$(PA)_2FeOFe(PA)_2$ (1) (eq 2 and 4, and Scheme Ia).

Scheme Ib outlines a proposed concerted, singlet-biradical mechanism for the selective ketonization of methylenic carbons and dioxygenation of acetylenes via a common reactive intermediate, species 3, which evolves $^1O_2$ in substrate-free DMF (Table IV). The ketonization of a methylene group in cyclohexene and 1,4-cyclohexadiene (Table II) is especially compelling evidence for a concerted selective process that is optimal for the geometry of methylenic carbons. In contrast, the dehydrogenation of 1,3-cyclohexadiene (Table II) probably is the result of selective reactivity with the precatalyst, species 2 (Scheme Ia).

The results for the $Fe(PA)_2$- and $(py)_4FeCl_2$-induced activation of $KO_2(s)$ to transform cyclohexane to cyclohexanone (Tables V) are closely similar to those for heterogeneous iron–dioxygen systems[1-6] and for the $Fe(PA)_2$/HOOH system in py/HOAc (Table I). The optimal efficiencies for the $KO_2(s)$ systems are achieved when the iron catalyst is in high concentration and the superoxide source is in suspension, which is consistent with a heterogeneous process. The irreversible two-electron reduction of dioxygen to hydrogen peroxide in py/HOAc (2:1 molar ratio)[18] confirms that free superoxide ion is not formed, which is incompatible with the protic matrix (the proton-induced disproportionation of superoxide ion to hydrogen peroxide and dioxygen is essentially a diffusion-controlled process).[22]

The reaction efficiencies and product profiles for the various organic substrates in combination with $KO_2(s)$ (Table V) or electro-reduced O_2 (Table VI) in 2:1 py/HOAc are almost identical with those for the $Fe(PA)_2$-induced activation of HOOH (Tables I and II). This and the instability of $O_2^{\bullet-}$ in py/HOAc matrices prompts the conclusion that the reactive intermediates are the same because of the in situ generation of HOOH (eq 8-11). Thus, species 3 transforms methylenic carbons directly

$$2Fe(PA)_2 + 2KO_2(s) + 2HOAc \longrightarrow \underset{\mathbf{1b}}{(PA)_2FeOFe(PA)_2} + 2KOAc(s) + O_2 \quad (8)$$

$$\mathbf{1b} + 4KO_2(s) + 4HOAc \longrightarrow \left|\mathbf{1b}(HOOH)_2\right| + 4KOAc(s) + 2O_2 \quad (9)$$

$$\left|\mathbf{1b}(HOOH)_2\right| \xrightarrow{[S]} \mathbf{3} + 2H_2O$$

$$2Fe(PA)_2 + O_2 + 2HOAc + 2e^- \longrightarrow \underset{\mathbf{1b}}{(PA)_2FeOFe(PA)} + H_2O + 2^-OAc \quad (10)$$

$$\mathbf{1b} + 2O_2 + 4e^- + 4HOAc \longrightarrow \mathbf{1b}(HOOH)_2 + 4^-OAc \quad (11)$$

$$\mathbf{1b}(HOOH)_2 \xrightarrow{[S]} \mathbf{3} + 2H_2O$$

to ketones, and acetylenes and arylolefins are dioxygenated to α-dicarbonyls and aldehydes, respectively.

The selective transformation of methylenic groups ($>CH_2$) to ketones via four heterogeneous iron–dioxygen systems[1-5] (a) iron powder/O_2 in 4:1 py/HOAc, (b) $Fe_3O(OAc)_6 \cdot 3.5py$/zinc dust/O_2 in 4:1 py/HOAc, (c) $(py)_4FeCl_2/KO_2(s)$ in 4:1 py/HOAc, and (d) $(py)_4FeCl_2/(O_2 + e^- \rightarrow O_2^{\bullet-})$ in 4:1 py/HOAc parallels the present results. This prompts the conclusion that for each of these systems the reactive intermediate is a binuclear dioxygen complex (3 or its equivalent) that results from two HOOH molecules per iron catalyst (1 or its equivalent).

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The Effects of Mg^{2+}, Hydrogen Bonding, and Steric Factors on Rate and Equilibrium Constants for Phosphoryl Transfer between Carboxylate Ions and Pyridines[1]

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Abstract: The reaction of bicarbonate ion with phosphorylated γ-picoline monoanion (PicP), which presumably gives carboxyphosphate, is faster than the reactions of acetate and carbonate by factors of 40 and 6, respectively (25 °C, I = 1.5). The rate increase is attributed to hydrogen bonding of the bicarbonate hydroxyl group to a phosphoryl oxygen atom; phosphate monoanion and dianion show similar increases. The reaction of acetate ion with PicP and the reverse reaction of γ-picoline with acetyl phosphate dianion are catalyzed ~20-fold by Mg^{2+}. The reaction of PicP with formate ion is 20-fold faster than with acetate ion and shows no catalysis by Mg^{2+}. This indicates that the rate increases with bicarbonate and with Mg^{2+} arise from overcoming unfavorable electrostatic, solvation, or steric interactions. Catalysis of the reaction of γ-picoline with acetyl phosphate (AcP), in both directions, is described by chelation of Mg^{2+} to the transition state, with $K_a^* = 97\ M^{-1}$, and binding to the ground states with $K_a = 5$ and 4.4 M^{-1} for PicP and AcP, respectively. Equilibrium constants are reported for phosphoryl transfer between pyridines, imidazole, ammonia, and acetate ion; the favorable transfer from pyridine to acetate, by ~100-fold, shows that the P–O bond is stronger than the P–N bond, relative to H–O and H–N bonds.

In this paper we analyze rate constants for nonenzymatic phosphoryl transfer from pyridines to carboxylate ions and related nucleophiles (eq 1) in order to identify factors that stabilize and destablize the transition state.

Carboxylate and nucleotide anions act as nucleophiles in a number of enzyme-catalyzed phosphoryl transfer reactions, many of which require Mg^{2+}. For example, aspartyl residues of the Ca^{2+} and Na^+/K^+ ATPases are phosphorylated in the transport cycles, and acetate and 3-phosphoglycerate ions are phosphorylated by

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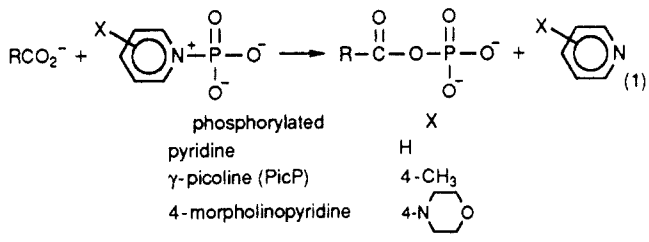


ATP in kinase reactions;[2] ADP is phosphorylated in the reverse reactions. Understanding the factors that cause rate enhancements and inhibition in the nonenzymatic reactions is important for understanding catalysis by enzymes. It is shown here that some obvious conclusions that might be reached concerning catalysis and effects of structure in these reactions are incomplete or misleading. For example, phosphoryl transfer is 40-fold faster to bicarbonate than to acetate ion because of intramolecular solvation through a hydrogen bond, but this rate increase is observed only because there are unfavorable interactions in the transition state of the reaction with acetate ion. It is concluded that it is important to evaluate unfavorable as well as favorable factors in order to characterize catalytic rate enhancements in enzymes and model reactions. Catalysis of the reaction by Mg^{2+} ion in both directions has been characterized and equilibrium constants for phosphoryl transfer between oxygen and nitrogen bases have been determined. The Mg^{2+} ion catalyzes these reactions by providing a template for the transition state.

Experimental Section

Materials. γ-Picoline, pyridine, and potassium hydrogen ethylphosphonate were purified by distillation or recrystallization. Aqueous solutions of phosphorylated pyridines and acetyl phosphate were prepared as described previously.[3-5] 4-Morpholinopyridine was a gift from Dr. Mark Skoog.

Reactions of Phosphorylated Pyridines. Reactions of 2×10^{-4} M phosphorylated γ-picoline, 1×10^{-4} M phosphorylated 4-morpholinopyridine, and 5×10^{-4} M phosphorylated pyridine at 25.1 ± 0.1 °C were followed at 256–258, 303, and 262 nm, respectively. These reactions were first order for >3 $t_{1/2}$; endpoints were determined after ≥10 $t_{1/2}$. The ionic strength was maintained at 1.5 with potassium chloride and the pH was determined at the end of each reaction.

