^{2-} + H_2O = CH_3COO^- + HPO_4^{2-} + H^+$$

A secondary reaction takes place under certain conditions

$$2HPO_4^{2-} + 2OH^- + 3M^{2+} = M_3(PO_4)_2\downarrow + 2\,H_2O$$

Experimental Procedures

Reagent grade metal perchlorates were employed and lithium acetyl phosphate (Worthington Biochemical Corp.) was used without further purification. The infrared spectrum of the latter is essentially the same as reported in the literature (Jencks *et al.*, 1960).

Reaction Media. Three aqueous media were em-

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[1] The following abbreviations are used: acetyl phosphate dianion, AcP^{2-}; divalent metal ions, M^{2+}; reaction products, RP.

TABLE I: Uncatalyzed Hydrolysis of AcP^{2-}, Ionic Strength 0.6.

pH	Buffer	Temp, °C	$k_u \times 10^3$ min^{-1}	E_a (kcal/mole)	ΔH^* (kcal/mole)	ΔS^* (eu)
7.7	HCO_3^-	26.4	0.69			
	HCO_3^-	33.0	1.8			
	HCO_3^-	39.0	4.1	25.4	24.8	1.6
7.8	Tris	25.0	0.55			
	Tris	26.4	0.65			
	Tris	39.0	4.0	25.9	25.3	3.2
7.2	Tris	26.4	0.61			
		39.0	4.0			
5.8	None	39.0	4.3			
6.8	None	39.0	4.6			
7.8	None	39.0	4.3	26.6	26.0	5.8
8.8	None	39.0	4.5			
7.8	None	26.4	0.68			

ployed, each with weighed quantities of sodium perchlorate added to give an ionic strength of 0.6. These media were 0.05 M $NaHCO_3$ buffer solution, 0.05 M Tris buffer solution, and an unbuffered solution where the pH was maintained constant with NaOH solution and the aid of a pH-Stat (Fisher titrimeter).

Kinetic Runs. Kinetic runs were made with 25- to 100-ml volumes of the medium maintained at the desired temperature and pH. For each milliliter of medium approximately 1 mg of cold, solid lithium acetyl phosphate was added, to give an initial acetyl phosphate concentration of 7×10^{-3} mole/l. It was possible to follow the reaction in all media by the indirect spectrophotometric procedure (Koshland, 1951). The reproducibility of the measurements was improved when the final solution for the analytical procedure was centrifuged before the absorbance at 540 mμ was measured with a Beckman Model B spectrophotometer. It was also possible to determine the rate of reaction in the unbuffered medium from the volume of standard base required at given times to keep the pH constant. Some rate constants were determined by both methods and they were in excellent agreement. The reactions were followed until 75–99% of the AcP^{2-} disappeared. Good pseudo-first-order plots were obtained. Variation of the initial acetyl phosphate concentration from 4×10^{-3} to 1.2×10^{-2} mole/l. for a given run gave the same rate constant within experimental error.

The pK_a values of acetylphosphoric acid were determined for solutions of 0.6 ionic strength at appropriate temperatures by the procedure reported in the literature (Lipmann and Tuttle, 1947). The pH measurements were made with a Beckman Model G pH meter.

Results

Ionization Constant of Acetylphosphoric Acid. The pK_a values for the second ionization of acetyl phosphoric acid in 0.6 M $NaClO_4$ were found to be 4.3 and 4.8 at 26.4 and 39.0°, respectively. A similar value, 4.8, was obtained at a lower temperature and lower ionic strength (Lipmann and Tuttle, 1947). All kinetic experiments, except one, were carried out in solutions where the dianion was the only significant species of acetyl phosphate present. At pH 2.7, where the monoanion is predominant, 0.05 M Zn^{2+} failed to catalyze the reaction.

The Uncatalyzed Reaction. The rate of the uncatalyzed hydrolysis of AcP^{2-} and the thermodynamic parameters that can be calculated from the data appear in Table I. Where comparison of the results with the literature is possible, the agreement is good (Koshland, 1952; Di Sabato and Jencks, 1961b). The pH dependence of the uncatalyzed reaction, typical plots for the kinetic runs, and the mathematical relationships which were used to calculate the thermodynamic quantities are available in these references.

