Evidence That Metaphosphate Monoanion Is Not an Intermediate in Solvolysis Reactions in Aqueous Solution[1]

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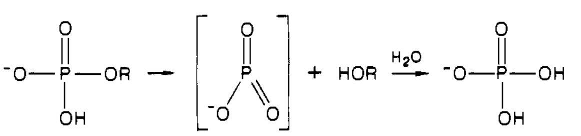
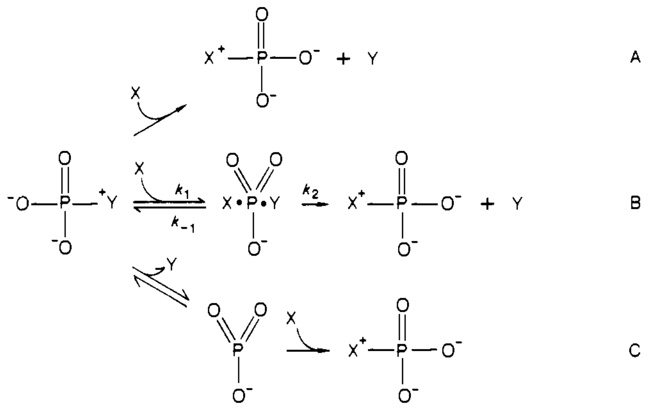
Abstract: The slope of plots of log k_2 against the pK_a of the leaving group for reactions of uncharged, monoanionic, and dianionic oxygen nucleophiles with three phosphorylated pyridine monoanions changes from $\beta_{lg} = -0.98$ to -0.79 as the pK_a of the oxygen nucleophile increases from 3.6 to 15.7 (aqueous solution; 25 °C; ionic strength, 1.5). This coupling between the strength of the nucleophile and the measure of bond cleavage is described by an interaction coefficient, $p_{xy} = \partial\beta_{lg}/\partial pK_{nuc} = \partial\beta_{nuc}/\partial pK_{lg} = 0.013$, and provides evidence for concerted phosphoryl transfer between pyridine and oxygen bases. The solvolysis of phosphorylated pyridines and of acetyl phosphate in aqueous solution also shows the behavior expected for a bimolecular substitution reaction with no metaphosphate intermediate: (1) The values of log k_2 for reactions of oxygen nucleophiles, including water, with phosphorylated γ-picoline monoanion follow a single line of slope 0.51 in a plot against log k_2 for the corresponding bimolecular reactions with methyl 2,4-dinitrophenyl phosphate monoanion (Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165). (2) Water behaves as expected for a nucleophile of its pK_a in a Brønsted-type plot of log k_2 for reaction with phosphorylated γ-picoline against the pK_a of oxygen nucleophiles. (3) The value of $\beta_{lg} = -1.02$ for the hydrolysis of phosphorylated pyridines is less negative than the value of $\beta_{eq}' = -1.25$ for complete breaking of the P–N bond. (4) The value of $\beta_{lg} = -1.02$ for the reaction of water with phosphorylated pyridines fits the correlation of β_{lg} against the pK_a of oxygen nucleophiles for concerted, bimolecular reactions, with a slope of $p_{xy} = \partial\beta_{lg}/\partial pK_{nuc} = 0.013$. (5) The reactions of acetyl phosphate monoanion and dianion with aqueous alcohols show selectivity that depends on the pK_a of the alcohol, with a larger selectivity for the less reactive dianion [50% aqueous alcohol (v/v), 55 °C].

Metaphosphate monoanion has been discussed as a possible reaction intermediate since 1955 when eq 1 was proposed in order to explain the rapid hydrolysis of phosphate monoester monoanions.[2] Phosphoryl-transfer reactions in aqueous solution exhibit a small dependence on the pK_a of the nucleophile, β_{nuc}, and a large dependence on the pK_a of the leaving group, $-\beta_{lg}$, which suggests that there is a small amount of bond making and a large amount of bond breaking in the transition state. They also exhibit near-zero values of the entropy and volume of activation and a large isotope effect with ^{18}O in the leaving group.[3-10] Although these data characterize the *transition state* as metaphosphate-like, they do not provide evidence for a metaphosphate *intermediate*. Indeed, we are aware of no evidence that requires the formation of a metaphosphate monoanion in aqueous solution.[11,12]



$$^-O\!-\!\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}\!-\!OR \longrightarrow \left[PO_3^-\right] + HOR \xrightarrow{H_2O} {}^-O\!-\!\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}\!-\!OH \qquad (1)$$

Scheme I



There are three types of evidence that a metaphosphate intermediate is not formed in the bimolecular reactions of phosphorylated pyridines with added amine nucleophiles in aqueous solution:[7,8]

(1) Three possible routes for phosphoryl transfer are shown in Scheme I: A is a concerted pathway; B is a stepwise pathway through a preassociation mechanism, in which a metaphosphate intermediate is formed but does not have a lifetime sufficient to allow diffusion; and C is a stepwise pathway involving a freely diffusing metaphosphate intermediate. The fact that reactions of phosphorylated pyridines **1** (X = H, 4-CH_3, 4-morpholino, 3-CH_3O) with added pyridine and amine nucleophiles are second order is consistent with concerted (A) or stepwise preassociation (B) mechanisms, but not with a free metaphosphate intermediate (C).[6-8] With a stepwise preassociation mechanism (B),

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[1] This research was supported in part by grants from the National Institutes of Health (GM20888 and 4-61271) and the National Science Foundation (PCM-8117816). D.H. was also supported by a fellowship from the Gillette Foundation.

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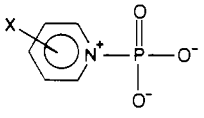

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Brønsted-type plots of log k_2 against pK_a are expected to give a break where the pK_a of the nucleophile and leaving group are equal, from a change in rate-limiting step. However, no break is observed in these plots for phosphoryl transfer between pyridines.[7,8]

(2) An increase in β_{nuc} with poorer leaving groups in these reactions can be described by an interaction coefficient[13] of p_{xy} = 0.014 (eq 2).

$$p_{xy} = \partial\beta_{lg}/\partial\,pK_{nuc} = \partial\beta_{nuc}/\partial\,pK_{lg} = \partial^2 \log k/\partial\,pK_{nuc}\partial\,pK_{lg} \qquad (2)$$

This interaction is evidence for coupling between bond formation to the nucleophile and bond breaking to the leaving group in a concerted reaction (A).[8]

(3) The rate constant for diffusion apart of an encounter pair is $\sim 10^{10}$ s^{-1}. This sets a lower limit on the rate constant for reaction of a nucleophile with a metaphosphate intermediate by the stepwise preassociation mechanism **B** because the reaction occurs, by definition, prior to diffusional separation of the metaphosphate intermediate and the nucleophile. The upper limit for this rate constant is the frequency of a bond vibration, $\sim 10^{13}$ s^{-1}, because collapse of an intermediate to form the product can be no faster than vibrational motion. Therefore, the most that the observed rate constant can vary with the preassociation mechanism B is 10^{13} s^{-1}/10^{10} s^{-1} = 10^3; this is less than the observed variation[8] of 10^5.

