The Effect of Metal Ions on Labile Phosphates. III. The Role of the Metal Ion*

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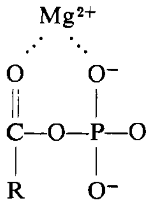
ABSTRACT: The acceleration of the hydrolysis of acetyl phenyl phosphate monoanion by magnesium and calcium ions has been found to be much more pronounced than in the case of the dianions derived from either acetyl or carbamyl phosphate. The rate of hydrolysis of acetyl phenyl phosphate is found to be strongly base dependent in the presence of metal ions. This was also found to be the case with other simple phosphate anions. Infrared and nuclear magnetic resonance (nmr) studies on D_2O solutions of acetyl phosphate and magnesium sulfate showed no characteristics which could be assigned to chelate structures involving the carbonyl group. Infrared studies on D_2O solutions of carbamyl phosphate led to the same conclusion. These results favor the view that the principal effect of the metal ion is to facilitate the attack of nucleophiles *via charge neutralization* rather than chelation, though they are consistent with the formation of a transient chelate ring in the transition state.

In the ground state these phosphate anions appear to be coordinated to the metal by oxygens of the phosphorus only.

Bivalent metal ions have been shown to greatly influence the decomposition of certain labile phosphates of biological importance. The nature of the interaction between these labile phosphate ions and metal ions has been a source of interest and speculation. Two proposals have been advanced to explain the role of the metal ion. The first emphasizes the importance of chelate formation (Koshland, 1952), *i.e.*, the formation of a cyclic intermediate by the metal ion and the labile phosphate. The second ascribes the predominant effect to charge neutralization (Kosower, 1962), *i.e.*, the reduction of the ionic charge of the phosphate anion by interaction with a metal ion of opposite charge. In this case chelate formation is not *required.*

The objective of the present investigation was to assemble evidence which could be used to support or eliminate either of the above proposals. In this way it was hoped that the role of the metal ion could be more explicitly defined. In papers I and II of this series[1] and references therein, it has been shown that magnesium ions exhibit a marked effect on the rate of decomposition of certain labile phosphate dianions,[2] AcP^{2-} and CAP^{2-}. Chelate formation would require participation of the carbonyl group as shown in structure I, but if the role of the metal ion is to neutralize the charge *only*, the carbonyl group need not participate as shown in structure II (R = CH_3, acetyl phosphate; R = NH_2, carbamyl phosphate). Some infrared and nuclear magnetic resonance (nmr) spectra have been examined in an effort to detect changes produced in these ligands by magnesium ions. Since magnesium phosphate (inorganic phosphate) is more soluble than other bivalent metal phosphates in neutral solutions, this metal ion was a convenient choice for these experiments.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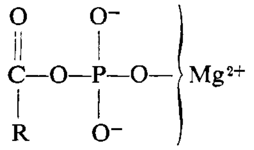
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Metal ions are known to affect these phosphate dianions but no effects with *monoanions* have been reported. Since such effects are base dependent and monovalent ions of many common labile phosphates exist only in acid solution, we sought a monoanion which would exist at higher pH. Acetyl phenyl phosphate exists as the monoanion in neutral solutions and the hydrolysis reaction was not reported to be acid or base catalyzed at pH 4–7 (DiSabato and Jencks, 1961). The effect of some metal ions on acetyl phenyl phosphate monoanion is reported herein.

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[1] See Oestreich and Jones (1966a,b) for the first two articles in this series.

[2] Abbreviations used: acetyl phosphate dianion, AcP^{2-}; carbamyl phosphate dianion, CAP^{2-}; acetyl phenyl phosphate monoanion, $\text{AcC}_6\text{H}_5\text{PO}_4^-$; acetyl phosphate monoanion, HAcP^-; bivalent metal ions, M^{2+}; observed rate constant, k_0; adenosine triphosphate, ATP; adenosine diphosphate, ADP; adenosine monophosphate, AMP.

