J. Am. Chem. Soc. 1989, 111, 7587–7596

Phosphoryl Transfer to Anionic Oxygen Nucleophiles. Nature of the Transition State and Electrostatic Repulsion[1]

Daniel Herschlag and William P. Jencks*

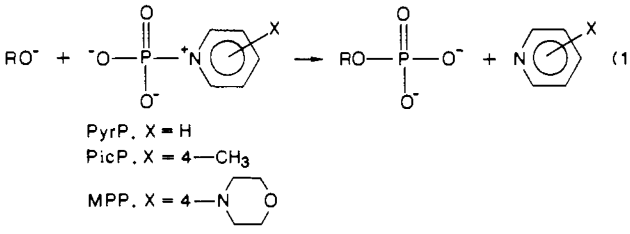
Contribution No. 1684 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254. *Received February 27, 1989*

Abstract: Bimolecular phosphoryl transfer to anionic oxygen nucleophiles from phosphorylated pyridine monoanions is observed in aqueous solution at 25 °C and ionic strength 1.5. The dependence on ionic strength of several reactions suggests that screening of charge repulsion with an increase in ionic strength from 0 to 1.5 causes only ~5-fold increases in rate for these reactions between monoanions. Comparisons with reactions of neutral substrates suggest that the electrostatic repulsion which remains at ionic strength 1.5 causes a decrease in rate of ~10-fold or less. The small values of $\beta_{nuc} \approx 0.3$ and the large negative values of $\beta_{lg} = -(1.0–0.8)$ for reactions of phosphorylated pyridines with anionic oxygen nucleophiles, and the small value of $\beta_{nuc} = 0.10$ for the reverse reaction of substituted pyridines with acetyl phosphate dianion, suggest that there is little bond formation to the oxygen nucleophile and much bond cleavage to the pyridine leaving group in the dissociative, metaphosphate-like transition state of a concerted displacement reaction. The absence of an effect of Mg^{2+} binding on the value of $\beta_{eq} = 1.05–1.1$ for the formation of phosphorylated pyridines shows that there is no significant charge/dipole interaction between Mg^{2+} and the substituent on the pyridine and that the effect of Mg^{2+} binding is less than the effect of protonation. The values of β_{nuc} for phosphoryl transfer to nitrogen nucleophiles are not significantly different with oxygen and nitrogen leaving groups of the same pK_a.

Phosphoryl transfer to anionic oxygen nucleophiles is an important biochemical reaction that usually involves a magnesium ion. Examples include the phosphorylation of AMP^{2-}, ADP^{3-}, acetate ion, and 3-phosphoglycerate ion.[2] Despite the biochemical importance of phosphoryl transfer to anionic nucleophiles, the transition state for the nonenzymatic reaction has not been characterized.

One reason that these reactions have seldom been examined may be a widely held belief that electrostatic repulsion is large in phosphoryl transfer to anionic oxygen nucleophiles; in fact, the possibility has been raised that enzymes can catalyze phosphoryl transfer by overcoming electrostatic repulsion.[2-4] However, the arguments that have been presented to support a large effect of electrostatic repulsion on the rate of phosphoryl transfer are inconclusive, as described in the Discussion.

We have observed and characterized phosphoryl transfer to anionic oxygen nucleophiles from pyridines (eq 1). The dependence of the rate on ionic strength suggests that electrostatic repulsion in the reaction of monoanionic oxygen nucleophiles with monoanionic phosphorylated pyridines is decreased by ~5-fold with an increase in ionic strength from 0 to 1.5, and comparisons with reactions of neutral substrates suggest that there is ≤10-fold electrostatic repulsion remaining at ionic strength 1.5. Linear free energy relationships suggest that the transition state is dissociative and metaphosphate-like, with decreasing dissociative character as the basicity of the oxygen nucleophile or the pyridine leaving group increases. There is no significant effect of Mg^{2+} on the structure of the transition state, as measured by linear free energy relationships in this and previous work.[5]



(1)

Experimental Section

Materials. γ-Picoline, pyridine, nicotinamide, 3-cyanopyridine, trifluoroacetic acid, cyanoacetic acid, chloroacetic acid, methoxyacetic acid, and glycolic acid were purified by distillation or recrystallization. Aqueous solutions of phosphorylated pyridines and acetyl phosphate were prepared as described previously.[5-7] 4-Morpholinopyridine was a gift from Dr. Mark Skoog. The concentration of hydrogen peroxide was determined by titration with potassium permanganate and by its absorbance at 260 nm, which was calibrated with standard solutions.

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 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 hydrogen peroxide ion were carried out with 70% free base. In general, second-order rate constants were obtained from four to eight data points.

Reactions of Acetyl Phosphate. The disappearance of acetyl phosphate was followed spectrophotometrically at 540 nm in aliquots from reaction mixtures containing 5×10^{-3} M initial 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.[8,9]

Results

There is a linear dependence of the observed rate constants for the disappearance of MPP,[10] PicP, and PyrP on the concentration of added oxygen nucleophiles. Plots of the observed rate constant against nucleophile concentration for the reactions of formate and cacodylate ions with PicP and for the reaction of acetate ion with PicP in the presence and absence of 0.33 M Mg^{2+} are shown elsewhere.[9,11] The slopes of these and similar plots in the absence and presence of 0.33 M Mg^{2+} give the second-order rate constants, k_2 and $(k_2^{Mg})_{obsd}$, for the reactions of PyrP, PicP, and MPP (Table I). The rate constants in parentheses in Table I represent observed rate increases of <25% at 1 M nucleophile and are included solely

---

[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.

[2] Walsh, C. *Enzymatic Reaction Mechanisms*; Freeman: San Francisco, CA, 1979; pp 210–239.

[3] Lowenstein, J. M. *Biochem. J.* **1960**, *75*, 269–274. Lloyd, G. J.; Cooperman, B. S. *J. Am. Chem. Soc.* **1971**, *93*, 4883–4889. Cooperman, B. S. In *Metal Ions in Biological Systems*; Sigel, H., Ed., Marcel Dekker: New York, 1976; Vol. 5, pp 79–125. Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw Hill: New York, 1969; pp 111–115.

[4] Westheimer, F. H. *Science* **1987**, *235*, 1173–1178.

[5] Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1987**, *109*, 4665–4674.

[6] Skoog, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 7597–7606.

[7] Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 7938–7946.

[8] Lipmann, F.; Tuttle, L. C. *J. Biol. Chem.* **1945**, *159*, 21–28.

[9] Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.*, preceding article in this issue.

[10] The following abbreviations are used: MPP, phosphorylated 4-morpholinopyridine monoanion; PicP, phosphorylated γ-picoline monoanion; PyrP, phosphorylated pyridine monoanion; MDPP, methyl 2,4-dinitrophenyl phosphate monoanion; CHES, 2-(cyclohexylamino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

[11] Herschlag, D.; Jencks, W. P., in preparation.