Reactions of Acetyl Phosphate. The disappearance of acetyl phosphate was followed spectrophotometrically at 540 nm in aliquots from reaction mixtures that contained 5×10^{-3} M acetyl phosphate, at 25.1 ± 0.1 °C and ionic strength 1.5 (KCl), by addition of hydroxylamine and conversion of the substrate to acetohydroxamic acid by the method of Lipmann and Tuttle with minor modification.[6,7]

Results

The Reaction of PicP with Acetate and Formate Ions and with Trimethylamine *N*-Oxide. Figure 1 shows that the observed pseudo-first-order rate constants for the disappearance of PicP (phosphorylated picoline monoanion)[8] increase linearly with the concentration of acetate ion in the presence of 0.33 M Mg^{2+} and give a second-order rate constant of $k_2 = 1.6 \times 10^{-3}$ M^{-1} s^{-1} (Table I); in the absence of Mg^{2+} there is little or no increase in rate with added acetate ion ($k_2 < 0.3 \times 10^{-3}$ M^{-1} s^{-1}). The rate constants are the same with potassium and sodium as the counterion.

The data in Figure 2 show that the reactions of PicP and phosphorylated 4-morpholinopyridine (MPP) with carboxylate ions are dependent upon one Mg^{2+} ion that complexes with the

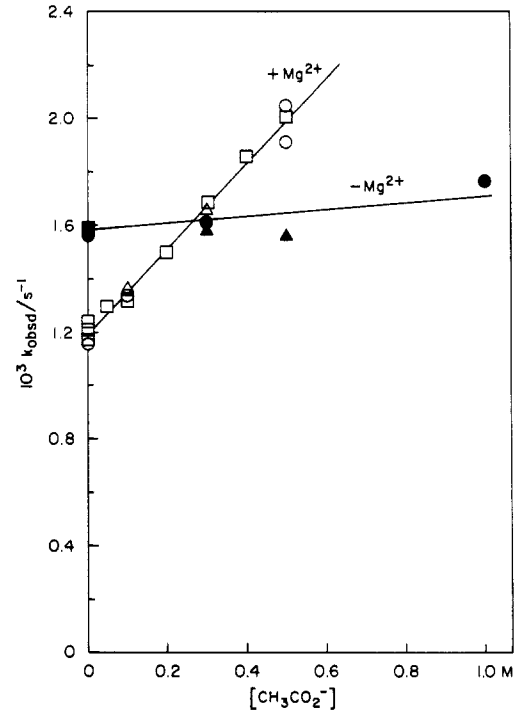


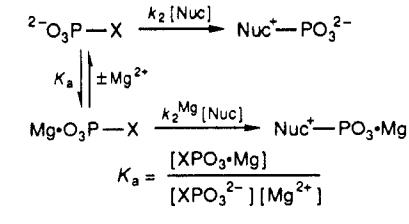
Figure 1. The effect of the concentration of acetate ion on the rate constant for disappearance of phosphorylated γ-picoline monoanion (PicP) with 0.33 M $MgCl_2$ (open symbols) and without $MgCl_2$ (closed symbols) in 0.05 M CHES buffer, pH 8.0, at 25 °C and ionic strength 1.5. Potassium acetate was added and the ionic strength was maintained with KCl (○, ●); sodium acetate was added and the ionic strength was maintained with NaCl (□, ■); and sodium acetate was added and the ionic strength was maintained with KCl (△, ▲). The line through the open symbols is a least squares fit to the data and the line through the closed symbols is for $k_2 = 0.13 \times 10^{-3}$ M^{-1} s^{-1} (Scheme II).

Table I. Second-Order Rate Constants for the Reaction of Phosphorylated γ-Picoline Monoanion and Acetate Ion and for the Reverse Reaction of Acetyl Phosphate Dianion with γ-Picoline in the Absence and Presence of Mg^{2+} [a]

PicP + acetate ion		AcP + γ-picoline	
substrate or conditions	$10^3 k_2$ (M^{-1} s^{-1})	substrate or conditions	$10^5 k_2$ (M^{-1} s^{-1})
PicP (k_2)	0.13[b]	AcP (k_2)	6.6[c]
PicP + 0.33 M Mg^{2+}	1.60[d]	AcP + 0.33 M Mg^{2+}	88[c]
PicP·Mg (k_2^{Mg})	2.5[b]	AcP·Mg (k_2^{Mg})	144[e]
k_2^{Mg}/k_2	19		22

[a] At 25 °C and ionic strength 1.5 (KCl). [b] Calculated from Scheme II as described in the Results section. [c] From the data of Figure 3. [d] From the observed increase in the rate constant for disappearance of PicP with 0–0.5 M acetate ion (15 rate measurements) in 0.05 M CHES buffer, pH 8.0. [e] Calculated from the above rate constants in the presence and absence of 0.33 M Mg^{2+} and the association constant for Mg^{2+} and the substrate (Table II) with use of Scheme I and eq 2 as described in the text.

Scheme I



$$K_a = \frac{[XPO_3{\cdot}Mg]}{[XPO_3^{2-}][Mg^{2+}]}$$

phosphorylated pyridine with an association constant of $K_a = 5$ M^{-1} (Scheme I, Table II). The observed rate constants for the reactions of PicP with 0.49 M bicarbonate ion and of MPP with acetate ion were corrected for the rate constants for hydrolysis in the absence of added nucleophile at each of several concen-

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(8) The following abbreviations are used: PicP, phosphorylated γ-picoline monoanion; PyrP, phosphorylated pyridine monoanion; MPP, phosphorylated 4-morpholinopyridine monoanion; AcP, acetyl phosphate dianion; CHES, 2-(cyclohexylamino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; MOPS, 3-morpholinopropanesulfonic acid.

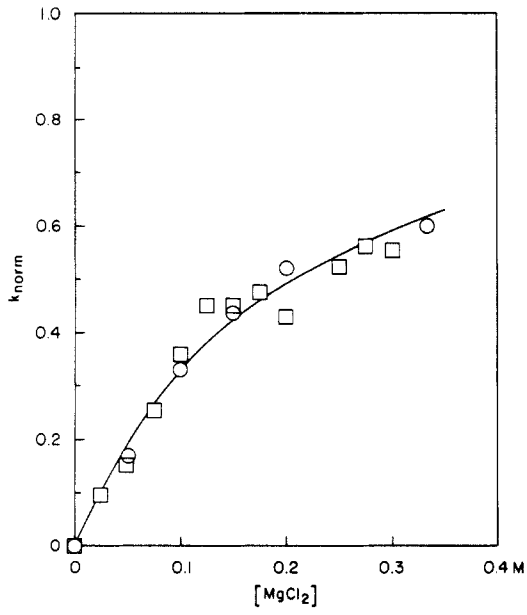

Figure 2. The dependence on the concentration of $MgCl_2$ of the rate constant for the reaction of phosphorylated γ-picoline (PicP) with 0.49 M HCO_3^-/CO_3^{2-}, 100/1 (circles), and for the reaction of phosphorylated 4-morpholinopyridine with 0.5 M acetate ion (squares) at 25 °C and ionic strength 1.5 (KCl). The reactions with phosphorylated 4-morpholinopyridine were buffered with 0.05 M Tris, pH 7.7. Each observed rate constant with added nucleophile (HCO_3^- or acetate ion) was corrected for the rate constant at the same pH with no added nucleophile. The resulting rate constants were normalized so that $k_{norm} = 0$ corresponds to the rate constant in the absence of Mg and $k_{norm} = 1$ corresponds to the rate constant for the Mg/substrate complex with use of the eq; $k_{norm} = (k_2^{Mg} - k_2)/k_2^{Mg}$. The line is the nonlinear least-squares fit to the data with $K_a = 5.0$ M^{-1} (Scheme I and eq 2).

trations of Mg^{2+}. The corrected rate constants from each set of data were fit by nonlinear least squares to eq 2 for the binding

$$k_2(\text{obsd}) = \frac{k_2[XPO_3^{2-}] + k_2^{Mg}[XPO_3{\cdot}Mg]}{[XPO_3^{2-}] + [XPO_3{\cdot}Mg]} = \frac{k_2 + k_2^{Mg}K_a[Mg^{2+}]}{1 + K_a[Mg^{2+}]} \quad (2)$$

of a single Mg^{2+} ion to the phosphorylated pyridine; eq 2 was derived from Scheme I. Each set of data gave $K_a = 5.0$ M^{-1}, and the line in Figure 2 is the calculated line for this association constant. This value of K_a at ionic strength 1.5 is the same as that determined by the same kinetic method for the reaction of MPP with 0.35 M pyridine at ionic strength 1.0;[5] it is also consistent with the dependence on $[Mg^{2+}]$ of the reaction of PicP with 0.5 M acetate ion, which shows an increase in rate of only 20% with 0.33 M Mg^{2+} (not shown).

Observed rate constants for the reaction of formate ion with PicP of $k = 3.0 \times 10^{-3}$ M^{-1} s^{-1} in the presence of 0.33 M Mg^{2+} and 2.8×10^{-3} M^{-1} s^{-1} without[7] Mg^{2+} correspond to a rate constant of 3.2×10^{-3} M^{-1} s^{-1} for reaction with the PicP·Mg complex. There is no significant catalysis by 0.33 M Mg^{2+} of the reaction of trimethylamine *N*-oxide with PicP (Table II).