The Catalyzed Reaction. The observed rate constants for the catalytic systems are presented in Table II. Similar data have been found to follow the equation $k_0 = k_u + k_c[Mg^{2+}]$, where k_c is a second-order catalytic constant (Koshland, 1952). This approach appears to be valid only at certain hydroxide ion concentrations. Reasonable catalytic constants can be calculated for Mg^{2+} at pH 7.7 and Ca^{2+} at pH 8.8. Since the observed rate is a function of hydroxide concentration, an equation which included base dependence was sought. A mechanism which may be considered is as follows.

$$AcP^{2-} \xrightarrow{k_u} RP$$

$$M^{2+} + AcP^{2-} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} MAcP$$

TABLE II: Rate Constants, Catalytic Hydrolysis of AcP^{2-}, Ionic Strength 0.6.

pH	Catalyst (M)	Buffer	Temp (°C)	$k_0 \times 10^3$ min^{-1}
7.7	0.100 Li^+	HCO_3^-	39.0	3.9[a]
	0.025 Mg^{2+}	HCO_3^-	26.4	1.6
	0.050			1.8
	0.100			3.2
	0.150			4.0
7.7	0.025 Mg^{2+}	HCO_3^-	33.0	4.0
	0.050			5.6
	0.100			7.1
	0.150			9.6
7.7	0.025 Mg^{2+}	HCO_3^-	39.0	7.4
	0.050			10.4
	0.100			17.0
	0.150			22.5
7.8	0.100 Mg^{2+}	Tris	25.0	2.2
7.8	0.100 Mg^{2+}	Tris	39.0	11.6
7.7	0.200 Li^+	None	39.0	4.2[a]
5.8	0.200 Mg^{2+}	None	39.0	9.2
6.8	0.050 Mg^{2+}	None	39.0	8.3
	0.100			11.2
	0.150			12.1
	0.200			14.6
7.2	0.200 Mg^{2+}	None	39.0	23.1
7.4	0.200 Mg^{2+}	None	39.0	34.6
7.6	0.200 Mg^{2+}	None	39.0	48.8
7.7	0.050 Mg^{2+}	None	26.4	6.2
7.7	0.100 Mg^{2+}	None	26.4	12.8
7.7	0.050 Mg^{2+}	None	39.0	14.7
	0.100			28.0
	0.150			41.0
	0.200			50.2
6.8	0.050 Ca^{2+}	None	39.0	11.8
	0.100			16.0
	0.150			17.4
	0.200			18.7
7.1	0.100 Ca^{2+}	None	39.0	16.6
7.2	0.100 Ca^{2+}	None	39.0	17.1
7.4	0.100 Ca^{2+}	None	39.0	19.2
7.7	0.100 Ca^{2+}	None	39.0	23.5
7.8	0.050 Ca^{2+}	None	39.0	17.7
	0.100			24.4
	0.150			31.3
	0.200			33.4
8.8	0.100 Ca^{2+}	None	26.4	22.2
8.8	0.200 Ca^{2+}	None	26.4	40.7
8.8	0.050 Ca^{2+}	None	39.0	39.4
	0.100			66.2
	0.150			110
	0.200			139
5.8	0.01 Zn^{2+}	None	39.0	52
6.0	0.05 Co^{2+}	None	39.0	64
	0.05 Mn^{2+}	None	39.0	40
	0.05 Ni^{2+}	None	39.0	38
	0.05 Ca^{2+}	None	39.0	9.6
	0.05 Mg^{2+}	None	39.0	6.2

[a] *Ca.* rate of uncatalyzed reaction in same medium.

$$\mathrm{MAcP} + \mathrm{H_2O} \xrightarrow{k_3} \mathrm{RP}$$

$$\mathrm{MAcP} + \mathrm{OH} \xrightarrow{k_4} \mathrm{RP}$$

RP = reaction products; MAcP = intermediate complex; $[AcP^{2-}]_f$ = free AcP^{2-} concentration; $[AcP^{2-}]_t$ = total AcP^{2-} concentration = $[AcP^{2-}]_f$ + [MAcP]; K_f (formation constant) = $[MAcP]/[M^{2+}][AcP^{2-}]_f$; k_u = uncatalyzed rate constant which is *not* a function of hydroxide ion concentration in this pH range; k_0 = observed rate constant. Conley and Martin (1965) have reported a similar mechanism for the copper-catalyzed hydrolysis of glycine esters.

