The Effect of Metal Ions on Labile Phosphates. II. The Elimination Reaction of Carbamyl Phosphate Dianion[*]

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ABSTRACT: The rate of decomposition of carbamyl phosphate dianion to cyanate and phosphate in water is affected by the presence of the alkaline earth ions, Mg^{2+}, Ca^{2+}, and Ba^{2+}. The rate of this reaction (known as the "elimination" reaction) is dependent upon the specific metal ions present, metal ion concentration, temperature, and pH. The rate is increased by calcium ions in the pH range 6.8–8.6, but the rate is decreased in neutral solutions of magnesium ion. The reaction in the presence of metal ions is catalyzed by base in all cases. Formation constants for the reaction of carbamyl phosphate dianion with Ca and Mg ions have been estimated to be 5.2 and 9.1, respectively. The mechanism postulated for the metal ion catalyzed hydrolysis of acetyl phosphate dianion is used in quantitatively evaluating metal ion and base dependence.

The broad objective of this research is to detect changes in labile phosphates which are brought about by metal ions and, if possible, to determine their role in pertinent reactions. The present study is concerned with the kinetics of elimination by carbamyl phosphate. The temperature dependence, base dependence, and the relation of the rate to metal ion concentration have been determined.

The reactions of carbamyl phosphate in aqueous solution have been the subject of two recent, extensive investigations (Halmann *et al.*, 1962; Allen and Jones, 1964). Carbamyl phosphate dianion,[1] CAP^{2-}, was shown to undergo an elimination reaction in solutions of intermediate pH. Since the reactant resembles acetyl phosphate dianion, AcP^{2-}, metal ions would be expected to alter the rate of elimination, but no report of this was found in the literature. Reported herein are effects which are similar to those reported for the AcP^{2-} reaction in the first paper of this series (Oestreich and Jones, 1966).

The following are the principal reactions examined in this investigation.

$$NH_2COOPO_3{}^{2-} \longrightarrow NCO^- + HPO_4{}^{2-} + H^+$$

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

Experimental Procedures

Reagent grade metal perchlorates were employed. The dilithium salt of carbamyl phosphate (Sigma Chemical Co.) was used without further purification. A Fisher titrimeter and a radiometer Model TTT1c served as pH-Stats. The pH values were checked with a Beckman Model G pH meter.

Kinetic Runs. Kinetic runs were made with 100-ml volumes of unbuffered salt solutions which were prepared with weighed quantities of the respective metal perchlorates and enough sodium perchlorate to give an ionic strength of 0.6. The proper temperature and the pH of the solution was established prior to the addition of approximately 40 mg of cold, solid, lithium carbamyl phosphate (3×10^{-3} mole/l.). The rate constants were determined from the volumes of standard sodium hydroxide solution required at given times to keep the pH constant. The reactions were followed essentially to completion and good pseudo-first-order rate plots were obtained for 3 half-lives. The same analytical method was utilized by Halmann *et al.* (1962) and in an acetyl phosphate study (Oestreich and Jones, 1966). No complications due to the formation of small quantities of inorganic phosphates have been observed in either laboratory. In a specific set of runs the initial carbamyl phosphate concentration was varied from 1 to 3×10^{-3} and the same rate constant (within experimental error) was obtained.

Results

The Uncatalyzed Reaction. The first-order rate constants for the elimination reaction of CAP^{2-} and the thermodynamic quantities which may be calculated from these data appear in Table I. A graphical representation of these rate data also appears in Figure 1. Similar data appear in the literature (Hal-

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

TABLE I: The Uncatalyzed Elimination Reaction of CAP^{2-}, Ionic Strength 0.6.

Unbuffered Solutions					
pH	Temp, °C	$k_u \times 10^3$ Min^{-1}	E_a (kcal/mole)	ΔH^* (kcal/mole)	ΔS^* (eu)
6.8	26.4	3.9			
7.2	26.4	3.7			
7.8	26.4	3.5			
6.8	37.0	16.8			
7.2	37.0	15.9			
7.8	37.0	15.2	25.2[a]	24.6[a]	2.3[a]
8.2	37.0	16.2			
8.6	37.0	19.5			
9.0	37.0	26.6			

[a] The values of K_f, k_3, and k_4 are not considered to be pH dependent. The K_f value, 5.2, is the mean value and it was used to calculate k_3 and k_4.