The mono- and dianion of acetyl phosphate react with inorganic phosphate monoanion in concentrated aqueous sodium perchlorate to give different yields of pyrophosphate; this proves that there is not a common free metaphosphate intermediate.[12] The second-order reactions of phosphorylated pyridine monoanions with inorganic phosphate anions in the absence of high concentrations of salt are also consistent with concerted phosphoryl transfer to phosphate.[14]

However, the intermediacy of metaphosphate monoanion in reactions with solvent is not excluded by these results because there could be a barrier for the reaction of metaphosphate with a weak nucleophile such as water; i.e., metaphosphate could have a significant lifetime in the presence of water, but not in the presence of the stronger pyridine nucleophiles. The inversion of configuration in solvolysis reactions of phosphoryl compounds in aqueous solution[15] and the absence of positional isotope exchange of the α–β bridge oxygen atom of [β-$^{18}O_4$]ADP with the nonbridge α-oxygen atoms concurrent with hydrolysis[16] show that there is not an intermediate with a lifetime long enough to allow diffusion or rotation, but do not distinguish between stepwise and concerted preassociation mechanisms.

In this and the accompanying paper we describe experiments that were designed to characterize the reactions of phosphorylating agents with anionic oxygen nucleophiles and water. This paper is concerned with the mechanism of these reactions; the following paper provides an evaluation of the role of electrostatic repulsion in reactions with anions and additional characterization of the nature of the transition state for phosphoryl transfer.

In this report we first provide evidence that phosphoryl transfer from pyridines to oxygen nucleophiles is concerted, like phosphoryl transfer between two pyridines. We then show that (1) the behavior of water in structure–reactivity correlations is like that of other oxygen nucleophiles that react through concerted, second-order processes, (2) positive rate deviations that arise from solvolysis by an additional pathway through a metaphosphate intermediate are not observed, and (3) the data are inconsistent with a stepwise preassociation mechanism with rate-limiting addition of water to a metaphosphate intermediate. Evidence for concerted phosphoryl transfer from acetyl phosphate to alcohols in aqueous solution is also described.

Experimental Section

Materials. γ-Picoline, pyridine, 2-methoxyethanol, 3-hydroxypropionitrile, acetohydroxamic acid, succinic acid, cacodylic acid, and trimethylamine *N*-oxide were purified by distillation or recrystallization. Aqueous solutions of phosphorylated γ-picoline (PicP),[17] phosphorylated 4-morpholinopyridine, phosphorylated pyridine, and acetyl phosphate were prepared as described previously.[8,12,18] 4-Morpholinopyridine was a gift from Dr. Mark Skoog.

Reactions of Phosphorylated Pyridines. Reactions of 2 × 10^{-4} M PicP, 10^{-4} M phosphorylated 4-morpholinopyridine, and 5 × 10^{-4} M phosphorylated pyridine at 25.1 ± 0.1 °C were followed spectrophotometrically at 256–258, 303, and 262 nm, respectively. These reactions were first order for $>3t_{1/2}$; end points were determined after $\geq 10t_{1/2}$. 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. Initial concentrations of acetyl phosphate in reaction mixtures were determined colorimetrically after conversion to acetohydroxamic acid by reaction with hydroxylamine by the method of Lipmann and Tuttle[19] with minor modification: 1 mL of 1:1 4 M $NH_2OH\cdot HCl$/3.5 M NaOH was added to a 1-mL aliquot; after $\geq$15 min at room temperature 4 mL of 10% $FeCl_3\cdot 6H_2O$ in 0.7 M HCl was added and the absorbance at 540 nm was compared to that with acetohydroxamic acid. Reactions of acetyl phosphate in 50% aqueous alcoholic solutions (v/v at 25 °C) were studied by product analysis after incubation overnight at 55 °C.

In general, the alkyl phosphate product was assayed as follows. Inorganic phosphate was precipitated by the addition of 2 mL of a solution of 0.27 M $MgCl_2$, 1.8 M NH_4Cl, and 1.5 M NH_4OH to a 2-mL aliquot of the reaction mixture and incubation at 4 °C for $\geq$3 h. After centrifugation, aliquots of the supernatant solution were removed to assay for small amounts (<10%) of remaining inorganic phosphate and for total phosphate; the amount of alkyl phosphate was given by the difference of these determinations. The amount of inorganic phosphate was determined colorimetrically by the method of Chen et al. without the ashing and hydrolysis steps;[20] the absence of an increase in absorbance with time in the presence of alkyl phosphates showed that the alkyl phosphates were stable under the conditions of the assay. Total phosphate was assayed as inorganic phosphate after conversion of the alkyl phosphate to inorganic phosphate by ashing with $Mg(NO_3)_2$ followed by acid hydrolysis of the pyrophosphate that was formed in the ashing step according to the method of Chen et al., except that the samples were dried prior to ashing.[20] In general, reactions were carried out in triplicate and assays for alkyl phosphates were carried out in quintuplicate.

The yield of ethyl phosphate from reactions of acetyl phosphate mono- and dianion in 50% aqueous ethanol that was determined by this procedure agreed within 1–5% with the yield that was determined by difference, by measuring inorganic phosphate[20] and total phosphate in the reaction mixture for the acetyl phosphate mono- and dianion reactions (data not shown). The total phosphate was measured as inorganic phosphate[20] after hydrolysis of acetyl phosphate in separate reaction mixtures, followed by addition of ethanol in order to match the assay conditions for the reactions that were carried out in the presence of ethanol. The precipitation and ashing method was used to determine yields of alkyl phosphate in the reactions of the other alcohols.

The following were shown to have no effect on the yield of alkyl phosphate for reactions of acetyl phosphate monoanion: a change in the ratio of formate buffer from 1:1 to 4:1 acid/base with each of the alcohols; variation in the concentration of formate buffer from 0.025–0.05 M in the ethanol and trifluoroethanol reactions; and variation of the initial concentration of acetyl phosphate from 2.5 to 20 mM and from 2.5 to 5.0 mM in the trifluoroethanol and 2-hydroxypropionitrile reactions, respectively. The following were shown to have no effect on the

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[17] The following abbreviations are used: PicP, phosphorylated γ-picoline monoanion; CHES, 2-(cyclohexylamino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

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Table I. Rate Constants for the Reactions of Oxygen Nucleophiles with Phosphorylated Pyridine Monoanions and a Phosphate Diester Monoanion^{a}

		phosphorylated compds			
nucleophile	pK_a^{b}	pyridine 10^2k/M^{-1} s^{-1}	γ-picoline 10^3k/M^{-1} s^{-1}	morpholinopyridine 10^6k/M^{-1} s^{-1}	methyl 2,4-dinitrophenyl phosphate^{c} 10^6k/M^{-1} s^{-1}
H_2O	−1.74	0.0193^{d}	0.0286^{d}	0.0541^{d}	0.00537
HCO_2^-	3.56^{e}		2.77		1.29
$(CH_3)_3NO$	4.6^{f}		0.4		0.42
$CH_3CO_2^-$	4.65^{g}		0.13^{h}		0.123
succinate^{2-}	5.35^{i}	0.34	0.6	1.6	
$(CH_3)_2AsO_2^-$	6.16^{g}	0.3	0.8	1.5	
CO_3^{2-}	9.78^{g}		0.92^{j}		1.23
$CF_3CH_2O^-$	12.4^{f}	0.7	1.8	11	
HO^-	15.74	1.5	4.5	31	842
HOO^-	11.6^{f}		480^{j}		1.73×10^5
F^-	3.2^{f}		1.59^{j}		32

^{a}Reactions of phosphorylated pyridines were carried out at 25.1 °C and ionic strength 1.5 (KCl). The reactions of trifluoroethoxide ion (see Results) and hydroxide ion were carried out without added buffer. The other reactions of phosphorylated pyridine and γ-picoline were carried out with 0.05 M CHES buffer, pH 8.0–8.5, and those of phosphorylated 4-morpholinopyridine were carried out with 0.05 M Tris buffer, pH 7.7; second-order rate constants were generally obtained from four to eight observed rate constants. ^{b}At 25 °C and ionic strength 1.0 (KCl) unless stated otherwise. ^{c}Reference 26; at 39 °C and ionic strength 1.0. ^{d}Reference 18. ^{e}Sayer, J. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1969**, *91*, 6353. ^{f}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). ^{g}Fox, J. B.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436. ^{h}Calculated in ref 14 from rate and equilibrium constants. ^{i}Wolfenden, R.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4390. ^{j}Reference 14.