Rate Law. The rate law for the above mechanism is

$$\text{rate} = k_u[AcP^{2-}]_f + k_3[MAcP] + k_4[MAcP][OH^-]$$

Rearrangement and substitution gives

$$k_0 = \frac{\text{rate}}{[AcP^{2-}]_t} = \frac{[AcP^{2-}]_f}{[AcP^{2-}]_t}(k_u + k_3K_f[M^{2+}] + k_4K_f[M^{2+}][OH^-])$$

and by further rearrangement, two other forms of this equation may be obtained, eq 1 and 2. These equations may be used to show base and metal ion dependence, respectively. K^* is defined by eq 3.

$$\frac{k_0 - k_u\dfrac{[AcP^{2-}]_f}{[AcP^{2-}]_t}}{[M^{2+}]} = \frac{[AcP^{2-}]_f}{[AcP^{2-}]_t}(k_3K_f + k_4K_f[OH^-]) \tag{1}$$

$$k_0(1 + [M^{2+}]K_f) = k_u + K^*[M^{2+}] \tag{2}$$

$$K^* = k_3K_f + k_4K_f[OH^-] \tag{3}$$

Equation 2 may be rewritten to give eq 4.

$$K^*[M^{2+}] = K_fk_0[M^{2+}] + k_0 - k_u \tag{4}$$

A plot of the left side of eq 4 *vs.* $k_0[M^{2+}]$ gives a line whose slope is the formation constant, K_f. Values of K^* which were needed for this determination of K_f were obtained from the intercepts of plots of k_c *vs.* $[M^{2+}]$, where k_c is the second-order catalytic constant of the equation, $k_0 = k_u + k_c[M^{2+}]$ (Koshland, 1952). Comparison of this equation with eq 2 suggested that $K^* = k_c$ at low metal ion concentrations. The k_c values at zero metal ion concentration, obtained by extrapolation, were used as K^* for the magnesium-catalyzed reaction at pH 6.8 and the calcium-catalyzed reaction at pH 6.8 and 7.8. Table III contains the data and the parameters which were used in the determination of K^* and K_f described above. The values of K^* were determined from the plots shown in Figure 1, and K_f values were determined from plots shown in Figure 2. $K^*_{Mg^{2+}}$ at pH 6.8 = 0.087, $K^*_{Ca^{2+}}$ at pH 6.8 = 0.17, $K^*_{Ca^{2+}}$ at pH 7.8 = 0.28, K_f for MgAcP

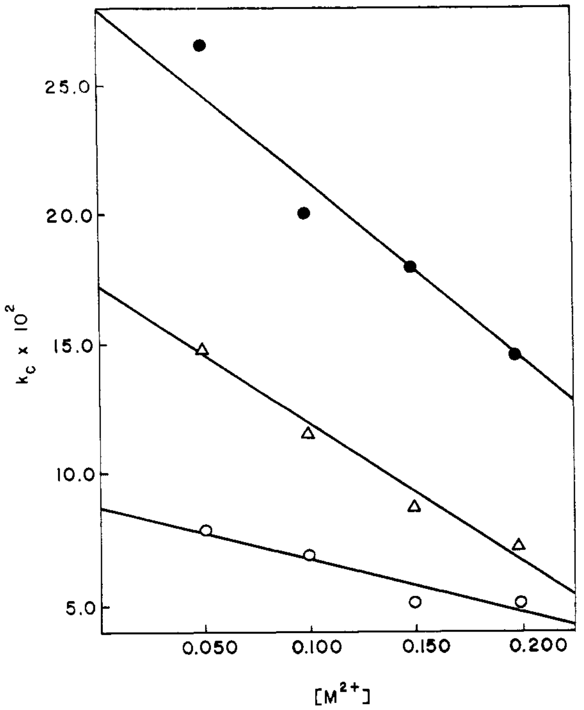

FIGURE 1: Determination of K^*. Open circles represent the k_c values for the magnesium-catalyzed system at pH 6.8. Triangles represent the k_c values for the calcium-catalyzed system at pH 6.8. Closed circles represent the k_c values of the calcium-catalyzed system at pH 7.8.