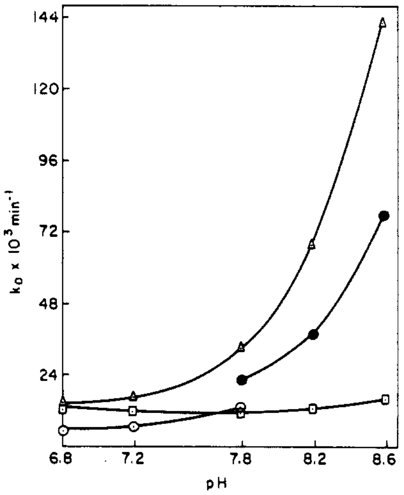

FIGURE 1: Observed rate constants as a function of pH, ionic strength 0.6 at 37.0°. Squares represent the uncatalyzed reaction. Open circles represent the magnesium-catalyzed reaction, $[Mg^{2+}] = 0.200$. Triangles represent the calcium-catalyzed reaction, $[Ca^{2+}] = 0.200$. Closed circles represent the calcium-catalyzed reaction, $[Ca^{2+}] = 0.050$.

mann *et al.*, 1962; Allen and Jones, 1964). Good agreement with the work of the latter is significant because quite different experimental procedures were employed. The rate of the uncatalyzed reaction is seen to be influenced to only a slight extent by hydroxide ion concentration in *this* pH range. The dianions, CAP^{2-} and AcP^{2-}, exhibit the same general characteristics in this pH range even though the rate of reaction of CAP is four times as great as the AcP^{2-} rate. No anions, except molybdate, have been shown to catalyze either reaction. The rate of elimination is not altered

TABLE II: Rate Constants, Catalyzed Elimination Reaction of CAP, Ionic Strength 0.6, and Unbuffered Solution.

pH	Catalyst (M)	Temp, °C	$k_0 \times 10^3$ Min^{-1}
7.8	0.200 Li^+	37.0	14.8[a]
6.8	0.100 Mg^{2+}	26.4	2.7
7.8	0.100 Mg^{2+}	26.4	4.4
6.8	0.100 Ca^{2+}	26.4	4.1
7.8	0.100 Ca^{2+}	26.4	8.0
6.8	0.015 Mg^{2+}	37.0	14.6
	0.025		13.9
	0.050		11.5
	0.100		8.0
	0.150		6.9
	0.200		7.2
7.2	0.050 Mg^{2+}	37.0	9.3
	0.100		8.7
	0.150		8.1
	0.200		8.5
7.8	0.050 Mg^{2+}	37.0	12.8
	0.100		13.8
	0.150		14.8
	0.200		16.8
6.8	0.050 Ca^{2+}	37.0	17.2
	0.100		17.3
	0.150		16.6
	0.200		17.4
7.2	0.050 Ca^{2+}	37.0	17.6
	0.100		16.9
	0.150		17.3
	0.200		20.3
7.8	0.050 Ca^{2+}	37.0	22.4
	0.100		29.5
	0.150		31.5
	0.200		32.4
8.2	0.050 Ca^{2+}	37.0	36.8
	0.100		49.4
	0.150		61.2
	0.200		66.7
8.6	0.050 Ca^{2+}	37.0	76.2
	0.100		92.5
	0.150		108.
	0.200		140.
7.8	0.200 Ba^{2+}	37.0	23.0
8.2	0.200 Ba^{2+}	37.0	34.3
8.6	0.200 Ba^{2+}	37.0	67.1

[a] Calculated from the relations: $\Delta H^* = E_a - RT$; $\Delta S^* = (\Delta H^* - \Delta F^*)/T$; $\Delta F^* = -RT \ln kh/k_B T$, where E_a is the Arrhenius energy of activation, h is the Planck constant, and k_B is the Boltzmann constant.

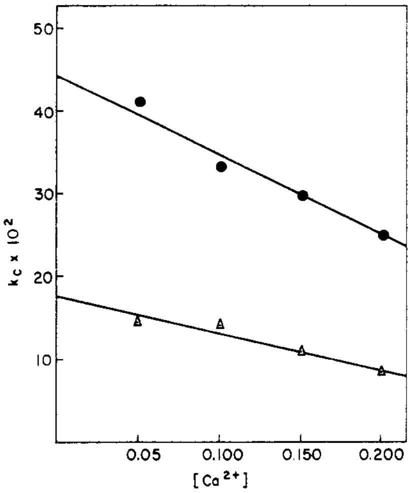

FIGURE 2: Determination of K^*. Triangles represent the k_c values of the calcium-catalyzed system at pH 7.8. Closed circles represent the k_c values of the calcium-catalyzed system at pH 8.2.