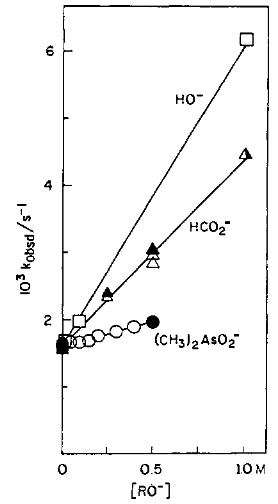

Figure 1. Effect of nucleophile concentration on the reactions of phosphorylated γ-picoline with cacodylate ion (○, ●), formate ion (△, ▲), and hydroxide ion (□) at 25 °C and ionic strength 1.5 (KCl). The reactions with cacodylate ion and formate ion were buffered with 0.05 M CHES, pH 8.0. The open and closed symbols represent data obtained in different experiments.

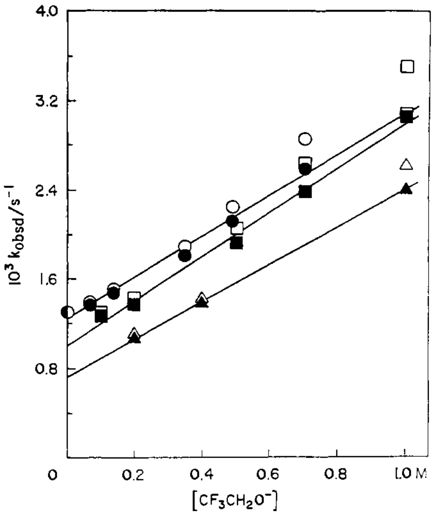

Figure 2. Effect of the concentration of trifluoroethoxide ion on the rate of disappearance of phosphorylated γ-picoline at 25 °C and ionic strength 1.5 (KCl), with total concentrations of 1.4 (○, ●), 2.0 (□, ■), and 4.0 M (△, ▲) trifluoroethanol. The open symbols represent the observed rate constants, and the closed symbols represent rate constants that were corrected for the decrease in trifluoroethanol inhibition and the increase in hydroxide ion catalysis with increasing concentration of trifluoroethoxide ion at each total trifluoroethanol concentration, as described in the text.

yield of alkyl phosphate for reactions of acetyl phosphate dianion: variation in the concentration of carbonate/bicarbonate buffer from 0.05 to 0.10 M in the reactions of ethanol and trifluoroethanol; and variation in the concentration of acetyl phosphate from 5 to 10 mM in the trifluoroethanol reaction.

Results

Figure 1 shows that the first-order rate constant for the disappearance of phosphorylated γ-picoline (PicP; **1**, X = CH_3) increases with increasing concentration of oxygen nucleophiles, such as hydroxide ion, formate ion, and cacodylate ion. Second-order rate constants obtained from these and similar data with other oxygen nucleophiles are listed in Table I; second-order rate constants for reactions of some of these nucleophiles with phosphorylated pyridine and phosphorylated 4-morpholinopyridine are also given. The observed rate constants for reactions of cacodylate ion with the phosphorylated pyridines were found to show upward curvature with increasing concentration of the nucleophile at >0.5 M, presumably from a specific salt effect; second-order rate constants were determined from data obtained with ≤0.5 M of this nucleophile (Figure 1).

The rate constants for reaction of trifluoroethoxide ion with the phosphorylated pyridines listed in Table I were determined as illustrated in Figure 2. The open symbols show the increase in the observed rate constant for the disappearance of PicP with increasing concentration of trifluoroethoxide ion at constant concentrations of total trifluoroethanol of 1.4, 2.0, and 4.0 M. The upward curvature at high pH is caused by (1) hydrolysis by hydroxide ion and (2) a small inhibition by trifluoroethanol of the pH-independent hydrolysis of PicP at low pH, which decreases as the trifluoroethanol is converted to its anion at high pH. The observed rate constants were corrected (<20%) for these factors, which were measured in separate experiments. The corrected rate constants are shown by the solid symbols and the same second-order rate constant of $(1.8 \pm 0.2) \times 10^{-3}$ M^{-1} s^{-1} was obtained from the lines through the solid symbols, for the three concentrations of total trifluoroethanol.

Figure 3 shows the dependence on the pK_a of the three pyridine leaving groups of the second-order rate constants for reactions of water, succinate dianion, cacodylate ion, trifluoroethoxide ion, and hydroxide ion with phosphorylated pyridines (Table I). The slopes of the lines in Figure 3 give values of $\beta_{lg} = -1.02, -0.96,$

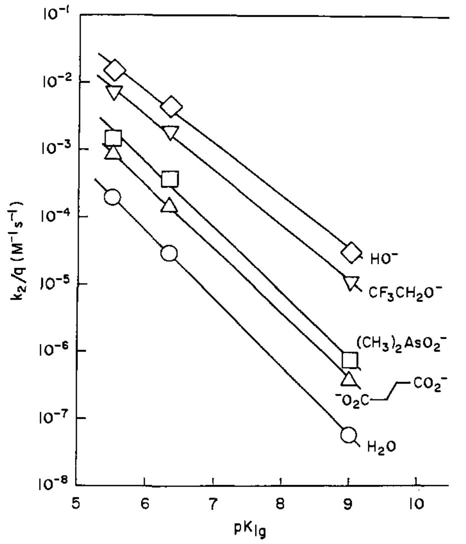

Figure 3. Brønsted-type plots of log k against the pK_a of the pyridine leaving group for the second-order reactions of phosphorylated pyridine, γ-picoline, and 4-morpholinopyridine with water, succinate dianion, cacodylate ion, trifluoroethoxide ion, and hydroxide ion (Table I). The rate constants were statistically corrected.

Table II. Ratios of the Rate Constants for Reaction of Alcohols and Water with Acetyl Phosphate Mono- and Dianions in 50% Aqueous Alcohol Solutions at 55 °C

		k_{ROH}/k_{HOH}[b]		
ROH	pK_{ROH}[a]	acetyl phosphate monoanion[c]	acetyl phosphate dianion[d]	ratio
CH_3CH_2OH	16.0	0.81	0.78	0.96
CH_3OCH_2OH	14.8	0.92	0.93	1.02
$NCCH_2CH_2OH$	14.0	0.83	0.74	0.89
CF_3CH_2OH	12.4	0.38	0.27	0.70

[a] 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. [b] Determined by product yields with use of eq 3 as described in the text. [c] In 0.025 M potassium formate buffer with ratios of 1:1 and 1:4 free base to acid. [d] In 0.05 M potassium carbonate buffer, extrapolated to zero [HO^-] (Figure 4).