0002-7863/89/1511-7587$01.50/0

Table I. Second-Order Rate Constants for Reactions of Oxygen Nucleophiles with Phosphorylated Pyridine Monoanions in the Absence and Presence of Mg^{2+}[a]

		PyrP			PicP			MPP		
nucleophile	pK_a[b]	k_2	$(k_2^{Mg})_{obsd}$[c] $10^2k/M^{-1}\,s^{-1}$	k_2^{Mg}[d]	k_2	$(k_2^{Mg})_{obsd}$[c] $10^3k/M^{-1}\,s^{-1}$	k_2^{Mg}[d]	k_2	$(k_2^{Mg})_{obsd}$[c] $10^6k/M^{-1}\,s^{-1}$	k_2^{Mg}[d]
HOH[e]	−1.74	0.0193	0.0144	0.0115	0.0286	0.0200	0.0148	0.054	0.032	0.0186
$CF_3CO_2^-$	0.23[f]						≤0.3[g]			
$CNCH_2CO_2^-$	2.23[b]	(0.11)	0.27	0.37	(0.22)	0.64	0.89	(0.69)	1.40	1.83
$ClCH_2CO_2^-$	2.65[b]					0.75				
$CH_3OCH_2CO_2^-$	3.33[b]				(0.27)	0.85	1.20			
$HOCH_2CO_2^-$	3.62[h]				0.48	1.89	2.74			
HCO_2^-	3.56[i]	1.56	1.64	1.69	2.77[j]	3.03[k]	3.19[k]	6.35	6.36	6.37
$CH_3CO_2^-$	4.65[l]	0.08[m]	0.88	1.4	0.13[m]	1.60[k]	2.46[k]	(0.71)	4.2	6.3
$CH_3CH_2CO_2^-$	4.70[n]				(0.12)	1.17	1.80			
$(CH_2CO_2^-)_2$	5.35[o]	0.34[j]	2.3	3.5	0.6[j]	4.5	6.9	1.7[j]	10.6	16
$(CH_3)_2AsO_2^-$	6.16[l]	0.3[j]	2.4	3.7	0.75[j]	4.9	7.4	1.6[j]	15	23
$(CH_3)_3NO$	4.6[f]				0.4[j]					
HCO_3^-[k]	3.8[f]	2.9			5.4			10.6		
CO_3^{2-}[k]	9.78[l]	0.6			0.92			4.5		
$H_2PO_4^-$[k]	1.72[l]	0.5			0.58			2.0		
HPO_4^{2-}[k]	6.49[l]	0.9			1.3			2.8		
PO_4^{3-}[k]	11.44[p]	1.0			1.4			4.3		
$CF_3CH_2O^-$[j]	12.4[f]	0.7			1.8			11		
HO^-[j]	15.74	1.5			4.5			31		
HOO^-[k]	11.6[d]				480					

[a] At 25.1 °C and ionic strength 1.5 (KCl); reactions of PyrP and PicP were buffered with 0.05 M CHES, pH 8.0–8.5 and reactions of MPP were buffered with 0.05 M Tris, pH 7.7. Values in parentheses are apparent second-order rate constants that represent observed catalysis of <25%. These small rate increases were not distinguished from specific salt effects and were used solely to obtain the values of k_2^{Mg} (see text). [b] At 25 °C and ionic strength 1.0 (KCl) unless noted otherwise. [c] Observed second-order rate constants in the presence of 0.33 M $MgCl_2$. [d] Second-order rate constants for the reaction of the complex of the phosphorylated pyridine with Mg^{2+} (Scheme I). [e] From ref 5. [f] 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. [g] Limit from the rate *decrease* of $0.2 \times 10^{-3}\ M^{-1}\ s^{-1}$ with trifluoroacetate ion and no Mg^{2+} and the rate increase of $<0.05 \times 10^{-3}\ s^{-1}$ with up to 1 M trifluoroacetate ion in the presence of 0.33 M Mg^{2+}. [h] Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *99*, 1206. [i] Sayer, J. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1969**, *91*, 6353. [j] Rate constants from ref 9. [k] Rate constants from ref 11. [l] Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436. [m] Calculated from rate and equilibrium constants in ref 11. [n] Sayer, J. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1973**, *95*, 5637. [o] Wolfenden, R.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4390. [p] At 30 °C, from Bruice, P. Y. *J. Am. Chem. Soc.* **1984**, *106*, 5959.

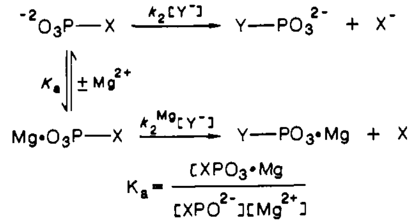
to obtain the second-order rate constants for the faster reaction of the nucleophile with the Mg^{2+}/phosphorylated pyridine complex (see below).

The following provide evidence that the oxygen bases react with phosphorylated pyridines by nucleophilic attack at phosphorus: (1) Formate ion reacts faster than the more basic acetate ion (Table I); this is consistent with an unfavorable nonbonding interaction with the methyl group of acetate, but not with general-base catalysis. (2) The large rate constants for the reactions of bicarbonate ion relative to those of carbonate and carboxylate ions (Table I) are consistent with facilitation of a nucleophilic reaction by an intramolecular interaction of the phosphoryl oxygen atoms and the hydroxyl group of bicarbonate ion, but not with general-base catalysis. (3) The α-effect nucleophiles, hydrogen peroxide ion and acetohydroxamate ion,[11] react rapidly with the phosphorylated pyridines. (4) Fluoride ion, which is known to be a strong nucleophile for attack at phosphorus, also undergoes a rapid reaction with the phosphorylated pyridines.[11,12] (5) UV spectra showed that γ-picoline is the product of the reactions of PicP with hydroxide and formate ions in the absence of Mg^{2+} and with acetate and formate ions in the presence of 0.33 M Mg^{2+}, and that 4-morpholinopyridine is the product of the reaction with formate ion in the presence and absence of 0.33 M Mg^{2+}.

The second-order rate constants for reaction of the oxygen nucleophiles with the complexes of Mg^{2+} and the phosphorylated pyridines in Table I (k_2^{Mg}) were obtained from the rate constants in the presence and absence of 0.33 M Mg^{2+} [Table I, $(k_2^{Mg})_{obsd}$ and k_2] and a value[11] of $K_a = 5\ M^{-1}$ according to eq 2, which was derived from Scheme I.

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

Scheme I



The dependence on Mg^{2+} concentration of the second-order rate constants for the reactions of acetate ion and bicarbonate ion with PicP and with MPP exhibits saturation behavior, which is consistent with reactions of the free and Mg^{2+}-bound phosphorylated pyridine according to Scheme I. This dependence was found to give $K_a = 5\ M^{-1}$ for the association of Mg^{2+} with PicP or MPP[11]; the same value of K_a was assumed for PyrP.