TABLE III: Values of Parameters for the Determination of K^* and K_f.

pH	Catalyst (M)	k_0 $\times 10^3$	k_c $\times 10^2$	$K^* \times$ $[M^{2+}]$ $\times 10^3$	$k_0 \times$ $[M^{2+}]$ $\times 10^4$
	None	4.4[a]	—	—	—
6.8	0.050 Mg^{2+}	8.3	7.8	4.4	4.2
	0.100 Mg^{2+}	11.2	6.8	8.7	11
	0.150 Mg^{2+}	12.1	5.1	13	18
	0.200 Mg^{2+}	14.6	5.1	17	29
6.8	0.050 Ca^{2+}	11.8	14.8	8.5	5.9
	0.100 Ca^{2+}	16.0	11.6	17	16
	0.150 Ca^{2+}	17.4	8.7	26	26
	0.200 Ca^{2+}	18.7	7.2	34	37
7.8	0.050 Ca^{2+}	17.7	26.6	14	8.9
	0.100 Ca^{2+}	24.4	20.0	28	24
	0.150 Ca^{2+}	31.3	17.9	42	47
	0.200 Ca^{2+}	33.4	14.5	56	67

[a] The average experimental value of the rate constant in the pH range 5.8–8.8, in the presence of 0.600 M $NaClO_4$.

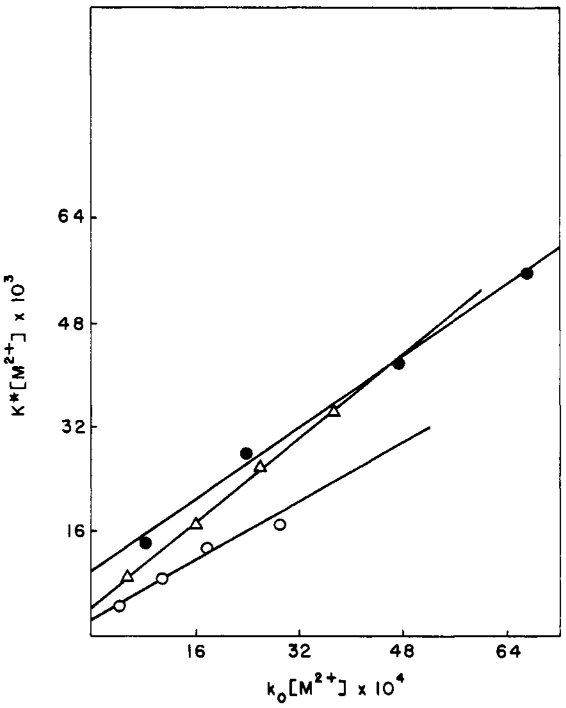

FIGURE 2: Determination of K_f. Open circles represent the magnesium catalysis at pH 6.8. Triangles represent the calcium catalysis at pH 6.8. Closed circles represent the calcium catalysis at pH 7.8. The parameters which are used in Figures 1 and 2 are presented in Table III.

= 5.7, average K_f for CaAcP = 7.7. To test eq 2 the K_f values and the observed rate constants k_0 (see Table II) for the magnesium system at pH 7.7 and the calcium system at pH 8.8 were used. Plots which constitute a test of eq 2 are shown in Figure 3. The $[AcP^{2-}]_f/[AcP^{2-}]_t$ ratio was calculated from the respective values of K_f where $[Mg^{2+}] = 0.200$ and $[Ca^{2+}] = 0.100$. Plots of the left side of eq 1 *vs.* $[OH^-]$ serve as a test of eq 1. The intercept and the slope from this plot were used to calculate k_3 and k_4 (see Figure 4). The values of k_3 and k_4 appear in Table IV.

TABLE IV: Summary of Measured and Calculated Constants at 39.0°.