TABLE I: Hydrolysis of Acetyl Phenyl Phosphate in Salt Solutions, Ionic Strength 0.6.[a]

	Cation (M)	Anion	pH	$k_0 \times 10^3$ (min^{-1})	Temp (°C)
Uncatalyzed reaction	0.60 Na^+	NO_3^-	6.5	0.21	39.0
		ClO_4^-	6.5	0.44	
		ClO_4^-	4.9	0.16[b]	
		NO_3^-	6.5	0.17[c]	
		ClO_4^-	6.5	0.17[c]	
		NO_3^-	6.8	0.26	
Effect of M^{2+}	0.05 Mg^{2+}	NO_3^-	6.5	1.0	39.0
	0.10 Mg^{2+}			1.3	
	0.20 Mg^{2+}			3.0	
	0.20 Ca^{2+}			1.8	
Effect of OH^-	0.20 Mg^{2+}	NO_3^-	6.2	1.8	39.0
	0.20 Mg^{2+}		6.5	3.0	
	0.20 Mg^{2+}		6.8	6.1	
Effect of temperature	0.20 Mg^{2+}	NO_3^-	6.5	0.82	28
	0.20 Mg^{2+}			12.1	50
	0.20 Ca^{2+}			5.7	50

[a] The initial $AcC_6H_5P^-$ concentration was 1×10^{-3} M. For the catalyzed reaction the ionic strength was brought up to 0.6 by the addition of a sufficient amount of sodium nitrate. For the solutions 0.2 M in Mg^{2+} or Ca^{2+} no such addition was necessary. The solutions 0.05 M in $Mg(NO_3)_2$ were also 0.45 M in $NaNO_3$ while those 0.10 M in $Mg(NO_3)_2$ were also 0.30 M in $NaNO_3$. [b] Phenyl phosphate buffer (0.05 M); DiSabato and Jencks (1961) reported a rate constant of 0.15×10^{-3} min^{-1}. [c] Disodium-EDTA was added in trace amount.

Experimental Procedures

Reagent grade inorganic salts were employed. Disodium phenyl phosphate was obtained from Nutritional Biochemicals Corp. Lithium acetyl phosphate was obtained from Worthington Biochemical Corp., and lithium carbamyl phosphate was obtained from Sigma Chemical Co. The solvent, D_2O, was obtained from Diaprep, Inc. All of these reagents were used without further purification. Sodium acetyl phenyl phosphate was prepared and purified by the method of Jencks and Carriuolo (1959). The product was dried under vacuum and its water content was reduced, but apparently water was not completely removed. (The infrared absorption at 2.9 μ was reduced but not eliminated.) *Anal.* Calcd for $NaC_8H_8O_5P$: C, 40.3; H, 3.4; P, 13.0. Found: C, 38.2; H, 3.8; P, 12.9. The discrepancies in the calculated and found values are attributed to the presence of water and the fact that some decomposition may have taken place in the drying operation. Jencks and Carriuolo (1959) experienced the same difficulties. Neither the decomposition products nor water was expected to complicate the kinetic measurements.

Kinetic Runs. Kinetics runs were made with 100-ml volumes of salt solutions which were prepared with the respective metal salts and weighed quantities of the sodium salt of the same anion. The ionic strength of the solutions was 0.6. The proper temperature and the pH of the solution were established prior to the addition of approximately 30 mg of solid acetyl phenyl phosphate (1×10^{-3} mole/l., initial concentration). The rate constants were determined from the volumes of standard base required at given times to keep the pH constant. Some rate constants were also determined simultaneously by a spectrophotometric method (Koshland, 1951). In this procedure acetyl phosphate is converted (by treatment with hydroxylamine) to acetylhydroxamic acid and this is estimated colorimetrically as its iron(III) complex. The latter method was required in the kinetic runs where phenyl phosphate buffer was employed. The pH of the unbuffered solutions was maintained constant with a pH-Stat. Fisher titrimeters and Radiometer titrators equipped with magnetic valves and microburets were utilized. Good pseudo-first-order plots were obtained for at least 2 half-lives. The initial concentration of acetyl phenyl phosphate was varied in a given run from 0.5 to 1.7×10^{-3} mole/l. and the same rate constant, within experimental error, was obtained. The errors in individual rate constants were of the order of 7%.