Reaction of AcP and γ-Picoline. Figure 3 shows that Mg^{2+} catalyzes the reaction of AcP and γ-picoline. The second-order rate constants in the presence and absence of 0.33 M Mg^{2+} (Table I) were obtained from the curved lines in Figure 3, which are nonlinear least-squares fits to the data that account for the apparent self-association of γ-picoline.[9]

The difference between the dependence of the rate constant for disappearance of AcP on the concentration of Mg^{2+} in the presence of 0.45 and 0.045 M pyridine (Figure 4, solid line) gives

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Table II. Comparison of Second-Order Rate Constants (M^{-1} s^{-1}) for the Reactions of Carboxylate Nucleophiles with Phosphorylated γ-Picoline Monoanion (PicP) and with Its Mg^{2+} Complex[a]

nucleophile	pK_a[b]	10^3k_2[c] (M^{-1} s^{-1})	$10^3k_2^{Mg}$[d] (M^{-1} s^{-1})	k_2^{Mg}/k_2	$k_2(\text{nuc})/k_2(CH_3CO_2^-)$
$CH_3CO_2^-$	4.65[e]	0.13[f]	2.5[f]	19	(1)
HCO_2^-	3.56[g]	2.8[h]	≤3.2[i]	1	22
HCO_3^-	3.8[j]	5.4[k]	10.9[l]	2	42
CO_3^{2-}	9.78[e]	0.92[k]	110[l]	120	5[m]
$(CH_3)_3NO^{\pm}$	4.6[j]	0.4[h]	0.4[n]	1	6[m]

[a] At 25 °C and ionic strength 1.5 (KCl). [b] The pK_a of the conjugate acid at 25 °C and ionic strength 1.0 (KCl) unless stated otherwise. [c] The second-order rate constants for reaction with PicP. [d] The second-order rate constants for reaction with the Mg complex of PicP (Scheme I). [e] Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436. [f] Determined from Scheme II as described in the Results. [g] Sayer, J. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1969**, *91*, 6353. [h] From ref 7. [i] Calculated from k_2 and k_2^{app} with 0.33 M Mg^{2+} with use of eq 2 (see text); the value of k_2^{app} was determined in 0.05 M CHES buffer, pH 8.0, from 8 observed rate constants with 0–0.5 M formate ion. [j] From Jencks, W. P.; Regenstein, J. In Physical and Chemical Data *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. 1, pp 305–351; not at ionic strength 1.0. [k] From the data of Figure 5 and Table III. [l] Calculated from k_2 and k_2^{app} with 0.33 M Mg^{2+} with use of eq 1 (see text); the value of k_2^{app} is from the data of Figure 6 and Table III. [m] Statistically corrected for the number of nucleophilic oxygen atoms. [n] From k_2 and k_2^{app} with 0.33 M Mg^{2+} with use of eq 2 (see text); the value of k_2^{app} was determined in 0.05 M CHES buffer, pH 8.0, from 7 observed rate constants with 0–0.75 M trimethylamine *N*-oxide.

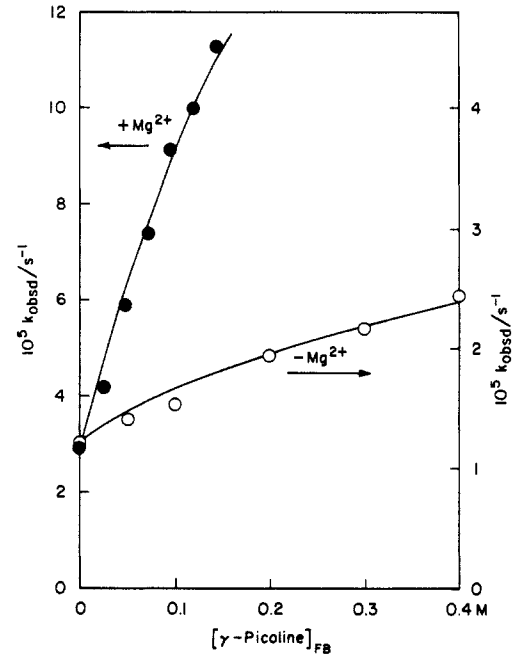

Figure 3. The dependence on the concentration of γ-picoline of the rate constant for disappearance of acetyl phosphate dianion in the presence (●) and absence (○) of 0.33 M $MgCl_2$ at 25 °C and ionic strength 1.5 (KCl). The reaction mixtures were buffered by 0.05 M MOPS, pH 8.1, and by γ-picoline (80% free base) in the absence and presence of $MgCl_2$, respectively. The lines are nonlinear least-squares fits to the data for the reaction of free γ-picoline that account for the apparent self-association of γ-picoline with $K_{SA} = [\gamma\text{-picoline}{\cdot}\gamma\text{-picoline}]/[\gamma\text{-picoline}]^2 = 3$ M^{-1} (39 °C, ionic strength 1.0 (KCl)).[9]

the dependence on Mg^{2+} of the pyridine-catalyzed reaction. The solid line in Figure 4 is a nonlinear least squares fit to these data for the reactions of pyridine with AcP and with the Mg^{2+} complex of AcP (Scheme I and eq 2). This fit gives an association constant for AcP and Mg^{2+} of $K_a = 4.4$ M^{-1} (Table II). The similar, but slightly larger values of $K_a = 6$ M^{-1} (30 °C, $I = 0.3$) and 8.2 M^{-1} (39 °C, $I = 0.6$) determined by competition with 8-hydroxyquinoline[10] for binding of Mg^{2+} and by a fit to kinetic data,[11]

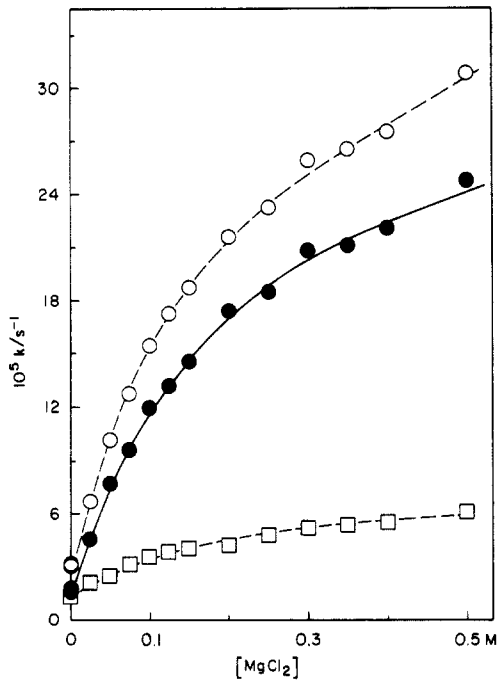

Figure 4. The dependence on the concentration of $MgCl_2$ of the rate constant for disappearance of acetyl phosphate dianion in the presence of 0.5 M (○) and 0.05 M (□) pyridine (90% free base) at 25 °C and ionic strength 1.5 (KCl), and the difference in these observed rate constants (●). The solid line is the best nonlinear least-squares fit to the data for association of Mg and AcP, according to Scheme I and eq 2 in the text.

respectively, are consistent with observed increases in the affinity of Mg^{2+} for phosphoryl compounds with decreasing ionic strength and increasing temperature.[5,10] These values are much smaller than a value of K_a = 75 M^{-1} (25 °C, I = 0.6) that was determined by competition with 8-hydroxyquinoline.[12]

The rate constant for the reaction of γ-picoline with the AcP·Mg complex in Table I was calculated from the observed second-order rate constants in the presence and absence of 0.33 M Mg^{2+} and the association constant for AcP and Mg^{2+} (Tables I and II) according to eq 2. The catalysis by Mg^{2+} of the reaction with γ-picoline by 22-fold (k_2^{Mg}/k_2, Table I) is similar to the catalysis by 15–20-fold of the reactions with 3-cyanopyridine, nicotinamide, and pyridine.[13]

The value of β_{nuc} = 0.10–0.14 for the reaction of substituted pyridines with AcP in the presence and absence of Mg^{2+} provides evidence for attack by pyridines at phosphorus rather than carbon.[13] This value is similar to values of β_{nuc} = 0–0.2 observed previously in nucleophilic reactions of pyridines at phosphorus,[4,5,9,14-16] but is much smaller than the values of β_{nuc} = ~0.8 that are typically obtained for reaction at the carbonyl carbon atom.[17,18] Previous data provide evidence for P–O bond cleavage in the reaction of AcP with pyridine and γ-picoline in the absence of Mg^{2+}: ^{18}O from $(^{18}O)H_2O$ is incorporated into inorganic phosphate during the reaction of AcP with pyridine;[19] methyl phosphate is formed in the pyridine and γ-picoline-catalyzed solvolysis of AcP in aqueous methanol;[17] and fluorophosphate is formed from AcP and fluoride ion in the presence of pyridine or