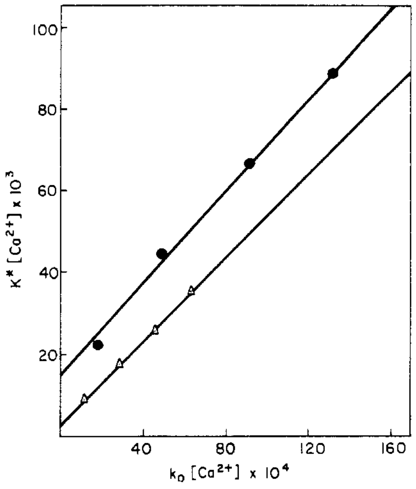

FIGURE 3: Determination of K_f. Triangles represent calcium catalysis at pH 7.8. Closed circles represent the calcium catalysis at pH 8.2. See eq 4.

significantly by the presence of lithium ions (see Table II). This was also found to be the case with AcP^{2-} (Oestreich and Jones, 1966).

The Catalyzed Reaction. The reaction in the presence of metal ions other than sodium is considered to be the catalyzed reaction. The effects of several ions (Li^+, Mg^{2+}, Ca^{2+}, and Ba^{2+}) are presented. The pronounced effect of base when bivalent ions are present and the retardation of the reaction by magnesium at low pH are the outstanding features. The rate data are tabulated in Table II. Some of the rate data from Table II are also presented in Figure 1. A general appreciation of the base dependence, metal dependence, and the relationships between the catalytic and noncatalytic systems can be obtained from Figure 1.

The reaction of CAP^{2-} with Ca^{2+} resembles the reaction of AcP^{2-} with Ca^{2+} and Mg^{2+} (Oestreich and Jones, 1966). A mechanism for the latter was proposed and a mathematical analysis of the data was carried out. A summary of this method and the equations which were employed follows. The objective in this case is the determination of the formation constant and metal dependence, and a quantitative evaluation of the role of the nucleophile, OH^-, for the calcium-catalyzed carbamyl phosphate elimination reaction.

Definitions. RP = reaction products; ML = intermediate complex; $[L^{2-}]_f$ = free ligand concentration (CAP^{2-} in this paper); $[L^{2-}]_t$ = total ligand concentration = $[L^{2-}]_f + [ML]$; K_f = formation constant = $[ML]/[M^{2+}][L^{2-}]_f$; k_u = uncatalyzed rate constant; k_o = observed rate constant; K^* is defined by eq 3; the term k_c is a second-order rate "constant" calculated with the aid of eq 5; $[M^{2+}]$ = free metal ion concentration; $[M^{2+}] > [ML]$ in the systems examined.

Proposed Mechanism.

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

$$M^{2+} + L^{2-} \rightleftharpoons ML$$

$$H_2O + ML \xrightarrow{k_3} RP$$

$$OH^- + ML \xrightarrow{k_4} RP$$

Rate Law.

$$\text{observed rate} = k_o[L^{2-}]_t$$

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

Base Dependence Equation.[2]

$$\frac{k_o - k_u\frac{[L^{2-}]_f}{[L^{2-}]_t}}{[M^{2+}]} = \frac{[L^{2-}]_f}{[L^{2-}]_t}[k_3K_f + k_4K_f[OH^-]] \qquad (1)$$

Metal Dependence Equations.[2]

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

$$K^* = k_3K_f + k_4K_f[OH^-] \qquad (3)$$

Equation 2 may be rewritten to give eq 4.

$$K^*[M^{2+}] = K_fk_o[M^{2+}] + k_o - k_u \qquad (4)$$

Equation 5 has been used at high pH (Koshland, 1952) to describe metal dependence. The values of k_c are

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[2] Equations 1 and 2 were derived from the rate law and the definitions.

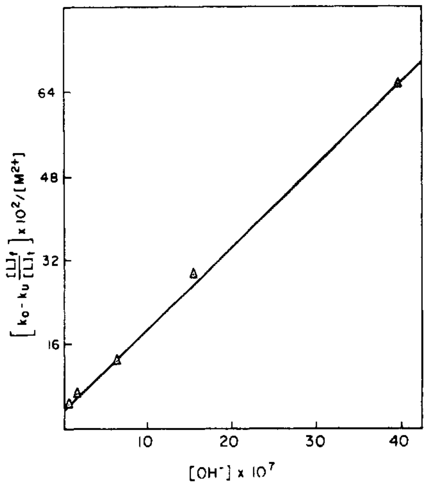


FIGURE 4: Graphical test of base dependence. See eq 1. Triangles represent the calcium-catalyzed system, $[Ca^{2+}] = 0.200$.