Catalyst	$k_u \times$ 10^3	K_f	$k_3 \times$ 10^2	$k_4 \times$ 10^{-4}
None	4.4	—	—	—
Mg^{2+}	4.4	5.7	1.4	17
Ca^{2+}	4.4	7.7	2.8	3.7

Discussion

The use of buffer media is less versatile than the pH-Stat method. Many metal ions of interest form

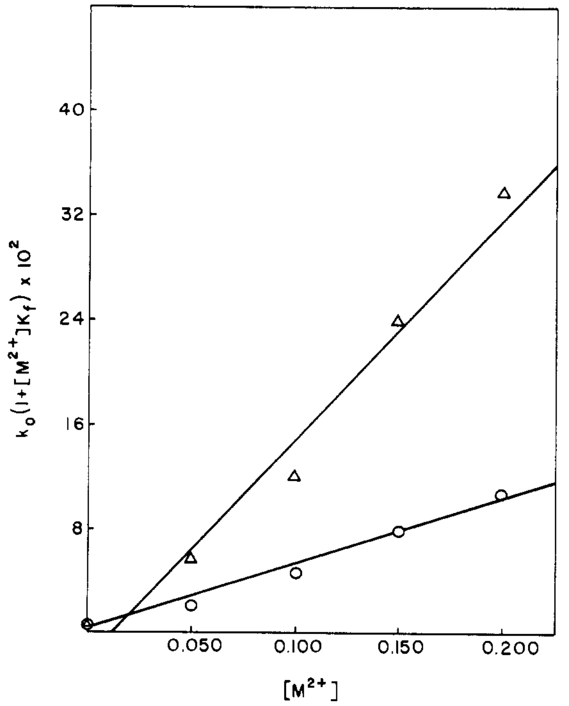

FIGURE 3: Test of eq 2. Open circles represent the data from the magnesium-catalyzed system at pH 7.7. Triangles represent the data from the calcium-catalyzed system at pH 8.8.

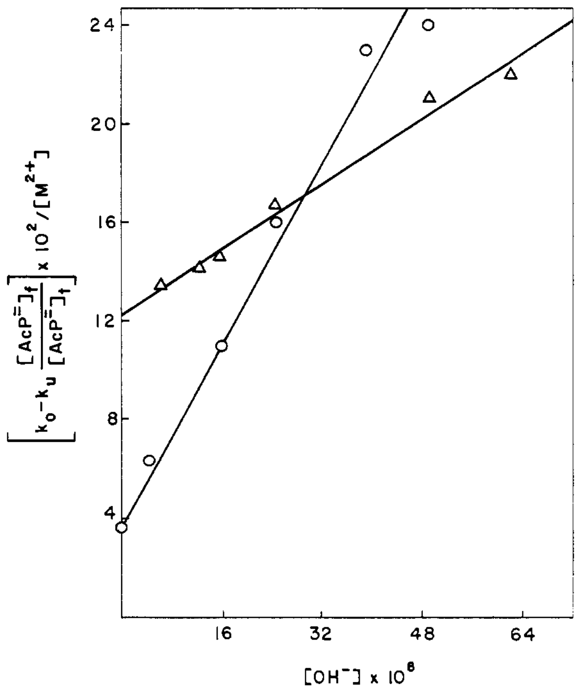

FIGURE 4: Test of eq 1. Open circles represent the magnesium-catalyzed system, $[Mg^{2+}]$ = 0.200, pH 5.8–7.7. Triangles represent the calcium-catalyzed system, $[Ca^{2+}]$ = 0.100, pH 6.8–7.8.

insoluble compounds with the common buffers, and the catalytic activity of the metal ions is reduced in the presence of buffer. The buffer Tris failed to keep the pH constant in the presence of calcium ions under the conditions of these experiments. When the pH-Stat method is used, some error is introduced since slight variations in pH undoubtedly take place. The formation of very small quantities of insoluble inorganic phosphates did not interfere with the determination of the rate constants by either method. Since the initial concentration of AcP^{2-} was small with respect to the catalyst concentration, the decrease in the latter due to precipitation was negligible.