Second-order rate constants for the reverse reaction of substituted pyridines with AcP dianion to give the phosphorylated pyridine[7] have also been obtained. Figure 1 shows the dependence on the concentration of the pyridine nucleophile of the rate constant for disappearance of AcP. The curved lines in Figure 1 for nicotinamide and pyridine are nonlinear least-squares fits to the data that account for the apparent self-association of the pyridines, with reported association constants of $K_{SA} = 0.35$ and $0.5\ M^{-1}$, respectively;[13] an analogous fit to the data for γ-picoline was obtained with $K_{SA} = 3\ M^{-1}$.[11] Self-association of 3-cyanopyridine does not appear to be significant in the concentration range that was examined. These data and similar data with 0.33 M Mg^{2+} give the second-order rate constants k_2 and $(k_2^{Mg})_{obsd}$ in Table II. The saturation behavior with increasing Mg^{2+} concentration of the rate constant for reaction of AcP dianion with pyridine[11]

---

(12) Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1970**, 1172–1182.

(13) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216.

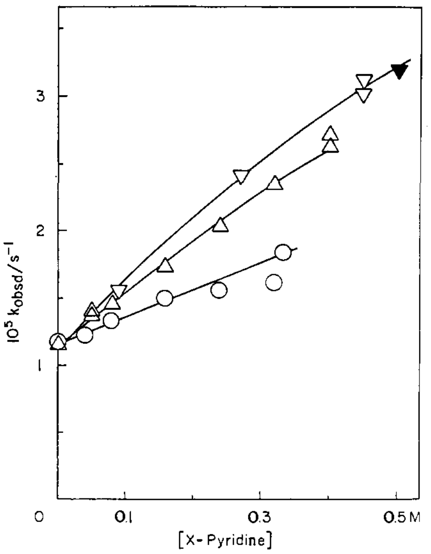


Figure 1. Dependence on the concentration of substituted pyridines of the rate constant for reaction with acetyl phosphate dianion (AcP) of 3-cyanopyridine (○), nicotinamide (△), and pyridine (▽) at 25.1 °C and ionic strength 1.5 (KCl). The reactions were carried out with 0.05 M MOPS, pH 6.7 (○, △), 90% pyridine as the free base, pH 6.5 (▽), or 0.05 M MOPS, pH 7.4 (▼). The lines are nonlinear least-squares fits to the data for reaction of the free pyridine base; the apparent self-association of nicotinamide and pyridine is accounted for with self-association constants of K_{SA} = 0.35 and 0.5 M^{-1}, respectively (39 °C, ionic strength 1.0; ref 13). No correction was made for self-association of 3-cyanopyridine.

Table II. Second-Order Rate Constants for Reaction of Substituted Pyridines with Acetyl Phosphate Dianion in the Absence and Presence of $Mg^{2+\,a}$

nucleophile	$pK_a{}^b$	K_{SA},[c] M^{-1}	k_2	$(k_2{}^{Mg})_{obsd}{}^d$ $10^5k/M^{-1}\ s^{-1}$	$k_2{}^{Mg\,e}$
3-cyanopyridine[f]	1.52[g]		2	19	31
nicotinamide[f]	3.55[h]	0.35	4.6	43	70
pyridine[i]	5.52[h]	0.5	5.9	72	118
γ-picoline[j]	6.33[h]	3	6.6	88	145

[a] At 25.1 °C and ionic strength 1.5 (KCl). [b] Determined at 25 °C and ionic strength 1.0 (KCl). [c] K_{SA} = [XPyr·XPyr]/$[XPyr]^2$; values at 39 °C and ionic strength 1.0 (KCl) from ref 13. [d] Observed second-order rate constant in the presence of 0.33 M $MgCl_2$. [e] Second-order rate constant for reaction of the Mg·acetyl phosphate complex, determined as described in the text, by using K_a = 4.4 M^{-1} for formation of the complex (ref 11). [f] With 0.05 M MOPS buffer, pH 6.7. [g] From ref 6. [h] From ref 26. [i] With 90% pyridine free base. [j] Rate constants from ref 11.

is consistent with reactions of free AcP and of a complex of Mg^{2+} and AcP, as described by Scheme I. The rate constants for reaction of the Mg·AcP complex ($k_2{}^{Mg}$) were obtained, as described above for the Mg^{2+} complexes of phosphorylated pyridines, from the values of k_2 and $(k_2{}^{Mg})_{obsd}$ (Table II) and a value[11] of K_a = 4.4 M^{-1} for formation of the Mg·AcP complex (Scheme I, eq 2); the calculated values of $k_2{}^{Mg}$ are listed in Table II.

The dependence on ionic strength of the second-order rate constant for reaction of the anions of hydrogen peroxide and PicP is shown by the circles in Figure 2. The observed rate constants are approximately the same in the presence of KCl and NaCl, (open and closed circles). The data were fit to the Debye–Hückel equation shown in eq 3, in which z_a and z_b are the charge on each

$$\ln (k/k_0) = \frac{1.02 z_a z_b I^{1/2}}{1 + (0.33\ \text{Å}^{-1})\alpha I^{1/2}} \quad 25\ °\text{C} \qquad (3)$$

reactant, I is the ionic strength, and α is treated as an adjustable parameter.[14] The upper line in Figure 2 is the fit to the data

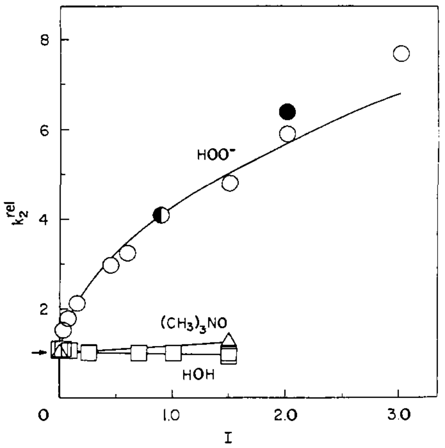


Figure 2. Dependence on ionic strength of the second-order rate constants for the reaction of phosphorylated γ-picoline monoanion (PicP) with hydrogen peroxide ion (○ (KCl), ● (NaCl); $10k_2/M^{-1}\ s^{-1}$], trimethylamine *N*-oxide [△ (KCl); $(3 \times 10^3)k_2/M^{-1}\ s^{-1}$], and water [□ (KCl); $(6 \times 10^4)k/s^{-1}$] at 25.1 °C. The upper line is a fit to the data according to the Debye–Hückel equation (eq 3) with the adjustable parameter α = 2.1 Å.

obtained with α = 2.1 Å. Figure 2 also shows the dependence on ionic strength of the rate constants for the reactions of PicP with trimethylamine *N*-oxide and water.