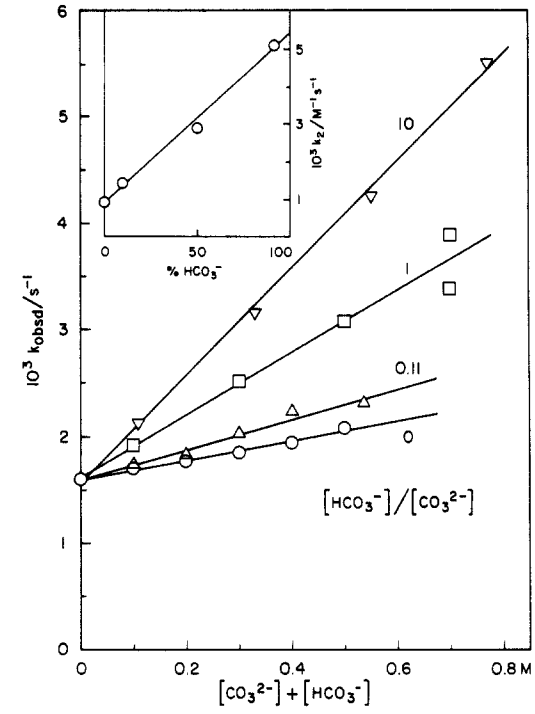

Figure 5. The effect of the concentration of potassium carbonate and bicarbonate on the rate constant for disappearance of phosphorylated γ-picoline monoanion (PicP) with ratios of $[HCO_3^-]/[CO_3^{2-}]$ = 10, 1, 0.11, and 0 at 25 °C and ionic strength 1.5 (KCl). The reactions without bicarbonate ion (ratio = 0) contained 0.01 M potassium hydroxide. Inset: the dependence on the fraction of bicarbonate ion of the apparent second-order rate constants for the reaction with PicP. The lines are least-squares fits to the data, and the slopes and intercepts give the second-order rate constants in Tables III and IV.

γ-picoline, but not in their absence.[17]

Reactions of PicP with Nucleophiles That Can Donate a Hydrogen Bond. Figure 5 shows the effect of the concentration of bicarbonate and carbonate ions, at varying ratios, on the rate of disappearance of PicP; the apparent second-order rate constants obtained from the slopes (Table IV) are plotted against the percentage of bicarbonate ion in the inset. The reaction with bicarbonate ion is 6-fold faster than that with carbonate ion, despite the much lower basicity of bicarbonate ion. The second-order rate constants in Table IV for reactions with bicarbonate and carbonate ions, k_2, were obtained from the slopes and intercepts in Figure 5 and from similar data for reactions with phosphorylated pyridine and phosphorylated 4-morpholinopyridine and for reactions of inorganic phosphate mono-, di-, and trianion and ethylphosphonate mono- and dianion with phosphorylated pyridines.

Figure 6 shows that the apparent second-order rate constants for the reaction of PicP with bicarbonate and carbonate ion in the presence of 0.33 M Mg^{2+} *decrease* with increasing fraction of bicarbonate. The intercept at 100% bicarbonate ion gives k_2 = 8.8 × 10^{-3} M^{-1} s^{-1} for the reaction of bicarbonate ion with PicP in the presence of 0.33 M Mg^{2+} and k_2^{Mg} = 10.9 × 10^{-3} M^{-1} s^{-1} for reaction with the Mg·PicP complex, from K_a = 5 M^{-1} and eq 2 (Tables III and IV). A long extrapolation gives k_2^{app} = 70 × 10^{-3} M^{-1} s^{-1} for reaction with carbonate dianion in the presence of 0.33 M Mg^{2+} and k_2^{Mg} = 110 × 10^{-3} M^{-1} s^{-1} for the Mg·PicP complex, as above. This rate constant is uncertain because precipitation prevented the determination of rate constants at high pH in the presence of Mg^{2+} and the dependence of k_2 on [Mg^{2+}] was not characterized completely. Similar catalysis by Mg^{2+} was observed for the reactions of bicarbonate and carbonate ions with phosphorylated pyridine monoanion (not shown).

Summary of Data for Phosphoryl Transfer between Acetate Ion and γ-Picoline in Both Directions. Scheme II shows the rate constants for phosphoryl transfer between acetate ion and γ-picoline in the presence and absence of bound Mg^{2+} and the binding

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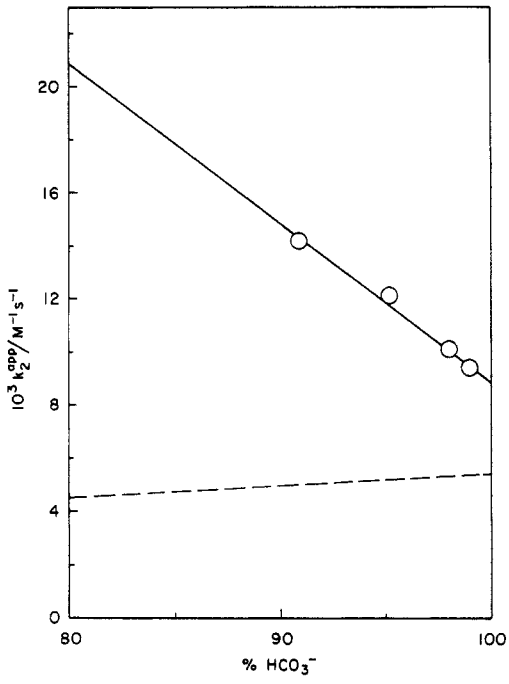
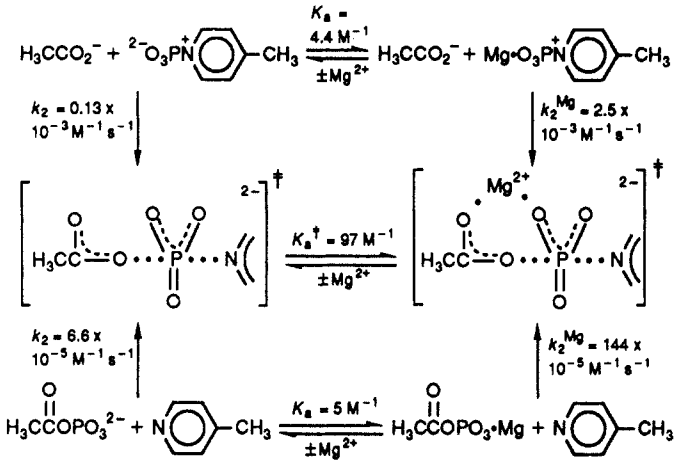


Figure 6. The effect of the ratio of bicarbonate/carbonate ion on the apparent second-order rate constants (Table III) for reaction with phosphorylated γ-picoline monoanion (PicP) in the presence of 0.33 M $MgCl_2$ at 25 °C and ionic strength 1.5 (KCl). The solid line is the least-squares fit to the data, and the intercepts at 100% bicarbonate and 100% carbonate ion (not shown) give the second-order rate constants for reaction in the presence of 0.33 M $MgCl_2$ (Table III). The dashed line is the least-squares fit for the reaction in the absence of $MgCl_2$, from the inset of Figure 5.

Scheme II



constants for Mg^{2+} with the reactants and the transition state. The rate constants in the bottom half of Scheme II for the reaction of AcP and AcP·Mg with γ-picoline are from Figures 3 and 4, as described above (Table I). These rate constants, the value of $K_a = 4.4\ M^{-1}$ (Table II), and eq 3, which was derived from Scheme

$$K_a k_2^{Mg} = K_a^{\ddagger} k_2 \qquad (3)$$

II, give $K_a^{\ddagger} = 97\ M^{-1}$ for binding of Mg^{2+} to the transition state. The rate constants for the reactions of PicP and PicP·Mg with acetate ion of $k_2 = 0.13 \times 10^{-3}\ M^{-1}\ s^{-1}$ and $k_2^{Mg} = 2.5 \times 10^{-3}\ M^{-1}\ s^{-1}$ were obtained by solving eqs 2 and 3 simultaneously, with values of $k_2^{app}(0.33\ M\ Mg^{2+}) = 1.6 \times 10^{-3}\ M^{-1}\ s^{-1}$ (Table I), $K_a(PicP{\cdot}Mg) = 5\ M^{-1}$ (Table II), and $K_a^{\ddagger} = 97\ M^{-1}$. The value of $k_2 = 0.13 \times 10^{-3}\ M^{-1}\ s^{-1}$ is consistent with the observed rate constants shown by the solid symbols in Figure 1. It should be noted that the value of k_2^{Mg} has little sensitivity to the value of k_2: values of $k_2 = 0$ and $0.3 \times 10^{-3}\ M^{-1}\ s^{-1}$ give $k_2^{Mg} = 2.6 \times 10^{-3}\ M^{-1}\ s^{-1}$ and $2.4 \times 10^{-3}\ M^{-1}\ s^{-1}$, respectively. The rate constants for reaction of PicP and PicP·Mg with acetate (Table III) correspond to catalysis by a factor of 19 in the Mg^{2+} complex, which is similar to the catalysis of ~10-fold for reactions of