Spectra. The infrared spectra were recorded by a Perkin-Elmer Model 621 infrared spectrophotometer and the nmr spectra were obtained with Varian Associates A-60 nmr spectrometer. The spectra were obtained with fresh, neutral D_2O solutions of 0.2 M carbamyl phosphate and 0.4 M acetyl phosphate. CaF_2 cells with D_2O in the blank cell were used for the infrared measurements. The path length of these cells was 0.05 mm. Magnesium was added in the form of anhydrous magnesium sulfate. The magnesium:acyl phosphate ratio was slightly greater than 1:1. The position of the proton resonance in acetyl phosphate was measured relative to the proton resonance in acetone which was used as an internal standard.

Results

The Hydrolysis of Acetyl Phenyl Phosphate. The reaction which takes place is described by $CH_3COOPO_3C_6H_5^- + H_2O \rightarrow CH_3COO^- + C_6H_5OPO_3^{2-} + 2H^+$. Acid (2 equiv) was observed to be generated for each mole of $AcC_6H_5PO_4^-$ which hydrolyzed. No precipitates of inorganic phosphate ($M_3(PO_4)_2$) were detected. The pK_a value of acetyl phenyl phosphoric acid was estimated to be 2.

The observed rate constants for the hydrolysis of acetyl phenyl phosphate are given in Table I. The data are arranged to illustrate the significance of the uncatalyzed reaction, and the substantial dependence of the rate on the presence of magnesium or calcium ions, metal ion concentration, pH, and temperature. The same rates were obtained for both nitrate and chloride salts but the rates with perchlorates present were slightly greater.

Spectra. The infrared absorption bands of AcP^{2-} and CAP^{2-} in the presence and absence of magnesium ions are listed in Table II. The nmr spectrum of AcP^{2-} in D_2O was unchanged by the addition of anhydrous magnesium sulfate.

Discussion

The kinetic data listed in Table I for the acetyl phenyl phosphate system show the same general rate features which were exhibited by AcP^{2-} and CAP^{2-} under similar conditions. The limited pH range which could be exploited with this compound by this method was unfortunate. The large metal dependence and the sensitivity of this ligand to impurities as indicated by the reduction in rate upon the addition of a trace of EDTA as well as possible anion effects complicate this system. Even though only a qualitative evaluation of these limited data is possible it is significant because the large metal dependence is apparently due to the effective neutralization of the charge of the monoanion by the bivalent metal ions. At pH 6.8 and at the same temperature the catalytic effect of magnesium ions on $AcC_6H_5PO_4^-$ is ten times larger than the effect on AcP^{2-}. (The catalytic rate with 0.2 M Mg^{2+} is 30 times the uncatalyzed rate in the former case and three times in the latter case.)

TABLE II: Infrared Absorption Bands[a] of Acetyl Phosphate and Carbamyl Phosphate (1200–1800 cm^{-1}) D_2O Solution.

AcP^{2-} [b]		CAP^{2-} [c]	
Mg^{2+} Absent	Mg^{2+} Present	Mg^{2+} Absent	Mg^{2+} Present
1275 s	1275 s		
1377 s	1375 m		
1420 w	1430 sh	1405 s	1407 sh
1548 w	1548 m		1429 s
1708 s	1711 s	1666 s	1670 s

[a] Abbreviations used: shoulder, sh; strong, s; medium, m; weak, w. [b] The concentration of AcP^{2-} (as dilithium salt) is 0.4 M; the concentration of $MgSO_4$ is 0.42 M. [c] The concentration of CAP^{2-} (as dilithium salt) is 0.2 M and that of $MgSO_4$ is 0.21 M.