Discussion

Electrostatic Repulsion. Figure 2 shows that there is a large increase with increasing ionic strength of the second-order rate constant for the reaction of hydrogen peroxide anion with PicP monoanion; there is little or no effect of ionic strength on the rate constants for hydrolysis and for reaction with trimethylamine *N*-oxide, a zwitterion. Surprisingly, the Debye–Hückel equation (eq 3) provides a reasonable fit to the rate constants for the reaction with hydrogen peroxide ion over the range of ionic strength 0.03–3.0 (Figure 2, upper line). Extrapolation of the rate constants to zero ionic strength gives $k_2{}^\circ$ = 0.11 $M^{-1}\ s^{-1}$ and a rough estimate for the effect of overcoming electrostatic repulsion by increasing ionic strength from 0 to 1.5 of 0.5 $M^{-1}\ s^{-1}$/0.11 $M^{-1}\ s^{-1}$ = ~5-fold.[15]

The rate constants for several reactions of anionic phosphoryl substrates with anions have a similar dependence on ionic strength: (1) The increase in the second-order rate constant for the reaction of hydrogen peroxide ion with MPP with an increase in the ionic strength from 0.04 to 0.45, 0.90, 1.5, and 3.0 was found to be the same, within 5%, as that with PicP (not shown). (2) The increases in rate constants with increasing ionic strength for the reactions of anions with PicP were found to be 0.48/0.21 = 2.3-fold for hydrogen peroxide (μ = 0.15–1.5) $(4.5 \times 10^{-3})/(2.1 \times 10^{-3})$ = 2.1-fold for hydroxide ion (I = 0.2–1.5),[16] and $(2.77 \times 10^{-3})/(1.6 \times 10^{-3})$ = 1.7-fold for formate ion (I = 0.26–1.5). (3) There are similar rate increases of 2–3-fold with an increase in the ionic strength from 0.2 to 1–3 for the reactions of hydroxide ion with phenyl methylphosphonate and *p*-nitrophenyl methylphosphonate monoanions.[17] In contrast, the increase in the second-order rate constant for the reaction of trimethylamine *N*-oxide with PicP is only ~40%, and the rate constants for the hydrolysis of PicP (Figure 2) and MPP[5] decrease by ~10 and 50%, respectively, as the ionic strength is increased from 0.002 to 1.5.

Electrostatic Repulsion at High Ionic Strength. Rate constants for the reactions of anionic oxygen nucleophiles with the mo-

---

(14) Moore, J. W.; Pearson, R. G. *Kinetics and Mechanism*; Wiley: New York, 1981; pp 272–276. *Lange's Handbook of Biochemistry*, 13th ed.; Dean, J. A., Ed.; McGraw Hill: New York, 1985; Section 5, pp 3–6.

(15) There is little change in $k_2{}^\circ$ with changes in the adjustable parameter α in eq 3; for example the values k° = 0.103, 0.106, and 0.109 $M^{-1}\ s^{-1}$ are calculated from eq 3 with α = 1, 2.1, and 4 Å, respectively, and the experimental value of k_2 = 0.152 $M^{-1}\ s^{-1}$ at ionic strength 0.03; in contrast, these calculated lines give rate constants that vary considerably at ionic strength 1.5, with values of k_2 = 1.19, 0.51, and 0.33 $M^{-1}\ s^{-1}$, respectively, which can be compared to the observed value of k_2 = 0.48 $M^{-1}\ s^{-1}$.

(16) Jameson, G. W.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 53–57.

(17) Behrman, E. J.; Biallas, M. J.; Brass, H. J.; Edwards, J. O.; Isaks, M. *J. Org. Chem.* **1970**, *35*, 3063–3069.

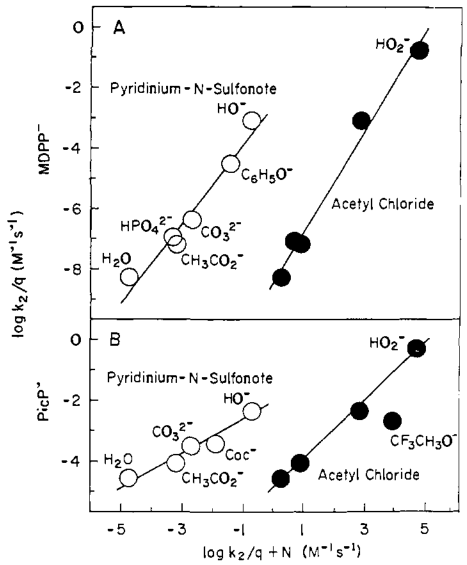


Figure 3. Logarithm of the second-order rate constants for reaction of oxygen nucleophiles with (A) methyl 2,4-dinitrophenyl phosphate monoanion (MDPP) or (B) phosphorylated γ-picoline monoanion (PicP) on the ordinate plotted against the corresponding rate constants for reaction with pyridinium-*N*-sulfonate (○; $N = 0$) or the corresponding rate constants for reaction with acetyl chloride (●; $N = -1$) on the abscissa. The rate constants for MDPP, PicP, pyridinium-*N*-sulfonate, and acetyl chloride were obtained at ionic strength 1.0 (39 °C),[24] 1.5 (25 °C, Table I), 1.0 (25 °C),[18] and 1.0 (22 °C, 2.5 vol % dioxane)[19] respectively, maintained with KCl (except for the rate constant for phenoxide ion and pyridinium-*N*-sulfonate, which was obtained at ionic strength 0.2). The lines are least-squares fits to the data. The second-order rate constants are statistically corrected. [Abbreviations: Cac⁻, cacodylate ion $((CH_3)AsO_2^-)$].

noanions of MDPP[10] and PicP at high ionic strength (1.0–1.5) are compared with those for the corresponding reactions of pyridinium-*N*-sulfonate[18] and acetyl chloride,[19] which have no net charge, in Figure 3. The absence of large deviations from the correlation lines for nucleophiles of varying charge type provides no indication of a large amount of rate inhibition from electrostatic repulsion. There are small positive deviations of $\leq$5-fold for uncharged water and small negative deviations of $\leq$2-fold for carbonate and phosphate dianions.[20] Water gives positive deviations of 3- and 20-fold from correlation lines determined with just the anionic nucleophiles for the comparisons of PicP and MDPP ions with pyridinium-*N*-sulfonate (not shown). These data suggest that a value of ~10-fold is a reasonable upper limit for the electrostatic repulsion between negative charges at high ionic strength in these reactions.

Comparison of the reactions of phosphorylated pyridine monoanions with those of the zwitterionic pyridinium-*N*-sulfonate should provide a reliable probe for electrostatic effects, because the transition states resemble pyridine and anionic PO_3^- or uncharged SO_3, respectively. Similar, dissociative transition states for these reactions are suggested by the similar small values of $\beta_{nuc} = 0.2$–0.3 for transfer of SO_3 and PO_3^- to oxygen and nitrogen nucleophiles and by the large negative values of β_{lg} for both SO_3 and PO_3^- transfer (see below).[18,21]

The plot of log k_2 for the reactions of oxygen nucleophiles with PicP monoanion at ionic strength 1.5 against log k_2 for the corresponding reactions of the phosphate diester monoanion, MDPP, at ionic strength 1.0, which is shown in Figure 8 of the previous paper,[9] has a single correlation line that is followed by uncharged, zwitterionic, monoanionic, and dianionic nucleophiles. This suggests that electrostatic repulsion at high ionic strength is similar in the reactions of the phosphorylated pyridine and the phosphate diester monoanions.