Table III. Second-Order Rate Constants for Reactions of the Monoanions of Phosphorylated Pyridines with Oxygen Nucleophiles[a]

nucleophile	pK_a[b]	fraction acid	k_2^{app}	k_2
Phosphorylated Picoline (10^3k)				
HCO_3^-	3.8[c]	1.0		5.4[d]
		0.91	5.1	
		0.50	2.9	
		0.10	1.43	
CO_3^{2-}	9.78[e]	0	0.92	0.92
$H_2PO_4^-$	1.72[e]	1.0		0.58[d]
		0.67	0.82	
		0.50	0.96	
		0.09	1.25	
HPO_4^{2-}	6.49[e]	0 (1.0)		1.32[d]
		0.5	1.36	
PO_4^{3-}	11.44[f]	0		1.4[d]
$CH_3CH_2PO_3H^-$	2.4[c]	1.0		0.2[d]
		0.70	0.32	
		0.50	0.40	
		0.25	0.58	
		0.10	0.50	
$CH_3CH_2PO_3^{2-}$	7.60[g]	0		0.6[d]
Phosphorylated Picoline + 0.33 M Mg^{2+} (10^3k)				
HCO_3^-	3.8[c]	1.0		8.8[d]
		0.99	9.4	
		0.98	10.1	
		0.95	12.1	
		0.91	14.2	
CO_3^{2-}	9.78[e]	0		70[d]
Phosphorylated Pyridine (10^2k)				
HCO_3^-	3.8[c]	1.0		2.9[d]
		0.91	2.8	
		0.50	1.6	
CO_3^{2-}	9.78[e]	0	0.6	0.6
$H_2PO_4^-$	1.72[e]	1.0		0.5[d]
		0.80	0.59	
		0.67	0.59	
		0.50	0.68	
		0.09	0.85	
HPO_4^{2-}	6.49[e]	0 (1.0)		0.9[d]
		0.91	0.71	
		0.67	0.92	
		0.50	0.87	
		0.20	0.99	
PO_4^{3-}	11.44[f]	0		1.0[d]
Phosphorylated 4-Morpholinopyridine (10^6k)				
HCO_3^-	3.8[c]	1.0		10.6[d]
		0.95	10.3	
		0.50	7.8	
CO_3^{2-}	9.78[e]	0	4.5	4.5
$H_2PO_4^-$	1.72[e]	1.0		2.0[d]
		0.91	2.38	
		0.50	2.45	
		0.09	2.75	
HPO_4^{2-}	6.49[e]	0 (1.0)		2.8[d]
		0.91	2.83	
		0.67	3.11	
		0.33	3.80	
		0.20	3.89	
PO_4^{3-}	11.44[f]	0		4.3[d]

[a] At 25.1 °C and ionic strength 1.5 (KCl); the rate constants have units of $M^{-1}\ s^{-1}$. Second-order rate constants based on total buffer concentration were generally obtained from four to six pseudo-first-order rate constants in the range of approximately 0–0.7 M buffer. [b] At 25 °C and ionic strength 1.0 (KCl) unless noted otherwise. [c] From Jencks, W. P.; Regenstein, J. In Physical and Chemical Data. *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. 1, pp 305–351. Not at ionic strength 1.0. [d] Extrapolated value, as described in the text. [e] Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436. [f] At 30 °C, from Bruice, P. Y. *J. Am. Chem. Soc.* **1984**, *106*, 5959. [g] From Sayer, J. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1973**, *95*, 5637.

Table IV. Equilibrium Constants for Phosphoryl Transfer and Hydrolysis Reactions[a]

A. Phosphoryl Transfer Reactions

reactions	K[b]	K_H[c]	reactions	K[b]	K_H[c]
$PicP^- + CH_3CO_2^- \rightleftharpoons AcP^{2-} +$ γ-picoline	2[d]		$PicP^- + NH_4^+ \rightleftharpoons {}^+H_3NPO_3^{2-} +$ γ-picoline-H^+		0.02
$PicP^- + CH_3CO_2H \rightleftharpoons AcP^{2-} +$ γ-picoline-H^+		96	$PyrP^- + NH_3 \rightleftharpoons {}^+H_3NPO_3^{2-}$ + pyridine	120[g]	
$PyrP^- + CH_3CO_2^- \rightleftharpoons AcP^{2-}$ + pyridine	13[e]		$PyrP^- + NH_4^+ \rightleftharpoons {}^+H_3NPO_3^{2-} +$ pyridine-H^+		0.02
$PyrP^- + CH_3CO_2H \rightleftharpoons AcP^{2-} +$ pyridine-H^+		98	$PyrP^- + NH_3 \rightleftharpoons H_2NPO_3^{2-} +$ pyridine-H^+		0.2
formyl phosphate(2−) + $CH_3CO_2^- \rightleftharpoons AcP^{2-} + HCO_2^-$	1[f]		$PicP^-$ + imidazole ⇌ phosphoimidazole(1−) + γ-picoline	140[i]	
formyl phosphate(2−) + $CH_3CO_2H \rightleftharpoons AcP^{2-} + HCO_2H$		0.1	$PicP^-$ + imidazole-H^+ ⇌ phosphoimidazole(1−) + γ-picoline-H^+		19
$PicP^- + NH_3 \rightleftharpoons {}^+H_3NPO_3^{2-} +$ γ-picoline	21[h]				

B. Hydrolysis of Phosphoryl Compounds

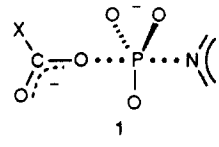
compound	K''[j]	compound	K''[j]	compound	K''[j]
AcP^{2-}	8×10^4[k]	$PicP^-$	8×10^6	$H_2NPO_3^{2-}$	4×10^7
formyl phosphate(2−)	1×10^4	$PyrP^-$	8×10^6	${}^+H_3NPO_3^{2-}$	4×10^8
phosphorylated imidazole(1−)	4×10^5			$HOPO_3^{2-}$	55[l]

[a] At 25 °C and ionic strength 1.5 (KCl), unless stated otherwise. [b] $K = k_+/k_- = [XPO_3^{2-}][Y^-]/[YPO_3^{2-}][X^-]$; determined from k_+ and k_-, the forward and reverse rate constants, respectively, for the specified reaction, except for the equilibrium between AcP^{2-} and formyl phosphate(2−), which was estimated indirectly (see text). [c] $K_H = [XPO_3^{2-}][YH]/[YPO_3^{2-}][XH]$; calculated from the values for K (Table IVA) and the following pK_a values for YH and XH at 25 °C and ionic strength 1.0 (KCl): acetic acid, 4.65; γ-picolinium ion, 6.33; pyridinium ion, 5.52; formic acid, 3.56; ammonium ion, 9.3; ${}^+H_3NPO_3^{2-}$, 8.3; and imidazolium ion, 7.2 (Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436. Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622; **1964**, *86*, 1410. Sayer, J. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1969**, *91*, 6353). The value of $pK_a = 8.3$ for ${}^+H_3NPO_3^{2-}$ is similar to that from the pH–rate profile for hydrolysis under slightly different conditions (Chanley, J. D.; Feageson, E. *J. Am. Chem. Soc.* **1963**, *85*, 1181). The difference of 1.0 between the pK_a values for ammonium ion and ${}^+H_3NPO_3^-$ is similar to that observed with other phosphoramidates (Benkovic, S. J.; Sampson, E. J. *J. Am. Chem. Soc.* **1971**, *93*, 4009). [d] From $k_+ = 0.13 \times 10^{-3}$ M^{-1} s^{-1} (Table I) and $k_- = 6.6 \times 10^{-5}$ M^{-1} s^{-1} (Table I). [e] From $k_+ = 7.8 \times 10^{-4}$ M^{-1} s^{-1} and $k_- = 5.9 \times 10^{-5}$ M^{-1} s^{-1} (ref 13). The value of $k_+ = 7.8 \times 10^{-4}$ M^{-1} s^{-1} was calculated by the analogous procedure used to calculate the second-order rate constant for the reaction of acetate ion with PicP: the rate constants for reaction of pyridine with AcP and with Mg·AcP of 5.9×10^{-5} and 118×10^{-5} M^{-1} s^{-1}, respectively (ref 13), and the value of $K_a = 4.4$ M^{-1} for formation of the Mg·AcP complex (Table II) give $K_a^* = 88$ M^{-1}, with use of eq 3; the rate constant of k_2^{app} (0.33 M Mg^{2+}) $= 7.2 \times 10^{-4}$ M^{-1} s^{-1} (ref 13), $K_a = 5$ M^{-1} for formation of the complex of Mg with phosphorylated pyridine, and $K_a^* = 88$ M^{-1} then give $k_+ = 7.8 \times 10^{-4}$ M^{-1} s^{-1} with use of eqs 2 and 3. [f] Estimated indirectly as described in the text. [g] From $k_+ = 0.67$ M^{-1} s^{-1} (Jameson, G. W.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 53) and $k_- = 5.6 \times 10^{-3}$ M^{-1} s^{-1} (Chanley, J. D.; Feageson, E. *J. Am. Chem. Soc.* **1963**, *85*, 1181); k_+ and k_- were determined at 25 °C and ionic strength 0.2. [h] From $k_+ = 0.15$ M^{-1} s^{-1} (Jameson, G. W.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 53) and $k_- = 7.2 \times 10^{-3}$ M^{-1} s^{-1} (Chanley, J. D.; Feageson, E. *J. Am. Chem. Soc.* **1963**, *85*, 1181); k_+ and k_- were determined at 25 °C and ionic strength 0.2. A 2-fold different value was calculated from rate constants for the forward and reverse reactions at two different temperatures (ref 15). [i] From $k_+ = 0.21$ M^{-1} s^{-1} ($I = 0.2$; Jameson, G. W.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 53) and $k_- = 1.5 \times 10^{-3}$ M^{-1} s^{-1}. The value of k_- is estimated from $k = 3.3 \times 10^{-3}$ M^{-1} s^{-1} for reaction of phosphorylated imidazole and pyridine at 40 °C and ionic strength 0.5 (Lloyd, G. J.; Hsu, C.-M.; Cooperman, B. S. *J. Am. Chem. Soc.* **1971**, *93*, 4889). This rate constant was corrected to that for a nucleophile of the pK_a of γ-picoline with use of $\beta_{nuc} = \sim 0.25$ (ref 4) to give $k = 5.2 \times 10^{-3}$ M^{-1} s^{-1}; this rate constant was then corrected to 25 °C with use of $E_a = \sim 15$ kcal/mol to give $k_- = 1.5 \times 10^{-3}$ M^{-1} s^{-1}. A 2-fold different value was estimated indirectly from forward and reverse rate constants obtained at different temperatures (ref 15). [j] $K'' = [HPO_4^{2-}][XH]/[XPO_3^{2-}]$, which is the equilibrium of eq 6, with a standard state of pure water. The values were calculated from K'' for AcP^{2-} and the values of K_H in Table IVA. [k] From ref 32. [l] From $K = 1$ for the identity reaction $K = [HOPO_3^{2-}][HOH]/[HOH][HOPO_3^{2-}]$ and the standard state of 55 M water.