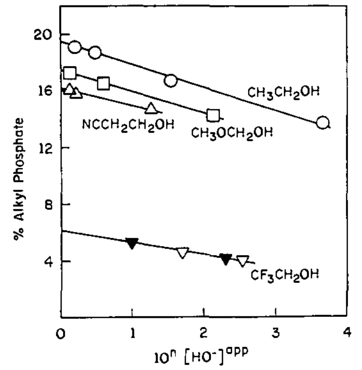

Figure 4. Yields of alkyl phosphates from acetyl phosphate dianion with varying concentrations of hydroxide and alkoxide ions in 50% aqueous alcohol for ethanol, methoxyethanol, 3-hydroxypropionitrile, and trifluoroethanol (open symbols with 5×10^{-3} M and closed symbols with 1.0×10^{-2} M acetyl phosphate). The values of n, for scaling the x axis, are 3, 3, 5, and 5, respectively. The apparent concentration of hydroxide ion was calculated from the observed pH at 25 °C.

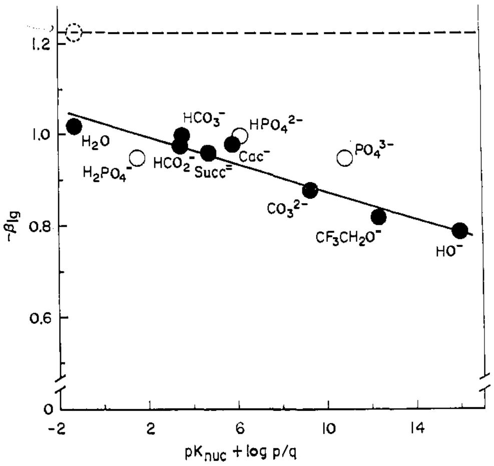

Figure 5. Plot of $\{-\beta_{lg}\}$ against the pK_a of the nucleophile for reactions of phosphorylated pyridine monoanions with oxygen nucleophiles (Figure 3).[14,21] The solid line is a least-squares fit to the data for the monoanionic nucleophiles. The dashed line represents the value of $\{-\beta_{lg}\}$ expected for complete bond breaking in the transition state and the dashed circle is the value expected for solvolysis by a stepwise preassociation mechanism with a metaphosphate intermediate (see text). Abbreviations: Succ$^=$, succinate dianion; Cac$^-$, $(CH_3)_2AsO_2^-$.

−0.98, −0.82, and −0.79, respectively. Evidence that the reactions of anionic oxygen nucleophiles with phosphorylated pyridines are nucleophilic is summarized in the following paper.[21]

The reactions of acetyl phosphate monoanion and dianion with alcohols in aqueous alcohol at 55 °C were studied by product analysis in order to determine ratios of the second-order rate constants for reaction with the alcohols (Table II). The ratios in Table II were determined from the fraction of phosphate from acetyl phosphate that was recovered as alkyl phosphate in 50% (v/v) aqueous alcohol solutions (27.75 M H_2O), according to eq 3.[22] The reactions of acetyl phosphate monoanion gave fractional

$$k_{rel} = k_{ROH}/k_{HOH} = \frac{(\text{frac ROPO}_3)}{[\text{ROH}]}\frac{[\text{HOH}]}{(1 - \text{frac ROPO}_3)} \quad (3)$$

yields of alkyl phosphate of 0.199, 0.174, 0.180, and 0.086 in 50% ethanol, methoxyethanol, hydroxypropionitrile, and trifluoroethanol, respectively. The reactions of acetyl phosphate dianion gave fractional yields of 0.194, 0.175, 0.163, and 0.062, respectively, after extrapolation to alkoxide ion independent yields, as described below. An increase in the ratio of carbonate to bicarbonate in the buffer resulted in a decrease in the yield of alkyl phosphate, presumably because of reaction of hydroxide and/or alkoxide ion at the carbonyl rather than the phosphate group of acetyl phosphate.[23] The yields of alkyl phosphate were corrected to the alkoxide-independent reaction by extrapolation to zero alkoxide/hydroxide concentration as shown in Figure 4. For each alcohol, the extrapolation is less than 15% from the lowest alkoxide concentration examined (with 9:1 bicarbonate/carbonate buffer); the extrapolation is greatest for trifluoroethanol because of its low pK_a.

Discussion

This discussion is divided into two sections that address the following: (1) whether or not there is a metaphosphate intermediate in the reactions of anionic oxygen nucleophiles with phosphorylated pyridines; and (2) whether or not there is a metaphosphate intermediate in the solvolysis of phosphorylated pyridines and acetyl phosphate in aqueous solution. Other aspects of the reactions of oxygen nucleophiles with phosphorylated pyridines are addressed in the following papers.[14,21]

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(21) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.*, following article in this issue.

(22) The formation of methyl phosphate (DiSabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4393–4400) and the alkyl phosphates described here from the solvolysis of acetyl phosphate mono- and dianions, and the incorporation of ^{18}O from [^{18}O]H_2O into inorganic phosphate that is formed in the hydrolysis of acetyl phosphate monoanion (ref 23) show that these solvolysis reactions proceed with P–O bond cleavage.

(23) Park, J. H.; Koshland, D. E., Jr. *J. Biol. Chem.* **1958**, *233*, 986–990.

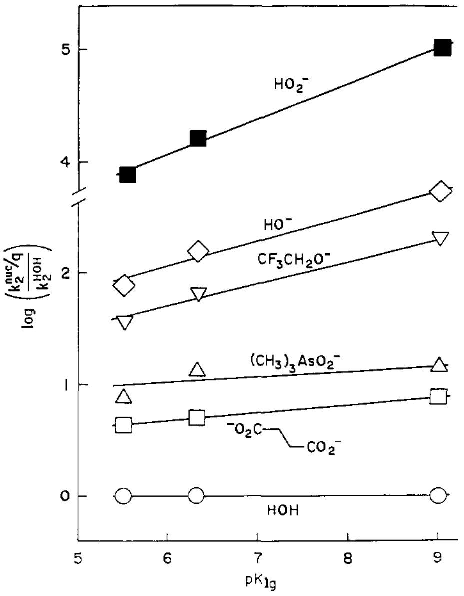

Figure 6. Brønsted-type correlation with the pK_a of the leaving group of the ratio of the second-order rate constants for reactions of phosphorylated pyridines with anionic nucleophiles and water. The rate constants (Table I and ref 21) are statistically corrected.

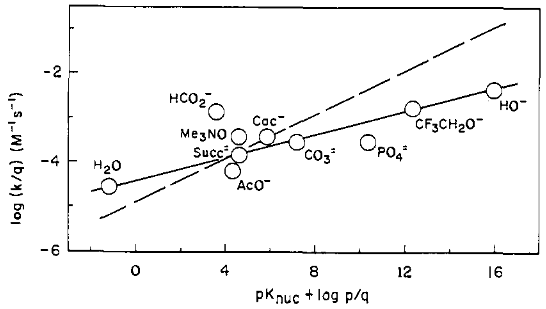

Figure 7. Brønsted-type plot of log k against the pK_a of oxygen nucleophiles for reactions of phosphorylated γ-picoline monoanion. The solid line of slope 0.13 and the dashed line of slope 0.25 are discussed in the text. The pK_a values and rate constants (Table I) are statistically corrected.

Reactions of Oxygen Nucleophiles with Phosphorylated Pyridines. The correlations shown in Figures 5–7 provide evidence for concerted phosphoryl transfer from pyridines to oxygen nucleophiles.