Metal ions are known to catalyze the hydrolyses of several types of esters bearing a negative charge including those of carboxylic (Bender, 1960) and phosphoric acids (Bender and Breslow, 1962; Cox and Ramsey, 1964). Ions where the charge varies from 1− to 4− have now been shown to be susceptible to metal effects. Polyphosphates, acyl phosphates, and substituted acyl phosphates, such as $AcC_6H_5PO_4^-$ and fluoroacetyl phosphate (Marcus and Elliot, 1958), are readily hydrolyzed in the presence of metal ions. The synthesis of 2,4-dinitrophenyl phosphate has been reported recently (Kirby and Varvoglis, 1966). This phosphate, which cannot form any reasonable chelate, is also strongly affected by metal ions. At 39° in Tris buffer solution (pH 8) the reaction rate is approximately doubled by 0.01 M Ca^{2+} or 0.01 M Mg^{2+} ions.[3] Chelate formation is, therefore, not a requirement for catalytic activity.

The coordination of metal ions with carbonyl groups produces large decreases in the infrared stretching frequencies of the C=O functional group. The spectra of salicylaldehyde and tropolone derivatives serve as good examples of these spectral changes (Cotton, 1960). Even when the carbonyl group participates in adduct formation such as in the complex $CH_3CH_2COCl \cdot SbCl_5$ (antimony is bonded to the oxygen) significant changes in the infrared spectra result (Olah *et al.*, 1962). The carbonyl group in AcP^{2-} and CAP^{2-} absorbs at 1708 and 1666 cm^{-1}, respectively. As shown in Table II, these frequencies are virtually unaltered by the presence of magnesium ions. The very slight increases which we have observed here are in sharp contrast with the large decreases mentioned above. Alexander and Busch (1966) have examined the changes in the infrared spectrum of D_2O solutions of *cis*-$[Co(en)_2(NH_2CH_2CO_2R)Cl]^{2+}$ during the course of the Hg^{2+}-catalyzed hydrolysis of the coordinated ester. They found that such changes involve the carbonyl stretching modes and that the change in the absorption at 1610 cm^{-1} can best be explained in terms of a chelate intermediate involving a $Co \cdots O{=}C$ linkage which is formed

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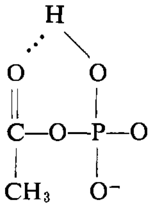
[3] Private communication from A. J. Kirby, University Chemical Laboratory, Cambridge, England.

during the first stages of the reaction and subsequently disappears as the hydrolysis proceeds. This sort of effect was sought in the present case, but without success.

We note that the carbonyl frequency of AcC$_6$H$_5$PO$_4^-$ and HAcP$^-$ is found near 1740 cm^{-1} but in the unsubstituted acyl phosphate (AcP^{2-}) it occurs at 1708 cm^{-1} (Jencks *et al.*, 1960). The carbonyl group of HAcP$^-$ could participate in intramolecular hydrogen bonding as shown in structure III; but if this were the case one would expect the frequency to be *lower* than in AcP^{2-}. This lowering of the frequency is well known; for example, the frequencies of the carbonyl group in acetophenone, *p*-hydroxyacetophenone, and *o*-hydroxyacetophenone are 1685, 1684, and 1640 cm^{-1} (Cullinane *et al.*, 1961). The large decrease in the frequency of the *ortho* derivative is due to intramolecular hydrogen bonding.



III

It should be noted that all of the spectra of the acyl phosphates discussed above were obtained with D$_2$O solutions, and where magnesium was present, all of the ligand would not be expected to be in the complexed form since the formation constants are approximately ten for these complexes. It is clear, however, that these data provide no support for the chelate intermediate. This was also found to be the case with the AcP^{2-} nmr spectra, but examples of simple magnetic resonance spectra for coordinated and uncoordinated ligands are not available in the literature, so the evaluation of this information is tenuous.

An infrared study of ATP in the presence of Mg^{2+} (Feldman and Keil, 1965) has indicated magnesium does not interact with the adenine moiety and the same conclusion has been reached in an ^{15}N nmr study (Happe and Morales, 1966). The magnesium–acyl phosphate system may be similar to the magnesium–ATP system in this respect.