phosphorylated pyridines with succinate dianion and cacodylate ion that was determined from the observed second-order rate constants in the presence and absence of 0.33 M Mg^{2+} and $K_a = 5$ M^{-1}.[13]

Discussion

Initial experiments on the breakdown of PicP led to the following preliminary conclusions. (1) The slower reaction of PicP with acetate ion than with formate ion, by a factor of ~20-fold (Table III), might be expected from a larger steric effect of the methyl group than of the hydrogen atom in the single transition state structure **1**. (2) The faster reaction of PicP with bicarbonate



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ion than with acetate ion, by a factor of ~40-fold (Table III), might be accounted for by stabilization from the favorable energy of an intramolecular hydrogen bond to the phosphoryl oxygen atoms in the transition state. (3) Catalysis by Mg^{2+} of the reactions of PicP with acetate ion and carbonate anion, by ~20- and 100-fold respectively (Table IV), might be accounted for by favorable electrostatic interactions with Mg^{2+} that remove an electrostatic barrier to approach of the anionic reactants.

However, experimental tests of these preliminary conclusions showed that all three are incorrect: (1) The catalysis of ~20-fold by Mg^{2+} of the reaction of acetate ion with PicP ion and the absence of catalysis of the reaction of formate ion (Table III) suggest that there must be more than one conformation of the transition state; a large change in the amount of catalysis by Mg^{2+} with a small change in the substituent X is not expected with the single transition state structure **1**. (2) Although bicarbonate ion, which can form an intramolecular hydrogen bond, reacts with PicP ~40-fold faster than acetate ion, formate ion also reacts faster, by ~20-fold, although it cannot form a hydrogen bond (Table III). Thus, most of the rate increase with bicarbonate ion could result from the removal of unfavorable interactions with acetate ion in the transition state, as is observed in the transition state with formate ion, rather than from a favorable interaction of an intramolecular hydrogen bond. (3) Although catalysis by Mg^{2+} of ~20-fold might be explained by overcoming electrostatic repulsion between the two anions, acetate and PicP, this does not explain the catalysis by 20-fold of the reaction between uncharged γ-picoline and AcP dianion in the reverse direction (Tables I and III; Scheme II). The catalysis in both directions shows that Mg^{2+} binds more strongly to the transition state than to either ground state, with $K_a^* = 97$ M^{-1} and $K_a = 4.4$ and 5 M^{-1} for AcP and PicP, respectively (Table II and Scheme II); the stronger binding of Mg^{2+} to the transition state can be ascribed to chelation in the transition state by oxygen atoms of both the phosphoryl and carboxyl group.[20]

(20) For most catalysts, the rate enhancement in the forward direction must be the same as in the reverse direction, since the catalyst does not change the equilibrium constant for the reaction. However, "catalysts" such as Mg^{2+} can form stable complexes with the reactants and the products at high concentrations and can thereby change the equilibrium, giving different rate enhancements for the forward and reverse reactions.

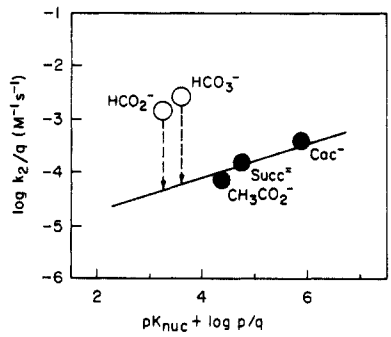
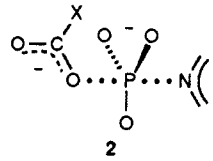


Figure 7. The dependence of log k_2 on the pK_a of the carboxylate ion nucleophile for reactions with phosphorylated γ-picoline monoanion (PicP) at 25 °C and ionic strength 1.5 (KCl). The line of slope 0.25 is the best value of β_{nuc} from ref 13 and is drawn through the closed symbols. The deviations of the rate constants for bicarbonate and formate ions (open symbols) of 43- and 28-fold, respectively, are discussed in the text. The rate constants are from Table III and ref 13; that for acetate ion is estimated with use of Scheme II. The rate constants and pK_a values are statistically corrected.

The factors that affect the rate of the reactions with PicP can be summarized with use of the reaction with acetate ion as a reference. Unfavorable steric, solvation, and electrostatic interactions that slow phosphoryl transfer to acetate ion can be overcome by (1) replacement of the methyl group by the smaller hydrogen atom; (2) replacement of the methyl group by the hydrophilic hydroxyl group; and (3) addition of Mg^{2+} to provide favorable electrostatic interactions in the transition state. Further analysis of these interactions is given in the following sections.

Steric and Solvation Effects. The rate constant for the reaction of formate ion has a positive deviation by a factor of 30-fold from the Brønsted correlation for the reaction of anionic oxygen nucleophiles with PicP in Figure 7; the slope of $\beta_{nuc} = 0.25$ is the best estimate of this value for carboxylate nucleophiles.[13] This difference may be attributed to unfavorable steric or solvation interactions with the phosphoryl oxygen atoms of the alkyl group of acetate in the transition state; other nucleophiles in Figure 7 may have similar unfavorable interactions. Acetate ion may have unfavorable solvation or electrostatic interactions with the phosphoryl oxygen atoms if it reacts through transition state **2** or **3**, and an unfavorable alignment of the sp^2 orbital in transition state **1**. Formate ion can react through transition state **2**, which



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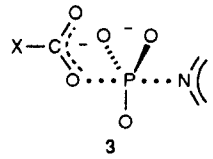
minimizes interaction between the carboxylate and phosphoryl oxygen atoms and aligns the approach to the phosphorus atom with the sp^2 orbital of the nucleophilic oxygen atom. The unfavorable interactions with acetate ion can be neutralized by the positive charges of Mg^{2+} chelated to transition state **3**, which gives



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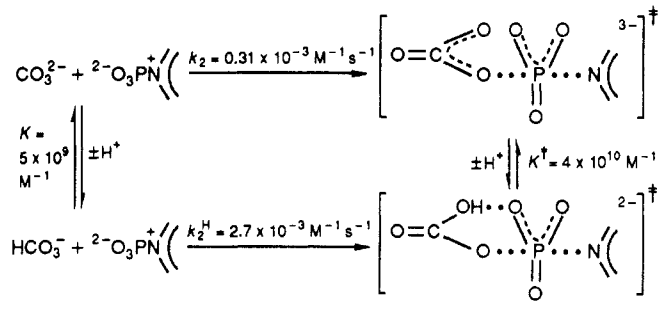
catalysis for acetate: an analogous structure may account for catalysis of the reaction with CO_3^{2-}. Formate ion may then show no catalysis because it can react rapidly through **2**.