The reactions of oxygen nucleophiles with phosphorylated pyridines are second order (Figure 1). This suggests that they represent concerted, bimolecular reactions (Scheme I, A), but the possibility must also be considered that they proceed through an intermediate pyridine/metaphosphate ion–molecule pair with rate-limiting collapse to give products (k_2, Scheme I, B). Figure 5 shows that the values of $\{-\beta_{lg}\}$ obtained from Figure 3 and from Table III of the following paper[21] decrease with increasing pK_a of the oxygen nucleophile. The slope of the line in Figure 5 gives an interaction coefficient of $p_{xy} = \partial\beta_{lg}/\partial\, pK_{nuc} = 0.013$, (eq 2).

The experimental evidence for the decrease in $\{-\beta_{lg}\}$ with an increase in the pK_a of the nucleophile is illustrated more clearly by the comparison in Figure 6, in which the ratios of the rate constants for reactions of nucleophiles and water with phosphorylated pyridines are plotted against the pK_a of the pyridine leaving group. The increase in the slopes with increasing basicity of the nucleophile that is shown in Figure 6 corresponds to the decrease in $\{-\beta_{lg}\}$ shown in Figure 5. The values of $\{-\beta_{lg}\} = 0.95$, 1.00, and 0.95 for inorganic phosphate mono-, di-, and trianions are similar so that they deviate from the correlation line of Figure 5 (open symbols). However, these values show only small deviations in a correlation of $\{-\beta_{lg}\}$ and log k_2 for reaction of the oxygen nucleophiles with phosphorylated 4-morpholinopyridine, which suggests that the value of $\{-\beta_{lg}\}$ reflects the reactivity rather than the basicity of the inorganic phosphate anion. In addition, the thermodynamic stability of the different ionic species of pyrophosphate varies less with the pK_a of the leaving group than that of different phosphate esters, so that less variation in β_{lg} might be expected with inorganic phosphate nucleophiles compared with other oxygen nucleophiles.[14,24] The slope for hydrogen peroxide ion in Figure 6 also reflects its rate constant, rather than its pK_a; this will be discussed subsequently.[14]

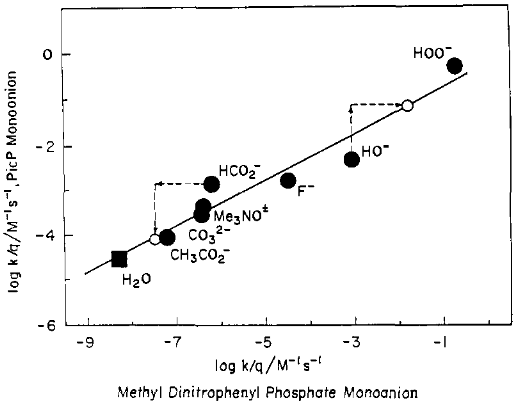

Figure 8. Correlation of the rate constants for the reaction of nucleophiles with phosphorylated γ-picoline monoanion and methyl 2,4-dinitrophenyl phosphate monoanion (Table I). The line of slope 0.51 is a least-squares fit to all of the data. The dashed lines and open symbols are described in the text. The rate constants are statistically corrected.

The decrease in $\{-\beta_{lg}\}$ with increasing strength of the nucleophile shows that there is an interaction between the nucleophile and the leaving group in the transition state. It provides evidence for coupling between the extent of cleavage of the P–N bond in the transition state and the reactivity of the nucleophile and, as required by eq 2, between the extent of bond formation to the oxygen nucleophile and the reactivity of the leaving group.[13] Such coupling is possible in a concerted reaction with a single transition state because there is simultaneous bonding to the nucleophile and to the leaving group; it is not consistent with a stepwise mechanism that proceeds through a metaphosphate intermediate. The value of $p_{xy} = 0.013$ is the same, within experimental error, as the value for concerted phosphoryl transfer between two pyridines[8] of $p_{xy} = 0.014$.

The decrease in $\{-\beta_{lg}\}$ with stronger nucleophiles is consistent with predictions from analysis of three-dimensional free energy–reaction coordinate diagrams for a concerted mechanism of phosphoryl transfer.[8,13,21] In contrast, an electrostatic interaction between the nucleophile and the leaving group, which might occur in either a concerted or a stepwise mechanism, would give an increase in $\{-\beta_{lg}\}$ with more basic nucleophiles.[13,25] An electrostatic interaction with an electron-donating substituent on a basic nucleophile would be more favorable for leaving groups with electron-withdrawing substituents; it would increase the rate of reaction for pyridine leaving groups of lower pK_a. This corresponds to an increase of $\{-\beta_{lg}\}$.

Finally, the small, but definite, positive slope of the Brønsted-type correlation of log k_2 with the pK_a of the oxygen nucleophiles for reactions of PicP (Figure 7) shows that there is nucleophilic participation in the transition state; positive slopes are also obtained for reactions of phosphorylated pyridine and 4-morpholinopyridine (Table I and ref 21; plots not shown). The solid line of slope 0.13 in Figure 7 provides a lower limit for β_{nuc} because nucleophiles of high pK_a tend to deviate negatively in Brønsted-type plots (see below and ref 14).

Solvolysis Reactions: A Metaphosphate Intermediate? The following data provide evidence that metaphosphate monoanion is not an intermediate in the hydrolysis of phosphorylated pyridines

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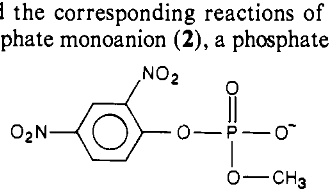
(24) Flodgaard, H.; Fleron, P. *J. Biol. Chem.* **1974**, *249*, 3465–3474. de Meis, L. *J. Biol. Chem.* **1984**, *259*, 6090–6097. Herschlag, D.; Jencks, W. P., unpublished calculations.

(25) Rothenberg, M. E.; Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1985**, *107*, 1340–1346.

and in the solvolysis of acetyl phosphate.

(1) Oxygen nucleophiles react with phosphorylated pyridines through a second-order, concerted reaction mechanism (Figures 5–7). If water reacts through an additional, stepwise mechanism with a metaphosphate intermediate, then the rate constant for the water reaction might be expected to show a positive deviation from structure–reactivity correlations for the concerted reactions. Figure 8 shows a correlation of the second-order rate constants for reactions of PicP monoanion with oxygen nucleophiles and fluoride ion and the corresponding reactions of methyl 2,4-dinitrophenyl phosphate monoanion (**2**), a phosphate diester.[26] The fit of the rate constant for water to the correlation (Figure 8, square) shows that the rate of solvolysis of PicP is equal to the rate expected for a bimolecular substitution reaction. The absence of a positive deviation for water from the correlation line of slope = 0.51 provides no evidence for a metaphosphate pathway in the reaction of phosphorylated γ-picoline with water. The phosphate diester reaction, which is used as the standard for nucleophilicity of the oxygen bases in this correlation, is believed to occur by nucleophilic displacement, not through a substituted metaphosphate intermediate.[27]



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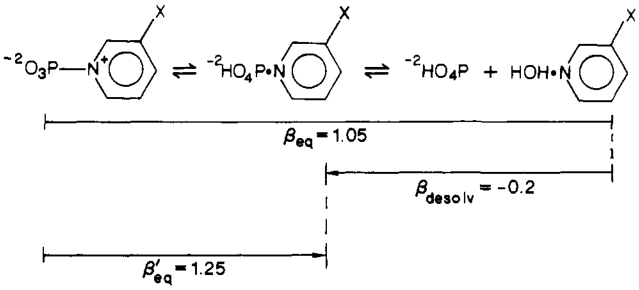
The correlation of Figure 5 is essentially linear over a range of reactivity of $\sim 10^8$ for the diester and 10^4 for PicP (correlation coefficient, 0.97). The 3-fold negative deviation for hydroxide ion and the 4-fold positive deviation of formate ion can be accounted for by solvation and steric effects, as described below. The behavior of the individual nucleophiles in this correlation is discussed elsewhere.[14,21]