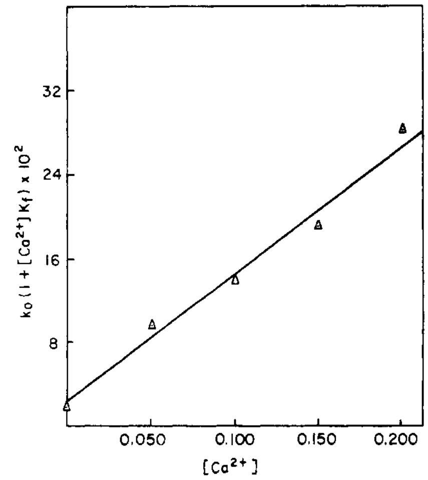


FIGURE 5: Graphical test of metal dependence. See eq 2. Triangles represent the calcium-catalyzed system at pH 8.6.

not constant at all pH values but they can be used in the evaluation K^*

$$k_o = k_u + k_c[M^{2+}] \qquad (5)$$

Mathematical Methods. To calculate k_3, k_4, and K_f it was necessary to first determine k_c and K^* values. The values of k_c were calculated with eq 5 and they were plotted against $[M^{2+}]$ (see Figure 2). The intercepts were used as K^* values (pH dependent). A mean value of the formation constant was obtained from the slopes of the lines in Figure 3. Equation 4 was the basis for this determination. The values of k_3 and k_4 were determined from the intercept and the slope of the line in Figure 4. Equation 1 was the basis of this determination. Figure 4 also serves as a test of eq 1. Figure 5 serves as a test of eq 2. Table III contains some parameters which were used in this sequence of calculations.

TABLE III: Values of the Parameters for the Determination of K^* and K_f at 37.0° for Calcium Carbamyl Phosphate.

pH	$[Ca^{2+}]$	$k_0 \times 10^3$ Min^{-1}	$k_c \times 10^2$	$K^* \times [Ca^{2+}] \times 10^3$	$k_0 \times [Ca^{2+}] \times 10^4$
7.8	0.000	15.2			
7.8	0.050	22.4	14.4	8.75	11.2
7.8	0.100	29.5	14.3	17.5	29.5
7.8	0.150	31.5	10.9	26.3	47.3
7.8	0.200	32.4	8.6	35.0	64.8
8.2	0.000	16.2			
8.2	0.050	36.8	41.2	22.1	18.4
8.2	0.100	49.4	33.2	44.2	49.4
8.2	0.150	61.2	30.0	66.3	91.9
8.2	0.200	66.7	25.3	88.4	133.

The present study is essentially confined to the pH range 6.8–8.6. Carbamyl phosphate undergoes another reaction and is also converted to the monoanion in more acidic solutions; the uncatalyzed reaction is strongly pH dependent in more alkaline solutions (Allen and Jones, 1964). Formation of hydroxy complexes limits the magnesium study even more and reactions with hydroxide ions preclude the use of other catalysts such as the zinc ion.

The formation constant for the magnesium carbamyl phosphate complex cannot be determined by the method described above because meaningful K^* values cannot be calculated (*i.e.*, useful connection between eq 2 and 5 is possible only when $[M^{2+}]$ is small and K^* is large). A plot (see Figure 6) of the observed rate constant, k_o, *vs.* $[M^{2+}]$, at pH 6.8 suggested another method, however. Equation 6 was obtained from the definitions and eq 7 was obtained from eq 1.

$$\frac{[L^{2-}]_t}{[L^{2-}]_f} = 1 + K_f[M^{2+}] \qquad (6)$$

$$k_o = \frac{[L^{2-}]_f}{[L^{2-}]_t}(k_u + k_3K_f[M^{2+}] + k_4K_f[M^{2+}][OH^-]) \qquad (7)$$

If the last two terms of eq 7 are negligible when $[M^{2+}]$ is small, eq 8 would be a useful approximation.

$$\frac{k_u}{k_o} = 1 + K_f[M^{2+}] \qquad (8)$$

A plot of the left side of eq 8 *vs.* magnesium ion con-

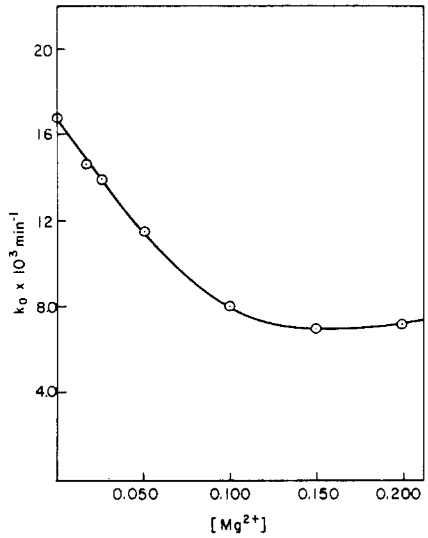


FIGURE 6: The stabilization of carbamyl phosphate dianion by magnesium ions. Open circles represent observed rate constants at pH 6.8 as a function of magnesium ion concentration.