The methyl group of acetate also appears to destabilize the ground state of acetyl phosphate. Acetate is 22 times less reactive toward PicP than formate, in spite of its greater basicity (Table III) and is expelled 27 times more slowly than formate upon hydrolysis of the corresponding acyl phosphate dianions.[21] The

Scheme III



dependence on the leaving group is probably similar for reaction of the acyl phosphates with picoline and with water, so that the equilibrium constant for eq 4 is expected to be close to 1. This is much smaller than the value of $K_{eq} = 30$ that is expected from the difference of 1.1 units in the pK_a of formic and acetic acids and the value[22] of $\beta_{eq} = 1.35$ for K_{eq} (eq 4).

$$H_3CCO_2^- + {}^{2-}O_3POCH \underset{}{\overset{K_{eq}}{\rightleftharpoons}} H_3CCOPO_3^{2-} + {}^-O_2CH \qquad (4)$$

Catalysis by Hydrogen Bonding. The bicarbonate ion reacts with PicP 9 times faster than carbonate dianion, although carbonate is 10^6-fold more basic (Table III), and shows a 40-fold positive deviation from the Brønsted plot of Figure 7; it is ~200 times more reactive than carbonate after correction for the difference in the basicity of the two nucleophiles. Scheme III and eq 5 show that the pK_a of bicarbonate in the transition state is

$$Kk_2^H = K^\ddagger k_2 \qquad (5)$$

10.6, which is higher than pK_a = 9.8 for bicarbonate in spite of the electron donation from the nucleophile in the transition state (the rate constants in Scheme III are statistically corrected). The rate constant for the reaction of inorganic phosphate monoanion with PicP (Table III) is 10-fold higher than predicted by the Brønsted correlation (not shown), and the rate constant for inorganic phosphate dianion is 2-fold larger than that for ethylphosphonate dianion, in spite of the higher pK_a of ethylphosphonate (Table III).

It might be concluded that these rate accelerations represent net stabilization of the transition state, by ~2 kcal mol^{-1}, from intramolecular hydrogen bonding to the HO group of bicarbonate and by smaller amounts for phosphate ions. However, this conclusion is incorrect, for the reasons that were described above. Bicarbonate is only slightly more reactive than formate, and the positive deviations of both compounds from the Brønsted correlation of Figure 7 reflect unfavorable interactions in the transition state of acetate and the other compounds that were used in the correlation. Bicarbonate can presumably react through transition state **2**, with a hydrogen bond to a phosphoryl oxygen atom. This avoids the unfavorable interactions that carbonate and acetate would have in this transition state, but there is no significant stabilization compared with the transition state for the reaction of formate, which may include a water molecule hydrogen bonded to the phosphoryl oxygen.

We conclude that there is no net stabilization of the transition state by intramolecular hydrogen bonding; the intramolecular hydrogen bonding of bicarbonate serves to avoid unfavorable

(21) The ratio of 27 is given by the ratio of rate constants for hydrolysis of formyl phosphate and of AcP at 25 °C of $k_w = 2.5 \times 10^{-4}$ and 9.3×10^{-6} s^{-1}, respectively (Di Sabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4400–4405). The rate constant for formyl phosphate is extrapolated to 25 °C from $k_w = 1.2 \times 10^{-4}$ s^{-1} at 20 °C (Smithers, G. W.; Jahansouz, H.; Kofron, J. L.; Himes, R. H.; Reed, G. H. *Biochemistry* **1987**, *26*, 3943–3948) with use of $E_a = 25$ kcal/mol, which is the energy of activation for hydrolysis of AcP (Di Sabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4400–4405), and the equation: $\log (k_1/k_2) = -E_a/2.3R(1/T_1 - 1/T_2)$ (Daniels, F.; Alberty, R. A. *Physical Chemistry*, 4th ed.; Wiley: New York, 1975; p 316).

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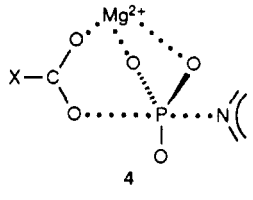
interactions in the transition state and is approximately as strong as the hydrogen bond to 55 M water. This conclusion underlines the point that it is difficult to evaluate, and sometimes even to define, the strength of a hydrogen bond in enzymes and other proteins, as well as in simple systems. Any such evaluation must include a complete analysis of factors that may destabilize the products of the reference reaction or the products that are formed when the hydrogen bond is broken, as well as the energy of the hydrogen bond itself.

It should be noted that the product of the reaction of bicarbonate or carbonate ion with phosphorylated pyridines is, presumably, carboxyphosphate. A carboxyphosphate intermediate is thought to be formed from bicarbonate ion and ATP in several enzymatic carboxylation reactions and in the carbamoyl synthetase reaction.[23]

Catalysis by Chelation of Mg^{2+} in the Transition State. The catalysis of ~20-fold for phosphoryl transfer in both directions between acetate ion and γ-picoline (Table I) means that Mg^{2+} binds more strongly to the transition state than to either ground state, with $K_a^{\ddagger} = 97\ M^{-1}$ and $K_a = 4\text{–}5\ M^{-1}$ for the transition and ground states, respectively (Table II and Scheme II). In contrast, phosphoryl transfer between pyridines is inhibited 2-fold by binding of Mg^{2+} because of the small charge density on the phosphoryl oxygen atoms that arises from the dissociative transition state for phosphoryl transfer.[5] The 40-fold stronger binding of Mg^{2+} to the transition state for the reaction with acetate suggests that the transition state is stabilized by chelation with the acetate and phosphoryl oxygen atoms as shown in **4**. The small value of β_{nuc}



4

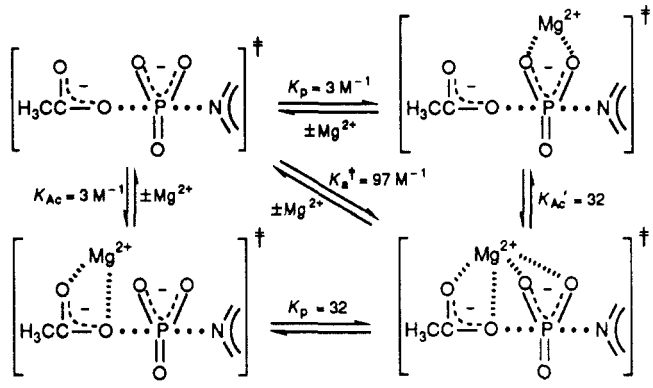
= 0.25 for the reaction of oxygen nucleophiles with PicP suggests that there is little bonding to phosphorus and considerable negative charge on the nonbonded oxygen atom of acetate that can interact with Mg^{2+} in the transition state.[13] The 30-fold difference between the binding of Mg^{2+} to acetate,[24] with $K_a = 3\ M^{-1}$, and to the transition state, with $K_a = 97\ M^{-1}$, shows that the phosphoryl oxygen atoms must also be involved in the binding.

Catalysis by Mg^{2+} of the reverse reaction of acetyl phosphate with substituted pyridines must involve the same interactions of Mg^{2+} with the phosphoryl oxygen atoms and the developing negative charge on the carbonyl oxygen atom in transition state **4**, although infrared and proton NMR spectra show no evidence for an interaction of Mg^{2+} with the carbonyl oxygen atom of the AcP·Mg complex in the ground state.[10,11] This interaction is consistent with catalysis by Mg^{2+} of the reaction of acetate with phosphorylated pyridines through transition state **3** and the absence of catalysis for the reaction of formate in transition state **2**.

The association constant of $K_a = 97\ M^{-1}$ for the chelation of Mg^{2+} in the transition state **4** is similar to $K_a = 79\ M^{-1}$ for binding to malonate dianion[25] and smaller than $K_a = 500\text{–}900\ M^{-1}$ for binding[26] to ADP^{2-}; these compounds can chelate Mg^{2+} in a six-membered ring.