The work of Conley and Martin (1965a,b) provides an additional test for chelation *via* their demonstration that *chelation* to a divalent metal atom makes a species far more susceptible to hydroxide ion attack (and presumably also attack by water) than does protonation. Thus a comparison of the rates of hydrolysis of AcPH$_2$ and AcPMg should allow chelation in AcPMg to be detected. For AcPMg, the second-order rate constant for attack by water is 1.4×10^{-2} l. mole^{-1} min^{-1}. Previously (Koshland, 1952) the value for what is presumably the corresponding process in AcPH$_2$ has been given as 8×10^{-3} l. mole^{-1} min^{-1}. The Mg^{2+} is thus more rapid by a factor of only 1.8, while in the case of the reaction of the copper(II)-catalyzed hydrolysis of glycine ethyl ester, the metal-catalyzed reaction has a greater rate constant by a factor of 3200! This again indicates that coordination to phosphate, but not necessarily chelation with the acetyl group, is involved in the magnesium-catalyzed hydrolysis of acetyl phosphate.

The relative magnitudes of the apparent formation constants for the metal complexes of phosphate ligands as shown in Table III, merit consideration. (Some

TABLE III: Apparent Formation Constants of Metal Phosphates.

Bivalent Metal	Ligand	Log K_f	Reference
Mg	Acetyl phosphate dianion	0.7	Oestreich and Jones (1966a)
Mg	Carbamyl phosphate dianion	1	Oestreich and Jones (1966b)
Mg	Creatine phosphate trianion	1.3	Smith and Alberty (1956)
Mn	Creatine phosphate trianion	2	Smith and Alberty (1956)
Ni	Methyl phosphate dianion	1.6	Brintzinger and Hammes (1966)
Mg	$P_2O_7^{4-}$	5.4	Lambert and Watters (1957)
Mg	$MgP_2O_7^{2-}$	2.3	Lambert and Watters (1957)
Mg	HPO_4^{2-}	1.9	Smith and Alberty (1956)
Mg	AMP dianion	1.7	Smith and Alberty (1956)
Mg	ADP dianion	1.4	Smith and Alberty (1956)
Mg	ADP trianion	3	Smith and Alberty (1956)
Mg	ATP trianion	1.5	Smith and Alberty (1956)
Mg	ATP tetraanion	3.5	Smith and Alberty (1956)

caution in the comparison must be exercised, however, since the numbers were obtained by a variety of methods under different conditions; diverse systems were examined.) These constants are generally small, particularly when the organic moiety is simple. As might be expected, Mn^{2+} and Ni^{2+} form slightly stronger complexes than Mg^{2+} does. The transition metal ions such as Mn^{2+} and Ni^{2+} were shown to be very effective catalysts in the hydrolysis of acetyl phosphate dianion (Oestreich and Jones, 1966a). This may be due to the increased strength of the complex, different coordination sites, and/or other chemical properties of these metal ions. It is also noted that where log K_f is 3 or greater the metal can form a six-membered ring with two *anionic* oxygens. This is not possible with acyl phosphates because the oxygen of the carbonyl group is electrically neutral.

From a consideration of a model of pyrophosphate tetraanion, Josse (1966), has shown this ion would be expected to form a six-membered chelate ring with *one* magnesium ion, but coordination of a second magnesium ion in the same manner is not possible. This is in agreement with the relative values of the stepwise formation constants of $MgP_2O_7^{2-}$ and $Mg_2P_2O_7$ (see Table III). Josse further suggests that neither magnesium is chelated in the latter case, but this merits careful consideration in view of large over-all formation constant (Lambert and Watters, 1957). Further studies of the effects of metal ions on substituted phenyl and benzoyl phosphates may aid in the understanding of the general pattern of phosphate hydrolysis.

Summary

While the evidence presented above does not constitute proof that the role of the metal ion is to neutralize the charge and thus favor nucleophilic attack, this does appear to be a more satisfactory explanation than chelate formation. We are aware of no experimental evidence that supports the chelate mechanism for the magnesium-catalyzed systems with acyl phosphates.

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