There is no large positive deviation for water or large negative deviation for dianionic oxygen nucleophiles compared with monoanionic nucleophiles in the Brønsted-type plot for reactions of PicP monoanion that is shown in Figure 7 of the previous paper.[9] This provides no indication of a large amount of electrostatic repulsion at ionic strength 1.5. The small negative deviations for carbonate dianion and phosphate trianion could result from strong solvation because carbonate and phosphate dianion also react slowly with neutral substrates.[22]

The absence of a large decrease in rate from electrostatic repulsion for phosphoryl transfer at high ionic strength could result from effective charge screening by nearby potassium ions, from interaction of the anions with a partial positive charge on the phosphorus atom in the transition state and, for carboxylate nucleophiles, from charge redistribution from the phosphoryl oxygen atom(s) that are near the nonreacting oxygen atom to distal phosphoryl oxygen atom(s). Analysis of X-ray crystal structures and spectroscopic studies have led to the conclusion that there is a redistribution of charge on the phosphoryl oxygen atoms upon ion-pair formation, which gives greater electron density on the atoms that interact with the cation.[23]

The conclusion that there is not a large amount of electrostatic repulsion from the negative charge of the phosphoryl group in reactions with oxyanions at high ionic strength contradicts a widely held belief that electrostatic repulsion is large for these reactions, even at high ionic strength. However, the arguments that have been proposed to support a large amount of electrostatic repulsion are inconclusive:

(1) It has been suggested that the absence of detectable second-order reactions of anionic oxygen nucleophiles with phosphate monoester dianions at ionic strength 1.0 results from a large amount of electrostatic repulsion.[24] However, the small values of β_{nuc} that have been observed in several related phosphoryl-transfer reactions (see Transition-State Structure and Table IV) can allow the reaction with water to mask that of other oxygen nucleophiles even if there is little or no electrostatic repulsion, because there is only a small increase in the second-order rate constant with increasing basicity of the nucleophile and the concentration of water is 55 M.

(2) The ratios of reactivities of an uncharged phosphate triester and a phosphate diester monoanion with anionic oxygen nucleophiles $(k_{tri}/k_{di})_O$ and with nitrogen nucleophiles $(k_{tri}/k_{di})_N$ have been measured at ionic strength 1.0. It has been suggested that the amount of electrostatic repulsion in the anion/anion reactions can be estimated from the ratio of these ratios, $\{(k_{tri}/k_{di})_O/(k_{tri}/k_{di})_N\}$, with the assumption that differences between P–O and P–N bonding in the transition state are not significant.[24] However, even if the difference between P–O and P–N bonding is ignored, these comparisons do not provide a clear-cut estimate of electrostatic repulsion. The values of $(k_{tri}/k_{di})_O$ are 60, 200, 1200, and 2000 for reactions of hydrogen peroxide, formate, hydroxide, and acetate anions, respectively, and the values of $(k_{tri}/k_{di})_N$ range from 2 to 100 for reactions of amines.[12,24] These data give ratios of $(k_{tri}/k_{di})_O/(k_{tri}/k_{di})_N$ that range from 0.6 (60/100) to 1000 (2000/2), so that no single value for electrostatic repulsion can be established.

(3) The difference of $\geq 10^5$ in the reactivity of hydroxide ion toward uncharged trimethyl phosphate compared with the phosphoryl group of dimethyl phosphate monoanion is a measure of the unfavorable electrostatic interaction in the reaction with the anion,[4] as well as the intrinsic difference in reactivity of the

---

[18] Hopkins, A.; Day, R. A.; Williams, A. *J. Am. Chem. Soc.* **1983**, *105*, 6062–6070.

[19] Palling, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 4869–4876.

[20] The second-order rate constant for the reaction of PicP with phosphate dianion is omitted because there is evidence that this reaction is catalyzed by intramolecular hydrogen bonding[11] so that the second-order rate constant gives a 3-fold positive deviation in these correlations. The deviation is in the direction opposite to that expected for an electrostatic effect.

[21] Bourne, N.; Hopkins, A.; Williams, A. *J. Am. Chem. Soc.* **1985**, *107*, 4327–4331.

[22] See, for example: Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 1778–1786.

[23] Spiro, T. G. In *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; Elsevier: Amsterdam, 1973; Vol. 1, pp 549–581. Baraniak, J.; Frey, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 4059–4060.

[24] Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165–1172.

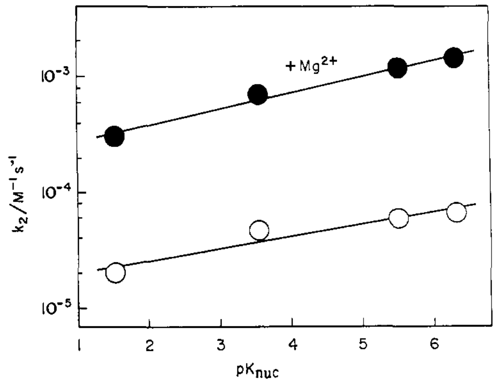


Figure 4. Brønsted-type plot for the reactions of substituted pyridines with acetyl phosphate dianion in the absence and presence of bound Mg^{2+} (Table II).

Table III. Values of β_{1g} for Phosphoryl Transfer from Substituted Pyridines to Oxygen Nucleophiles in the Absence and Presence of Bound $Mg^{2+\,a}$

		$\{-\beta_{1g}\}$	
nucleophile	$pK_a{}^b$	without Mg^{2+}	with Mg^{2+} bound
HOH	−1.74	1.02	1.08
$H_2PO_4^-$	1.72	0.95	
$NCCH_2CO_2^-$	2.23		0.98
HCO_2^-	3.56	0.98	0.99
HCO_3^-	3.8	1.00	
$CH_3CO_2^-$	4.65		0.96
$(CH_2CO_2^-)_2$	5.35	0.96	0.97
$(CH_3)_2AsO_2^-$	6.16	0.98	0.92
HPO_4^{2-}	6.49	1.00	
CO_3^{2-}	9.78	0.88	
PO_4^{3-}	11.44	0.95	
$CF_3CH_2O^-$	12.4	0.82	
HO^-	15.74	0.79	

[a] Obtained from the slopes of plots of log k_2 and log $k_2{}^{Mg}$ against the pK_a of the pyridine leaving group by using the second-order rate constants from Table I and the pK_a values from Table IV. [b] From Table I.

two esters. Hydroxide ion reacts 1.2×10^3 fold faster with the triester 2-(2,4-dinitrophenoxy)-2-oxydioxaphosphorinane than with the diester methyl 2,4-dinitrophenyl phosphate monoanion at ionic strength 1.0. The corresponding ratio for reactions with water is 30, which is 40-fold smaller than the ratio with hydroxide ion.[12,24] This factor of 40 is not much larger than the upper limit of ~10-fold given above for inhibition from electrostatic repulsion at high ionic strength for phosphoryl transfer between monoanions relative to transfer from monoanions to neutral nucleophiles. Furthermore, part of the 40-fold difference in the ratio of reactivities may be accounted for by a higher selectivity of the triester than of the diester toward nucleophiles.[12]