Scheme IV shows that the chelation of Mg^{2+} in the transition state can be described by an "effective molarity" of ≥10 M (from $32/\leq 3\ M^{-1}$). The effective molarity is the molarity of a reagent

Scheme IV



that would be required in a bimolecular reaction to achieve the same effect as is observed in the corresponding intramolecular reaction. The value of 10 M is a lower limit because acetate in the transition state is less basic than in the ground state and will have a value of $K_{Ac} < 3\ M^{-1}$. The value of K_p was calculated from eq 3, the value of $K_a = 5\ M^{-1}$ for binding of Mg^{2+} to the phosphorylated pyridine in the ground state, and the 2-fold inhibition of phosphoryl transfer between pyridines with bound Mg^{2+}.[5]

Catalysis of the reaction of acetate with PicP by Mg^{2+} might be "explained" by facilitation of the reaction when the reactants are brought together in a complex with Mg^{2+}, so that their effective concentration is increased and the reaction is more probable. However, this explanation is unacceptable because no such facilitation is possible for the reaction of acetyl phosphate dianion with picoline and the amount of catalysis by Mg^{2+} is the same in both directions of the reaction. This comparison emphasizes the dangers of attempting to characterize catalysis by the behavior of metastable intermediates; catalysis is described most clearly and simply by comparison of only the ground and transition states.[27] It is appropriate to describe the role of Mg^{2+} as providing a template that stabilizes the assembled transition state by chelation, as in **4**. Although there is no chelation to picoline, the picoline provides electron density which helps the leaving group to depart, with the development of negative charge on the carbonyl oxygen atom that can interact with Mg^{2+} in the transition state.

Interaction of Mg^{2+} with the single, more basic oxygen atom of the leaving *p*-nitrophenolate ion gives a 4-fold rate increase from catalysis of the hydrolysis of *p*-nitrophenyl phosphate dianion, in spite of the less favorable geometry for chelation of Mg^{2+} in this reaction.[5]

The Mg^{2+} ion presumably acts in a similar manner by helping to provide a template for the transition state at the active site of enzymes that catalyze phosphoryl transfer between ATP and carboxylate groups, such as carboxylate kinases and the Ca^{2+} and Na^+K^+ ATPases. The effective molarity of ~10 M for the reacting group that is induced by Mg^{2+} shows that there is a significant loss of entropy in the transition state for phosphoryl transfer, even though the transition state for phosphoryl transfer is dissociative. Interactions with several groups at the active site have the potential to bring about much more exact fixation with a larger loss of entropy that could contribute significantly to catalysis. The maximum advantage for phosphoryl transfer that could be obtained from this induced intramolecularity is not known.

Equilibrium Constants for Phosphoryl Transfer between Nitrogen and Oxygen Bases. The ratio of the second-order rate constants for phosphoryl transfer from PicP to acetate ion and for the reverse reaction (Table I) gives an equilibrium constant of $K = 2$ for this phosphoryl transfer reaction, according to eq 6. The equilibrium

$$X^n\text{-}PO_3^{2-} + Y^{m-1} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Y^m\text{-}PO_3^{2-} + X^{n-1} \qquad (6)$$

(23) Reference 2, pp 150–154.

(24) *Stability Constants of Metal-Ion Complexes: Part B Organic Ligands*, 2nd ed.; Perrin, D. D., Ed.; Pergamon: Oxford, 1979; p 42. The association constant for $AcO^- + Mg^{2+}$ was determined at $\mu = 0.4$ ($NaNO_3$). No indication of an effect of this association on the kinetics of the reactions with AcO^- was observed.

(25) *Stability Constants of Metal-Ion Complexes: Part 1 Organic Ligands*; Bjerrum, J.; Schwarzenbach, G.; Sillén, L. G., Eds.; Chemical Society: London, 1975; Special Publication No. 6, p 11.

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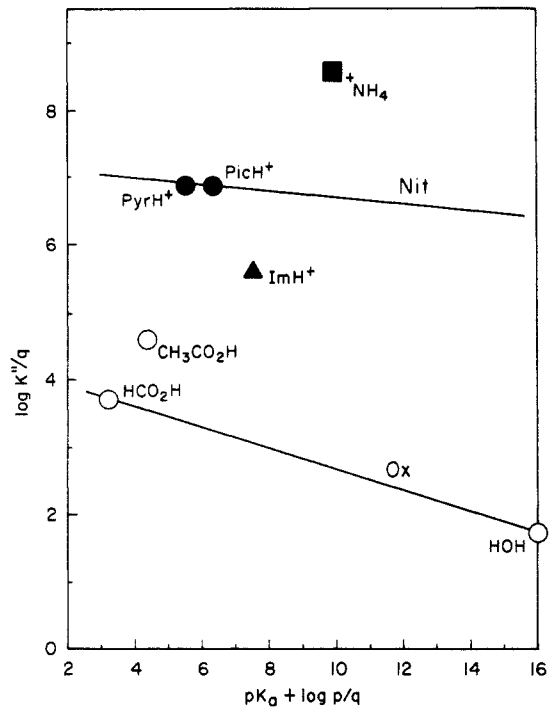

Figure 8. The log of the equilibrium constant, K'', for the hydrolysis reaction of eq 8 of the phosphorylated nitrogen (closed symbols) and oxygen (open symbols) compounds plotted against the pK_a of the nitrogen and oxygen bases (Table IVB). The log K'' and pK_a values are statistically corrected.

constant for phosphoryl transfer from phosphorylated pyridine, obtained in the same way, is $K = 13$ (Table IV). The fact that phosphorylation of acetate ($pK_a = 4.7$) is favored over phosphorylation of the more basic pyridines ($pK_a = 5.2$ and 6.0) shows that the P–O bond is stronger than the P–N bond, relative to their proton affinities.

This is demonstrated more directly by the equilibrium constant, K_H, for the exchange of a phosphoryl group and a proton according to eq 7, which removes the effect of basicity toward the proton from the comparison. The values of $K_H = \sim 100$ for phosphoryl

$$X\text{-}PO_3^{2-} + YH \overset{K_H}{\rightleftharpoons} Y\text{-}PO_3^{2-} + XH \qquad (7)$$

transfer from PicP and PyrP to acetate (Table IV) show that this reaction is strongly favorable. The value of $K_H = 0.1$ for phosphoryl transfer from formic to acetic acid reflects the destabilization in acetyl phosphate that was described above.

The larger affinity of oxygen than of nitrogen bases toward the phosphoryl group is also shown by the values of log K'' in Table IVB and Figure 8 for the hydrolysis of phosphorylated nitrogen and oxygen bases to give the protonated oxygen or nitrogen leaving group, according to eq 8. There is some uncertainty in the value

$$X\text{-}PO_3^{2-} + HOH \overset{K''}{\rightleftharpoons} XH + HOPO_3^{2-} \qquad (8)$$

of K'' for AcP,[28] which was used to obtain the other values in Table IVB, but this does not affect the relative values of K''. The large stabilization by oxygen compared with nitrogen is apparent from the small values of K'' for the two acyl phosphates, even though these compounds are acid anhydrides. The phosphorylated amines decrease in stability over a range of 10^3 with different leaving groups, in the order imidazole, pyridines, H_2N- and NH_3. The difference in stability of the P–O and P–N compounds is consistent with the difference of ~7 kcal/mol per bond that is calculated from bond dissociation energies for the equilibrium of eq 9, in

$$P(N(CH_3)_2)_3 + 3ROH \rightleftharpoons P(OR)_3 + 3HN(CH_3)_2$$
$$\Delta E/3 = -7 \text{ kcal/mol} \qquad (9)$$

which the P–N and O–H bonds are replaced by P–O and N–H bonds. The difference between the P–OR and the P–$N(CH_3)_2$ bond dissociation energies is 90 – 70 = 20 kcal mol^{-1}, which is larger by 7 kcal mol^{-1} than the difference of 105 – 92 = 13 kcal mol^{-1} between the RO–H and the R_2N–H bond energies.[29]

The greater stability of the oxygen compounds compared with $H_2NPO_3^{2-}$ (Figure 8) may be attributed to more favorable p–d orbital overlap between phosphorus and two electron pairs of oxygen, compared with the single electron pair of nitrogen. The large electronegativity of OR and F may also provide stabilization by facilitating electron donation to phosphorus by p–d orbital overlap.[30-35] The greater stability of phosphorylated pyridines and imidazole relative to $H_2NPO_3^{2-}$ might represent resonance interaction between the aromatic ring and d orbitals of phosphorus, but we are not able to estimate the contribution of such stabilization.

Stabilization by p–d orbital overlap is supported by P–O and P–N bond lengths that are shorter than predicted for single bonds; protonation results in an increase to the length expected for a normal P–N single bond in $^+H_3NPO_3^{2-}$.[33,34,36] The difference between the drop in pK_a of 15.7 – 12.3 = 3.4 units for HOH and 9.3 – 8.2 = 1.1 for NH_4^+ when a proton is replaced by PO_3^{2-} is consistent with a larger amount of electron donation to phosphorus by oxygen than by nitrogen through resonance. The electrostatic interaction with the two negative charges of XPO_3^{2-} is expected to cause an increase in the absolute values of the pK_a of both compounds; the amount of this increase is ~4–5 pK units for single charges on two atoms that are separated by a carbon atom.[37] The large equilibrium constant of $K = 1.7 \times 10^5$ for transfer of the $(EtO)_2PO$ group from imidazole to the *p*-nitrophenolate ion[38] suggests that p–d orbital overlap from oxygen is more important to this uncharged group than to the PO_3^{2-} group.

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