The slope of 0.51 for the line in Figure 8 shows that the reaction of PicP is less sensitive to nucleophilicity than the reaction of the phosphate diester. This suggests that the transition state for the reaction of PicP has less bond formation to the nucleophile and more metaphosphate character than that for the phosphate diester reaction. A similar result has been obtained for reactions of 2,4-dinitrophenyl phosphate dianion and 2,4-dinitrophenyl methyl phosphate monoanion with substituted pyridine nucleophiles; the observed values of β_{nuc} are 0 and 0.34 for the mono- and diester, respectively.[4,28] After correction for desolvation of the pyridine nucleophile,[29] these values correspond to $\beta_{nuc}{}^{cor} = 0.17$ and 0.45, respectively, or a ratio of 0.4 for the sensitivities of the monoester and diester to the basicity of the nucleophile.

(2) The Brønsted-type correlation of log k against pK_{nuc} for the reaction of oxygen nucleophiles with PicP monoanion in Figure 7 shows no large positive deviation for water that would suggest a change to a metaphosphate mechanism for solvolysis. The solid line of slope 0.13 provides a reasonable fit to the rate constants for the nucleophiles including water. The dashed line of slope 0.25 is based on the value of $\beta_{nuc} = 0.25$ for the Mg^{2+}-catalyzed reaction of substituted acetate ions with PicP.[21] The small positive deviation of water from the line of slope 0.25 could result from the absence of electrostatic repulsion that decreases the reaction rate of the anionic nucleophiles.[21] It should be noted, however, that it is possible to have a change in mechanism with little or no positive deviation in rate near the point at which the mechanism changes.[30]

Scheme II



The positive deviation of the rate constant for formate ion of 20-fold in Figure 7 can be accounted for by decreased steric hindrance to nucleophilic attack for formate ion relative to acetate ion.[14,31] If it is assumed that the steric effect of 20-fold is the same for the reactions of formate ion with PicP and methyl dinitrophenyl phosphate, then the rate constants corrected for these steric effects fall on the correlation line in Figure 8 (open circle).

A factor that affects both the ordinate and abscissa terms of a linear free energy correlation by the same amount will cause a deviation from the correlation when the slope of the linear free energy relationship is less (or greater) than 1.0, because the deviation is the same for both axes and corresponds to a slope of 1.0. Thus, the dashed arrows in Figure 8 that account for the deviation of formate ion give a corrected rate constant that fits on the correlation line of slope 0.51.

The negative deviation of the rate constant for hydroxide ion in Figure 8 can be accounted for in the same way if partial desolvation of hydroxide ion is required prior to nucleophilic attack (eq 4). It has been suggested previously that negative deviations

$$HO^-(H_2O)_n \overset{K_d}{\rightleftharpoons} HO^-(H_2O)_{n-1} + H_2O$$

$$K_d = \frac{[HO^-(H_2O)_{n-1}][HOH]}{[HO^-(H_2O)_n]} \qquad (a_{HOH} = 1) \tag{4}$$

for hydroxide and alkoxide ions that have been observed for the deprotonation of carbon acids result from a requirement for desolvation.[33,34] A requirement for desolvation of hydroxide ion should, to a first approximation, slow the reactions with PicP and the phosphate diester to the same extent. Correction of the two rate constants by a factor of 16 gives a point that falls on the correlation line in Figure 8, as shown by the dashed arrows and open circle.[35] This correction corresponds to a value of K_d for desolvation of 1/16 = 0.06 (eq 4). A value of $K_d = 0.02$ has been estimated for desolvation of hydroxide ion in proton abstraction from thiazolium ions.[34] Hydroxide and alkoxide ions, in contrast to amines, can be solvated by several water molecules and do not require complete desolvation for reaction, so that different amounts of desolvation can occur in different reactions.

(3) The value of $\beta_{lg} = -1.02$ for the hydrolysis of phosphorylated pyridines (Figure 3) is less negative than the value of $\beta_{lg} = \beta_{eq}' = -1.25$ that is expected for complete P–N bond breaking in a stepwise preassociation mechanism through a metaphosphate intermediate. This limiting value of $\beta_{lg} = -1.25$ is shown by the dashed line in Figure 5, which is well above the value of $\beta_{lg} = -1.02$ for the hydrolysis reaction.

In order to form a metaphosphate intermediate, the P–N bond to the pyridine leaving group must break completely prior to

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(26) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165–1172.

(27) See, for example: Benkovic, S. J.; Schray, K. J. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York; 1973; Vol. 8, pp 201–238.

(28) The value of $\beta_{nuc} = 0.34$ for reactions of pyridines and the diester was determined from a least-squares fit to the rate constants of ref 26 with use of the pK_a values of ref 8.

(29) The values of β_{nuc} for attack by pyridines were corrected for desolvation by using the equation $\beta_{nuc}{}^{cor} = (\beta_{nuc} - \beta_{desolv})/(1 - \beta_d)$, with $\beta_{desolv} = -0.2$, from ref 9. This correction accounts for an initial desolvation of the pyridine prior to nucleophilic attack with the equilibrium for desolvation dependent on the pK_a of the pyridine (see Scheme II).

(30) Gandler, J. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 1937–1951. Amyes, T. L.; Jencks, W. P., unpublished experiments.

(31) See, for example: Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1373–1383.

(32) Jencks, W. P. In *Nucleophilicity*; Advances in Chemistry 215; Harris, J. M., McManus, S. P. Eds.; American Chemical Society: Washington DC, 1987; pp 155–167.

(33) Pohl, E. R.; Hupe, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 5634–5640. Bernasconi, C. F. *Tetrahedron* **1985**, *41*, 3219–3234.

(34) Washabaugh, M. W.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 683–692.

(35) Jencks, W. P.; Brant, S. R.; Gandler, J. R.; Fendrich, G.; Nakamura, C. *J. Am. Chem. Soc.* **1982**, *104*, 7045–7051.

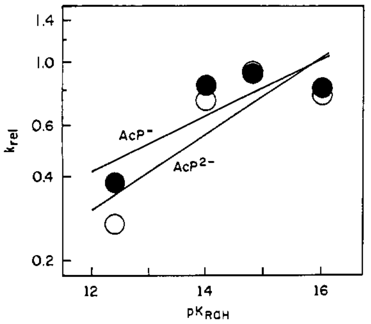

Figure 9. Dependence of the relative rate constants, k_{ROH}/k_{HOH} (Table II), for the reactions of alcohols with acetyl phosphate monoanion (●) and dianion (○) on the pK_a of the alcohol. The lines are least-squares fits to the data.

initiation of P–O bond formation to water. Because pyridines are stronger nucleophiles than water, the metaphosphate intermediate of a stepwise preassociation mechanism would react very rapidly with pyridine to regenerate reactants so that the addition of water would be rate limiting (k_2, Scheme I, B). Therefore, the value of β_{lg} would reflect complete breaking of the P–N bond in the rate-limiting transition state if there were a metaphosphate intermediate.