Reported electrostatic effects on reaction rates in aqueous solution at high ionic strength span a large range, which includes factors of ~10^3 for the acceleration of NAD^+ hydrolysis by a 2′ anion,[25] 40-fold for the reaction of anionic nucleophiles with 1-acetoxy-4-methoxypyridinium cation,[26,27] and 2-fold for reactions of alkyldiamine cations with *p*-nitrophenyl phosphate dianion.[7,13]

Table IV. Values of β_{nuc} for the Reactions of Oxygen Nucleophiles with Phosphorylated Pyridine Monoanions[a]

		β_{nuc}	
leaving group	$pK_a{}^b$	without Mg^{2+}	with Mg^{2+} bound
		Oxygen Nucleophiles	
pyridine	5.52	0.24^c	0.25^d
γ-picoline	6.33	0.27^c	0.25^d
4-morpholino pyridine	9.01^e	0.32^c	0.29^d
		Pyridine Nucleophiles	
acetate ion	4.65	0.10^f	0.14^f

[a] Determined from the rate constants in Table I obtained at 25.1 °C and ionic strength 1.5 (KCl). [b] At 25 °C and ionic strength 1.0 (KCl), from Tables I and II. [c] Estimated values determined by comparison with the rate constants for reaction of MDPP and the estimated value of β_{nuc} for reactions of oxygen nucleophiles with MDPP as described in ref 33. [d] Determined from the slopes of the Brønsted-type plots of Figure 5. [e] From ref 6. [f] Determined from the slopes of the Brønsted-type plots of Figure 4.

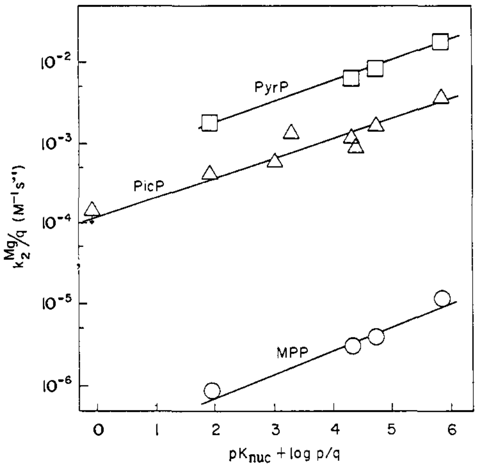


Figure 5. Brønsted-type plot for the reaction of substituted acetate ions and cacodylate ion with the Mg^{2+} complexes of PyrP, PicP, and MPP at 25.1 °C and ionic strength 1.5 (KCl). The rate constants ($k_2{}^{Mg}$) and pK_a values are from Table I; the rate constants are statistically corrected.

The slope of 1.74 in the correlation line relating rate constants for reactions of MDPP and acetyl chloride in Figure 3A and the estimated value of $\beta_{nuc} = 0.53$ for the reaction of oxygen nucleophiles with MDPP[28,29] give an estimate of $\beta_{nuc} = 0.53/1.74 = 0.30$ for the reaction of oxygen nucleophiles with acetyl chloride. A value of β_{nuc} could not be obtained directly from Brønsted-type plots for this reaction because a series of structurally similar oxygen nucleophiles was not investigated. The value of $\beta_{nuc} = 0.30$ is similar to the value of $\beta_{nuc} = 0.24$ for the reaction of pyridines with acetyl chloride.[19]

Transition-State Structure. The Brønsted-type correlations of log k_2 and pK_{nuc} in Figure 4 give slopes of $\beta_{nuc} = 0.10$ and 0.14 for phosphoryl transfer from AcP to pyridine nucleophiles in the absence and presence of bound Mg^{2+}, respectively (Table IV).[30] These small values of β_{nuc} and the large negative values of $\beta_{1g} = -(1.08\text{–}0.79)$ for the reverse reactions of phosphorylated pyridines with oxygen nucleophiles with and without bound Mg^{2+} (Table III)[31] suggest that there is only a small amount of P–N bonding in the transition state for phosphoryl transfer between pyridine and oxygen bases. The absence of significant increases in these values of β_{1g} and β_{nuc} upon binding of Mg^{2+} confirms the previous

---

(25) Johnson, R. W.; Marschner, T. M.; Oppenheimer, N. J. *J. Am. Chem. Soc.* **1988**, *110*, 2257–2263.

(26) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622–2637.

(27) A correlation like those of Figure 3 of log k_2 for the reaction of MDPP monoanion with oxygen nucleophiles[24] and log k_2 for the corresponding reactions of 1-acetoxy-4-methoxypyridinium cation[26] gives a positive deviation of ~400 for the reaction of water with MDPP from the correlation line for anionic oxygen nucleophiles, acetate, *p*-chlorophenoxide, phenoxide, hydroxide, and hydrogen peroxide ions. This deviation of 400-fold and the factor of 40-fold from electrostatic attraction in the reaction of the cationic ester with oxyanions[26] leaves a factor of ~10 for electrostatic repulsion in the reaction between the phosphate diester anion and oxyanionic nucleophiles; this value agrees with the upper limit for electrostatic repulsion given in the text.

(28) A value of $\beta_{nuc} \approx 0.53$ was estimated from $\beta_{1g} = -1.2$ for the intramolecular displacement of phenoxide ions by carboxylate ion in 2-carboxyphenyl phosphates (Khan, S. A.; Kirby, A. J.; Wakselman, M.; Horning, D. P.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 1182. Steffens, J. J.; Siewers, I. J.; Benkovic, S. J. *Biochemistry* **1975**, *14*, 2431) and $\beta_{eq} = 1.73$ for formation of a phosphate diester (ref 29) with use of eq 5: $\beta_{nuc} = 1.73 + (-1.2) = 0.53$.

(29) Bourne, N.; Williams, A. *J. Org. Chem.* **1984**, *49*, 1200–1204.