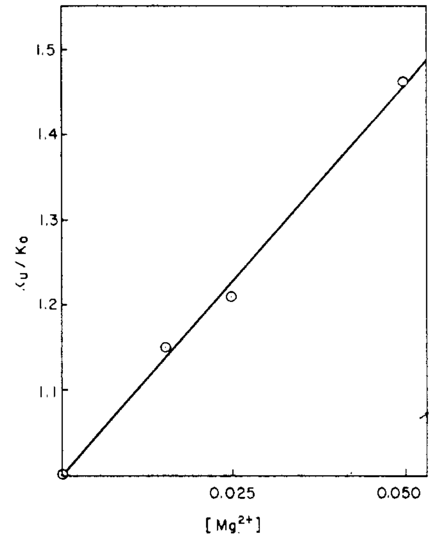


FIGURE 7: Determination of K_f for the reaction of magnesium with CAP^{2-}. The slope of the line is K_f. See eq 8.

centration is linear as shown in Figure 7. The K_f value determined from the slope of the line is 9.1. It was not possible to calculate values of k_3 and k_4 for the magnesium system, since data for more basic solutions could not be obtained. Table IV is a summary of the important constants calculated from these data.

TABLE IV: Summary of Calculated Constants,[a] Carbamyl Phosphate Solutions at 37.0°.

Metal Ion	pH	K^* $\times 10^2$	K_f	k_3 $\times 10^2$	k_4 $\times 10^{-4}$
Ca^{2+}	7.8	17.5	4.9		
Ca^{2+}	8.2	44.2	5.5		
Ca^{2+}	8.6		5.2	1.3	6.1
Mg^{2+}	6.8		9.1		

[a] This is essentially the rate of the uncatalyzed reaction.

Discussion

All of the divalent cations which were tested influenced the rate of decomposition of carbamyl phosphate dianion. Calcium ions are the most effective but the influence of magnesium and barium ions is also substantial.

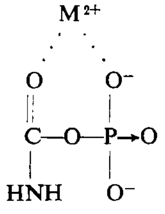
The first explanation of the effect of metal ions on acyl phosphates was given by Koshland (1952). The quantitative effects were described with eq 5. The present treatment is an expansion of his explanation. Three features which have been added are obvious when eq 7 is compared with eq 5: (1) retardation of the reaction due to a decrease in the free ligand concentration; this is reflected in the free ligand to total ligand ratio; (2) resolution of the metal dependence term; (3) incorporation of a base dependence term. All of the experimental data collected to date are consistent with this mechanism and explanation.

The unusual effect of magnesium ions does not require (or exclude) another mechanism. This retardation by magnesium ions at lower pH values appears to be due to the decrease in the free ligand concentration. In the presence of barium and calcium ions retardation was also probable, but the attack by water and/or base produced *net* catalysis. One mathematical description applies to the calcium and magnesium systems in the presence of both ligands CAP^{2-} and AcP^{2-}. The pH profiles for the calcium and magnesium complexes are quite similar as shown in Figure 1. These considerations support the idea of a common mechanism. The effect of water and base on MgCAP is simply less than in the other three cases. Magnesium is known to form complexes more readily than barium or calcium, and it is also possible that a different type complex was formed with magnesium.

In the mechanism which has been proposed, water is included in the equation: $H_2O + ML \rightarrow RP$. It is also possible that simple decomposition of the metal complex without participation of the solvent could take place. The data presented here do not exclude either possibility.

The activation parameters, E_a, ΔH^*, ΔS^*, were determined for the uncatalyzed reactions of AcP^{2-} (Oestreich and Jones, 1966) and CAP^{2-} in the same medium and by the same method. The magnitudes of these quantities are very similar. Since the decomposition of CAP^{2-} does not require water, the similarity of these values supports the monomeric metaphosphate mechanism which has been proposed for AcP^{2-} (Di Sabato and Jencks, 1961).

The structure of this metal complex, ML, has been a subject of speculation (Kosower, 1962). For the ligand CAP^{2-}, three structures merit consideration.



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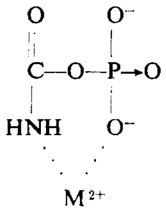

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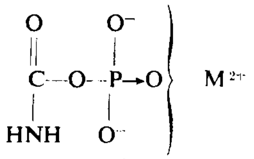

III

Structure II seems less probable in view of the very similar reaction of MAcP where this structure is not possible. Experiments which may provide information relative to the true structure are in progress.

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