The value of $\beta_{eq}' = -1.25$ for P–N bond breaking to give the desolvated pyridine, which would be formed in a stepwise preassociation mechanism, was obtained as described by Scheme II. The overall reaction, to form solvated pyridines, has a value[8] of $\beta_{eq} = -1.05$ and the desolvation of tertiary amines[9] follows $\beta_d = -0.2$. Therefore, the equilibrium constant for formation of the unsolvated pyridine follows $\beta_{eq}' = -1.05 + (-0.2) = -1.25$.

It might be objected that an electrostatic interaction of the pyridine with a metaphosphate-like transition state for the addition of water could give a less negative value of β_{lg} than −1.25. However, negative values of β_{nuc} have been observed for the bimolecular reactions of amines with phosphorylated pyridines and a phosphate ester when nucleophilic attack of the pyridine is occurring in the rate-limiting transition state.[9] This is caused by the requirement for desolvation of the pyridine before reaction and shows that the pyridine interacts with phosphorus in the transition state for bond formation more weakly than it interacts with solvating water; the pyridine–phosphorus interaction would be still weaker if there were no bonding to phosphorus.

(4) The more negative values of β_{lg} for reactions of phosphorylated pyridines with weaker oxygen nucleophiles (Figure 5) show that there is coupling between bond formation and bond cleavage and provide evidence for a concerted phosphoryl-transfer reaction, as described above. The value of $\{-\beta_{lg}\} = 1.02$ with water as the nucleophile is within experimental error of the value of $\{-\beta_{lg}\} = 1.04$ that is predicted from the correlation of pK_{nuc} with $\{-\beta_{lg}\}$ for concerted nucleophilic reactions in Figure 5. This shows that water behaves like the other oxygen nucleophiles and provides evidence that hydrolysis occurs with coupling between bond formation and bond cleavage in a concerted second-order reaction.

(5) The selectivity for alcohols of increasing pK_a in the solvolysis of acetyl phosphate monoanion and dianion and the greater selectivity between alcohols for reaction with the dianion compared with the monoanion are consistent with nucleophilic involvement in concerted solvolysis reactions. Figure 9 shows that the relative rate constants, k_{ROH}/k_{HOH}, for the reactions with alcohols in the solvolysis of acetyl phosphate dianion and monoanion in aqueous alcohol solutions are consistent with slopes of 0.14 and 0.10, respectively, for a correlation of log k with the pK_a of the alcohol in water. These correlations depend on the rate constants for reaction with trifluoroethanol, but the slopes are certainly larger than zero. The positive slopes suggest that there is nucleophilic involvement by the alcohol in the transition state. These results confirm previous observations of selectivity between alcohols in phosphoryl-transfer reactions: the solvolysis of 2,4-dinitrophenyl phosphate dianion gives a yield of ethyl phosphate in 50% ethanol (v/v) that is 4-fold larger than the yield of trifluoroethyl phosphate in 50% trifluoroethanol,[10] and the solvolysis of acetyl phosphate dianion and monoanion in 1:1:1:1:1 trifluoroethanol/ethanol/methanol/2-propanol/water gives more ethyl phosphate than trifluoroethyl phosphate, as determined by ^{31}P NMR spectroscopy.[36]

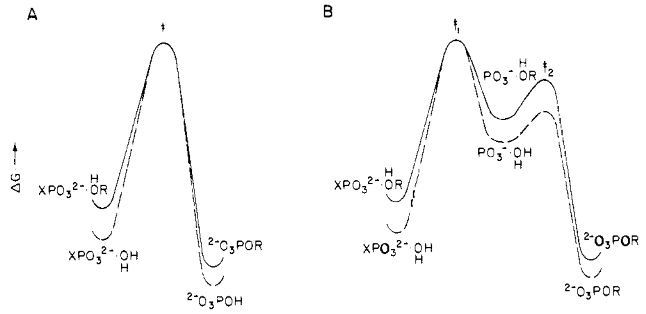

Figure 10. Free energy–reaction coordinate profile for phosphoryl transfer to water and an alcohol, HOH and ROH, in which the substrate, XPO_3^{2-}, is selectively solvated by water. The transition states are of equal energy for the case without (A) and with (B) a metaphosphate intermediate. In the reaction with a metaphosphate intermediate (B), the energy of the transition state for formation of products (‡2) is of lower energy for the reactions with water than for the reaction with the alcohol.

The small slopes of 0.10 and 0.14 for the reactions of acetyl phosphate monoanion and dianion in Figure 9 indicate a small amount of bond formation in the metaphosphate-like transition state of the reaction.[21] The difference in the slopes, which is beyond experimental error, is indicated more clearly by the decreasing product ratios for the mono- and dianions with increasing acidity of the alcohols, in Table II. This difference corresponds to a positive p_{xy} coefficient and is consistent with coupling between the alcohol nucleophile and the leaving group in the transition state of a concerted reaction.[8] The leaving group is much better in the monoanion than in the dianion reaction because there is complete or almost complete proton transfer to the leaving acetate ion in the transition state.[5]

It is of interest that Chanley and Feageson suggested in 1963 that the observed selectivity for reaction of phosphoramidates with different alcohols results from a bimolecular reaction mechanism.[3b]

Selective Solvation. Different product ratios have frequently been observed with different phosphorylating agents in mixed alcohol/water solvents, which would seem to be inconsistent with a common metaphosphate intermediate. These observations have been rationalized by the proposal that different substrates will have different solvent environments in the ground state, so that when an unstable metaphosphate intermediate is formed it will react rapidly with the surrounding solvent and give different products with different starting materials.[10,37] However, Figure 10 shows that ground-state solvation is irrelevant to product composition and that the product yield is determined only by the relative stability of the transition states for reaction with different solvents, in accord with the Curtin–Hammett principle.[38]

Figure 10A shows that selective solvation of the reactant by water will give equal yields of ester and alcohol products if the transition states for the two reactions are of equal energy, because the selective solvation results in a larger barrier for the reaction with water. Figure 10B shows that the same result is obtained if an unstable metaphosphate intermediate is formed in the rate-limiting step and the intermediate is too unstable to discriminate between the solvent components. However, this situation could give different product yields from different reactants if the first transition state had different preferential solvation of the two reactants. A common metaphosphate intermediate would give identical product yields that are determined by the energy of the second transition state if it could become diffusionally equilibrated

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(36) Satterthwait, A., personal communication.

(37) Bunton, C. A.; Chaimovich, H. *Inorg. Chem.* **1965**, *4*, 1763–1766. Haake, P.; Allen, G. W. *Bioorg. Chem.* **1980**, *9*, 325–341.

(38) Hammett, L. P. *Physical Organic Chemistry; Reaction Rates, Equilibria, and Mechanism*, 2nd ed.; McGraw Hill: New York; 1970; pp 119–120. Seeman, J. I. *Chem. Rev.* **1983**, *83*, 83–134.

with the solvent components, but it is known that several phosphoryl-transfer reactions to water do not proceed through a diffusionally equilibrated metaphosphate intermediate.[15]

When Could Metaphosphate Be an Intermediate? There are three circumstances in which a monomeric metaphosphate ion could be a reaction intermediate: (1) Metaphosphate exists in the gas phase as the free ion and as a complex with one or two water molecules; thermochemical data suggest that it forms phosphate in the presence of three or more water molecules.[39] It should exist in nonnucleophilic or very weakly nucleophilic solvents. (2) Stabilization of the metaphosphate species by substitution of sulfur or nitrogen for phosphoryl oxygen atoms allows substituted metaphosphate intermediates to be formed in nucleophilic solvents. (3) A metaphosphate intermediate might be formed if bond breaking and bond formation are uncoupled by a change to a weaker nucleophile or a better leaving group. However, crude calculations suggest that the solvent must be much less nucleophilic than water or unhindered alcohols in order to achieve such uncoupling in reactions of phosphorylated pyridines.