(30) The small values of β_{nuc} suggest that these reactions occur with attack at phosphorus and not at the carbonyl carbon atom.[11]

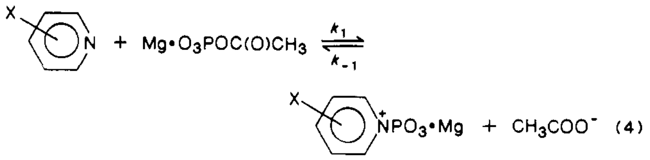
(31) See Figure 3 of ref 9 for representative plots.

conclusion that Mg^{2+} does not increase the associative character of the transition state for phosphoryl transfer.[5]

The Brønsted-type correlations of log k_2^{Mg} and pK_{nuc} in Figure 5 for reactions of carboxylate nucleophiles with the Mg^{2+} complexes of PyrP, PicP, and MPP give shallow slopes of $\beta_{nuc} = 0.25$, 0.25, and 0.29, respectively (Table IV),[32] which also show that bound Mg^{2+} does not give rise to large values of β_{nuc}. These small values of β_{nuc} provide upper limits for the values of β_{nuc} in the absence of Mg^{2+} and suggest that there is little P–O bond formation in the transition state in the presence and in the absence of bound Mg^{2+}. The values are upper limits because Mg^{2+} does not change the nature of the transition state[5] and a simple electrostatic interaction of Mg^{2+} with anionic oxygen nucleophiles should be more favorable with electron-donating substituents and increase the value of β_{nuc}. The average value of $\beta_{nuc} = 0.28$ in the absence of Mg^{2+}, from two-point Brønsted-type plots for the reactions of succinate and cacodylate ions with three phosphorylated pyridines, is consistent with these small values of β_{nuc}.[33] The slope of a Brønsted-type correlation[9] for a heterogenous series of oxygen nucleophiles with different charges and structures also gives a small slope of 0.1. Finally, the value of $\beta_{lg} = -1.03$ for the reaction of substituted phenyl phosphate monoesters with pyridine is also consistent with a small amount of P–O bonding in the transition state.[34]

These linear free energy relationships suggest that there is little P–N and little P–O bonding in the transition state for phosphoryl transfer between oxygen and pyridine bases; the transition state is dissociative and resembles metaphosphate monoanion. The same conclusion has been reached from similar results for other phosphoryl-transfer reactions, including values of $\beta_{nuc} = 0.15$–0.28 and 0–0.18 and $\beta_{lg} = -(0.97$–$0.73)$ and -1.03 for phosphoryl transfer to amines from pyridine and phenolate leaving groups, respectively, and values of β_{lg} in the range -1.0 to -1.2 for the hydrolysis of phosphate monoester dianions, benzoyl phosphate dianions, phosphoramidate monoanions, and phosphorylated pyridine monoanions.[5,6,13,16,34-39]

Charge/Dipole Interactions with Bound Mg^{2+}. The values of $\beta_{nuc} = 0.14$ for the reaction of substituted pyridines with the Mg^{2+} complex of AcP (k_1, eq 4) and $\beta_{lg} = -0.96$ for the reverse reaction



(k_{-1}, eq 4) (Tables III and IV) give a value of $\beta_{eq} = 0.14 - (-0.96) = 1.1$ (eq 5) for the transfer of a phosphoryl group to substituted

$$\beta_{eq} = \partial \log K_{eq}/\partial pK_a = \beta_{nuc} - \beta_{lg} \qquad (5)$$

pyridines at equilibrium. This value agrees closely with the previously reported values[6,39] of $\beta_{eq} = 1.05$ and 1.07 for the same reaction in the absence of Mg^{2+}. The observation that Mg^{2+} has the same affinity for phosphorylated pyridines formed from pyridines of $pK_a = 6.3$ and 9.0 also shows that the value of β_{eq} is similar with and without bound Mg^{2+}.[11] The similarity of the substituent effect in the presence and absence of bound Mg^{2+} shows that there is no significant charge/dipole interaction between the charges of the Mg^{2+} and the dipole of the pyridine substituent at high ionic strength. In contrast, the addition of a proton to a phosphate monoester dianion increases β_{eq} by 0.38 unit.[29] This is another example of a smaller effect from the ionic interactions with a metal ion than from the covalent interactions with a proton.[5,40]

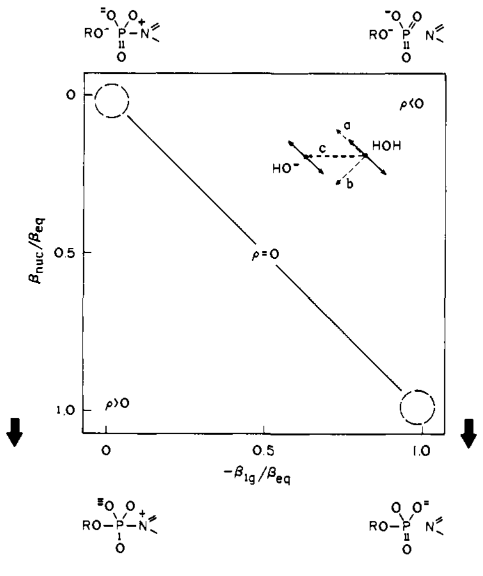


Figure 6. Reaction coordinate–energy contour diagram for phosphoryl transfer defined by structure–reactivity correlations. The position of the transition states for reaction of phosphorylated γ-picoline (PicP) with water and with hydroxide ion are indicated by H_2O and HO^-, respectively. The bold arrows show the perturbation to the energy surface from a change to a stronger nucleophile, such as the change from water to hydroxide ion. Dotted arrows a and b show the Hammond and anti-Hammond effects, respectively, from this perturbation, and the resultant arrow c shows the motion of the transition state that is predicted.

Changes in Transition-State Structure. The interaction coefficient $p_{xy} = \partial\beta_{lg}/\partial pK_{nuc} = 0.013$ (eq 6) describes the decrease in $\{-\beta_{lg}\}$ with increasing pK of the oxygen nucleophiles for the

$$p_{xy} = \partial\beta_{lg}/\partial pK_{nuc} = \partial\beta_{nuc}/\partial pK_{lg} = \partial^2 \log k/\partial pK_{nuc}\partial pK_{lg} \qquad (6)$$

reactions with phosphorylated pyridines.[9] The values of $\{-\beta_{lg}\}$ that fit this correlation are listed in Table III. The previously reported values of $p_{xy} = 0.014$ for phosphoryl transfer between pyridines and $p_{xy} = \sim 0.02$ for transfer from pyridines to other amines[6,41] are similar to the value of $p_{xy} = 0.013$.

These changes in β_{lg} and β_{nuc} suggest that the transition state becomes less dissociative as the pK_a of the nucleophile or leaving group increases. This is shown for the reactions of PicP with water and hydroxide ion in the reaction coordinate–energy contour diagram of Figure 6. This diagram has separate coordinates for bond formation (y axis) and for bond breaking (x axis), and the energy may be represented by contours that describe the dimension perpendicular to the plane of the diagram.[6,42] The position of

---

(32) The values of $\beta_{nuc} = 0.24$, 0.24, and 0.26 for reactions of substituted acetate nucleophiles with PyrP, PicP, and MPP, respectively, determined from the observed second-order rate constants in the presence of 0.33 M Mg^{2+} [$(k_2^{Mg})_{obsd}$] do not differ significantly from the values of β_{nuc} determined with the second-order rate constants, k_2^{Mg}, that were corrected for the apparent second-order rate constants obtained in the absence of Mg^{2+} (k_2) as described in the text. The reaction of cacodylate ion is included in the correlations to obtain β_{nuc}, but that of formate ion is not included because of the large positive deviation of its rate constant in the reaction without Mg^{2+}.