Catalysis by divalent metal ions appears to be a fairly general property since all the divalent ions tested did catalyze the hydrolysis reaction *of the dianion.* The importance of the dianion is most convincingly demonstrated by consideration of the rate of reaction in the presence of zinc ion. At pH 2.7, where the monoanion exists, 0.05 M Zn^{2+} failed to catalyze the reaction. At pH 5.8, where the dianion is present, 0.01 M Zn^{2+} produced a reaction 12 times as fast as the uncatalyzed reaction (see Table II). The hydrolysis of the monoanion is not catalyzed by Mg^{2+} (Koshland, 1952) or by Ca^{2+} (Marcus and Elliott, 1958). Monovalent lithium ions did not catalyze the hydrolysis reaction of the dianion under the conditions used in these experiments.

The relationship between k_c and $[M^{2+}]$ is not as simple as it was assumed to be in previous work. A more accurate expression has been derived, and it was utilized in the determination of K^* which preceded the determination of K_f. An eq (5) which reveals the relationship clearly has been derived from eq 1 and the definitions which have been presented. This is

$$k_c = \left(\frac{[AcP^{2-}]_f}{[AcP^{2-}]_t} - 1\right)\frac{k_u}{[M^{2+}]} + \frac{[AcP^{2-}]_f}{[AcP^{2-}]_t}K^* \qquad (5)$$

Since the $[AcP^{2-}]_f/[AcP^{2-}]_t$ ratio is small at high metal ion concentration and since $[M^{2+}]$ appears in the denominator on the right side of the equation, k_c reaches a minimum at high metal ion concentration and a maximum at low metal ion concentration. Figure 1, which is a plot of k_c *vs.* $[M^{2+}]$, illustrates this.

It has been suggested that the role of metal ions in this reaction is to form *chelates* with the ligand, AcP^{2-}. The data presented here neither confirm nor reject the occurrence of a chelate ring in the intermediate complex. It should be noted, however, that metal ions which form coordination compounds readily are shown here to be excellent catalysts. Substantial and significant interaction of the metal ions with AcP^{2-} is highly probable.

The oxygen bond which is broken in the hydrolysis of acetyl phosphate has been a subject of great interest. The acid- and base-catalyzed reactions have been shown to take place by cleavage of the C–O bonds, while the uncatalyzed reaction and the pyridine catalyzed reaction proceed by O–P bond split (Park and Koshland, 1958). Unfortunately, no ^{18}O studies have been carried out with the metal ion catalyzed systems. Conceivably, either bond could be split by either catalytic reaction we have proposed.

The enzymatic synthesis of acetyl phosphate has been reported in a recent publication (Raijman and Grisolia, 1964). The pH optimum for the formation of acetyl phosphate in the presence of Mn^{2+} was found to be between six and seven. It can be shown from the data presented here that this is the pH range where the dianion is less susceptible to catalytic hydrolysis. It is possible that their product was formed at a higher pH but it hydrolyzed.

Another objective of this research was the determination of the energy of activation of the catalytic reaction. With the data presented in Table II it would be possible to estimate an apparent value, but this would be a composite term involving both equilibrium and rate factors. Evaluation of such a term would be tenuous. More meaningful values can be calculated when k_3 and k_4 are known at several temperatures. An experimental determination of K_f would simplify the approach to this problem. The reactivity of AcP^{2-} in the presence of metal ions complicates the direct determination of K_f, however. The magnitude of the formation constants seems reasonable when one considers that the constants for $MgHPO_4$ and $CaHPO_4$ are 76 and 50, respectively. A lower value (5–10) for a ligand in which the acetyl group has replaced a hydrogen appears to be in agreement with the ideas presented by Kosower (1962).

Di Sabato and Jencks (1961a) have also observed the calcium-catalyzed hydrolysis of AcP^{2-}, and they suggested that two catalytic reactions were involved. The present findings provide considerable support for their suggestion. Marcus and Elliot (1958) compared the catalytic activity of magnesium and calcium ions but their comparison was limited in scope. It is evident from data reported here that the relative catalytic activity is complex and it is dependent upon pH (see Table II). The mechanism and the mathematical analysis presented here reflect this complex behavior and provide the first detailed explanation for it. As can be seen in Table IV, the calcium complex is more susceptible to attack by water and thus at low pH the catalytic activity of calcium is greater. The magnesium complex is more susceptible to base attack and thus magnesium has the greater catalytic activity at high pH.

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