(1) Racemization of the phosphoryl group of *p*-nitrophenyl phosphate dianion upon solvolysis and the positional isotope exchange of adenosine 5′-[α,β-^{18}O]diphosphate in the weakly nucleophilic solvent *tert*-butyl alcohol provide evidence for a reaction intermediate in this solvent.[40,41] Metaphosphate could exist as an intermediate in *tert*-butyl alcohol because of steric hindrance to nucleophilic attack or because it is not in a position to react immediately with a solvent molecule when it is formed. It has been suggested that constraints may be imposed by a requirement for proton transfer from the bridge oxygen atom of *tert*-butyl phosphate to solvent or to a phosphoryl oxygen atom. Indeed, it is conceivable that this proton transfer is rate limiting, so that racemization occurs by several concerted phosphoryl transfers between molecules of *tert*-butyl alcohol before proton transfer takes place.[40,42] Molecular models suggest that removal of the proton from the attacking *tert*-butyl alcohol molecule by another molecule of *tert*-butyl alcohol is difficult because of steric hindrance.

Partial racemization of the phosphoryl moiety of an asymmetrical pyrophosphate derivative upon reaction with 2-O-benzylpropane-1,2-diol in dichloromethane also suggests that a metaphosphate intermediate is formed,[43] but partial racemization through initial reaction with the ether oxygen atom of the nucleophile cannot be rigorously excluded.

(2) There is evidence for sulfur- and nitrogen-substituted metaphosphate intermediates from the partial racemization (80%) of the phosphoryl moiety in the solvolysis of *p*-nitrophenyl thiophosphate in ethanol and from racemization in the alkaline solvolysis of methyl *N*-cyclohexylphosphoramidothioic chloride in aqueous dimethoxyethane.[44] Replacement of a phosphoryl oxygen atom by a sulfur atom or a nitrogen substituent may stabilize the metaphosphate structure because of the polarizability of sulfur and the ability of nitrogen to form double bonds.

(3) The interaction coefficient of $p_{xy} = \partial\beta_{\mathrm{lg}}/\partial \mathrm{p}K_{\mathrm{nuc}} = \partial\beta_{\mathrm{nuc}}/\partial \mathrm{p}K_{\mathrm{lg}} = 0.013$ (eq 2) for phosphoryl transfer between oxygen and nitrogen bases shows that bond formation in the transition state, as measured by β_{nuc}, decreases as the leaving group becomes better. This relationship predicts that with a sufficiently good leaving group a point should be reached at which there is no longer any nucleophilic involvement in the transition state for bond breaking, so that breaking of the bond to the leaving group and formation of the bond to the nucleophile become uncoupled. A metaphosphate intermediate could then exist if there is a diffusional barrier for addition of a nucleophile in addition to the activation barrier for addition of the leaving group, which is the reverse of the bond-breaking reaction. Analogous uncoupling could result from the $\partial\beta_{\mathrm{lg}}/\partial \mathrm{p}K_{\mathrm{nuc}}$ term of p_{xy} when the nucleophile becomes so weak that the bond to the leaving group breaks completely prior to activation-limited addition of the nucleophile. With both a very poor nucleophile and a very good leaving group there could be activation barriers for both addition of the nucleophile and addition of the leaving group to a metaphosphate intermediate.

The observed interaction coefficient of $p_{xy} = 0.013$ may be used to predict the amount of change in the $\mathrm{p}K_{\mathrm{a}}$ of the leaving group or nucleophile that would uncouple bond formation and bond cleavage in phosphoryl transfer. With γ-picoline as the leaving group ($\mathrm{p}K_{\mathrm{a}} = 6.3$) the value of β_{nuc} is $\sim$0.25,[21] so that the $\mathrm{p}K_{\mathrm{a}}$ of the leaving group must change by $\Delta \mathrm{p}K_{\mathrm{lg}} = \partial\beta_{\mathrm{nuc}}/p_{xy} = (0.25 - 0)/0.013 = 19$ $\mathrm{p}K_{\mathrm{a}}$ units in order to give $\beta_{\mathrm{nuc}} = 0$. Thus, a leaving group of $\mathrm{p}K_{\mathrm{a}} = 6.3 - 19 = -13$ is calculated to give uncoupling of bond formation to the nucleophile from bond breaking of the leaving group.[45] With water as the nucleophile ($\mathrm{p}K_{\mathrm{a}} = -1.7$) the value of β_{lg} is -1.02 for pyridine leaving groups, so that the $\mathrm{p}K_{\mathrm{a}}$ of the nucleophile must change by $\Delta \mathrm{p}K_{\mathrm{nuc}} = \partial\beta_{\mathrm{lg}}/p_{xy} = (1.25 - 1.02)/0.013 = 18$ $\mathrm{p}K_{\mathrm{a}}$ units in order to give $\beta_{\mathrm{lg}} = \beta_{\mathrm{eq}}' = -1.25$ and complete bond breaking in the transition state (Scheme II). Thus, a nucleophile of $\mathrm{p}K_{\mathrm{a}} = -1.7 - 18 = -20$ is predicted to give uncoupling.[45]

The values of $\mathrm{p}K_{\mathrm{nuc}} = -20$ and $\mathrm{p}K_{\mathrm{lg}} = -13$ that are required for uncoupling of bond formation and bond cleavage suggest that a transition to a stepwise mechanism will not occur with ordinary reagents or solvents. It is unlikely that there is a large difference between oxygen and nitrogen bases in this respect because p_{xy} is the same for phosphoryl transfer between two nitrogen bases and between an oxygen and a nitrogen base, and β_{nuc} is the same for nitrogen and oxygen leaving groups of the same $\mathrm{p}K_{\mathrm{a}}$.[8,21]

Registry No. **1** (X = 4-morpholino), 26322-06-5; **1** (X = H), 26322-03-2; **1** (X = 4-CH_3), 26322-04-3; PO_3^-, 15389-19-2; acetyl phosphate monoanion, 19926-70-6; acetyl phosphate dianion, 19926-71-7.

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(45) The assumption of a constant value of p_{xy} presumably gives upper limits for the estimated values of $\mathrm{p}K_{\mathrm{lg}}$ and $\mathrm{p}K_{\mathrm{nuc}}$ that give uncoupling because of the following. The interaction coefficient, $p_{xy} = \partial\beta_{\mathrm{lg}}/\partial \mathrm{p}K_{\mathrm{nuc}} = \partial\beta_{\mathrm{nuc}}/\partial \mathrm{p}K_{\mathrm{lg}}$, must change to a limiting value of zero at the edge of a reaction coordinate–free energy contour surface because the transition state cannot go over the edge. If this change in p_{xy} is continuous, then the amount of motion in the transition state toward the edge for a given perturbation in energy (change in $\mathrm{p}K_{\mathrm{a}}$) will decrease as the transition state approaches the edge: Ta-Shma, R.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 8040–8050. Thus, a larger decrease in $\mathrm{p}K_{\mathrm{lg}}$ or $\mathrm{p}K_{\mathrm{nuc}}$ would be required to reach the edge than is calculated in the text.