(33) Values of $\beta_{nuc} = 0.22$, 0.27, and 0.32 for the reactions of oxygen nucleophiles with PyrP, PicP, and MPP, respectively, can be estimated from the slopes of 0.41, 0.51, and 0.61 for plots of log k_2 for reactions of oxygen nucleophiles with PyrP, PicP, and MPP, respectively, against the value of log k_2 for the corresponding reactions with the phosphate diester methyl 2,4-dinitrophenyl phosphate and the value of $\beta_{nuc} \simeq 0.53$ for reaction of oxygen nucleophiles with a phosphate diester;[28] e.g., $0.41 \times 0.53 = 0.22$. The Brønsted-type correlations were statistically corrected.

(34) Kirby, A. J.; Varvoglis, A. G. *J. Chem. Soc. B* **1968**, 135–141.

(35) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415–423.

(36) DiSabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4400–4405.

(37) Benkovic, S. J.; Sampson, E. J. *J. Am. Chem. Soc.* **1971**, *93*, 4009–4016.

(38) Hsu, C.-M.; Cooperman, B. S. *J. Am. Chem. Soc.* **1976**, *98*, 5652–5657. Cooperman, B. S.; Hsu, C.-M. *J. Am. Chem. Soc.* **1976**, *98*, 5657–5663.

(39) Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1984**, *106*, 7591–7596.

(40) Breinig, J. B.; Jones, M. M. *J. Org. Chem.* **1963**, *28*, 852–854. Kluger, R.; Wasserstein, P.; Nakaoda, K. *J. Am. Chem. Soc.* **1975**, *97*, 4298–4303.

(41) Jencks, W. P.; Haber, M. T.; Herschlag, D.; Nazaretian, K. L. *J. Am. Chem. Soc.* **1986**, *108*, 479–483. Brønsted β values for amines can be corrected for desolvation according to $\beta_{nuc}^{cor} = (\beta_{nuc}^{obsd} - \beta_{desolv})/(1 - \beta_{desolv})$ with $\beta_{desolv} = -0.2$.

(42) Jencks, D. A.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 7948–7960. Jencks, W. P. *Chem. Rev.* **1985**, *85*, 511–527.

the transition state for the hydrolysis of PicP was obtained as follows: the value of $\beta_{lg} = -1.02$ for hydrolysis of phosphorylated pyridines (Table III) and the value[9] of $\beta_{eq}' = -1.25$ suggest that the P–N bond behaves as if it is 1.02/1.25 = 0.82 broken in the transition state (x axis), and the value of $\beta_{nuc} = \sim 0.25$ for reaction of oxygen bases with PicP (Table IV and above) and the corresponding value[29] of $\beta_{eq} = 1.35$ suggest that the P–O bond behaves as if it is 0.25/1.35 = 0.19 formed in the transition state (y axis). For the reaction of the stronger nucleophile, hydroxide ion, the value of $\beta_{lg} = -0.79$ for reaction with phosphorylated pyridines (Table III) suggests that the P–N bond behaves as if it is only 0.79/1.25 = 0.63 broken in the transition state (x axis); the value of β_{nuc} is also taken as 0.25 because there is no evidence for curvature in correlations of log k and pK_{nuc} for phosphoryl transfer reactions (Figures 4 and 5).[6,39] These linear free energy relationships place the transition states near the metaphosphate structure in the upper right corner of Figure 6, but the transition state for reaction of the weaker base, water, is nearer to this corner.

This change to less dissociative transition-state structures with increasing basicity of the nucleophile is predicted by Hammond and anti-Hammond effects. A change to a stronger oxygen base will stabilize the product in the lower right and the hypothetical pentavalent species in the lower left corner of Figure 6. The resulting decrease in energy at the lower edge of the diagram is indicated by the bold arrows. The effect of this perturbation on the position of the transition state can be resolved into movements parallel and perpendicular to the direction of the reaction coordinate at the transition state: The Hammond effect from the perturbation along the reaction coordinate causes the transition state to move toward the position of higher energy (dotted arrow a), while the anti-Hammond effect from the perturbation perpendicular to the reaction coordinate causes the transition state to slide downhill in energy (dotted arrow b). The net movement of the transition state, shown by arrow c, gives the observed change to a less negative value of β_{lg} with the stronger nucleophile.

The same reaction coordinate–energy contour diagram also predicts that the dissociative character of the transition state should decrease in the order: phosphate monoesters > phosphate diesters > phosphate triesters, and phosphate monoesters > phosphonate monoesters. Increasing substitution on the phosphoryl oxygen atoms of the phosphate monoester to give the di- and triesters or replacement of a phosphoryl oxygen atom with an alkyl group to give the phosphonate ester is expected to destabilize the metaphosphate structure in the upper right corner of Figure 6 and stabilize the pentavalent phosphate structure in the lower left corner. The transition state is predicted to slide downhill in energy, from an anti-Hammond effect, in the direction of arrow b toward a less dissociative and more associative structure. This change is described by a positive p_{xy}' coefficient, eq 7, which describes

$$p_{xy}' = \frac{\partial \beta_{nuc}}{\partial \sigma} = \frac{\partial \rho}{\partial pK_{nuc}} \tag{7}$$

interactions between the effects of substituents on the nucleophile and on the central atom;[42] substitution on oxygen that removes a negative charge is equivalent to an increase in σ.

Previously reported linear free energy relationships are consistent with this change in transition-state structure. The values of β_{nuc} = 0, 0.34, and 0.61 for the reactions of pyridines with 2,4-dinitrophenyl phosphate, methyl 2,4-dinitrophenyl phosphate, and 2-(2,4-dinitrophenoxy)-2-oxydioxaphosphorinane, respectively, suggest that there is an increase in the amount of bond formation to the nucleophile with increasing substitution on the phosphoryl oxygen atoms.[24,34,43,44] The extent of bond breaking in the transition state as measured by $\{-\beta_{lg}/\beta_{eq}\}$ is 1.05/1.35 = 0.76 and 1.06/1.73 = 0.61 for the reactions of pyridine with aryl phosphate monoesters and methyl aryl phosphate diesters, respectively, which corresponds to more bonding to the leaving group in the transition state of the diester reaction than the monoester reaction.[24,29,34] A better estimate of changes in the amount of bonding in the transition state is provided by β_{lg} than by β_{nuc} values because there is no change in net charge between the phosphate ester and the transition state. The value of β_{nuc} may be influenced by an electrostatic interaction between the charge on the ester and the substituent on the pyridine. The value of β_{lg} for the triester reaction with a pyridine nucleophile has not been determined because of the tendency of pyridines to act as general-base catalysts for the attack of water in these reactions.[12]

A change to a more associative transition state is also consistent with the structure–reactivity coefficients for three other reaction series, but the contribution of simple charge–dipole interactions to these coefficients has not been evaluated.

(1) The rate of intramolecular transfer of a diaryl phosphate group to a carboxylate group in a salicylate derivative, with endocyclic cleavage, is accelerated by electron-withdrawing substituents in the group being transferred.[45]

(2) There is an increase in the rate of the reactions of diethyl alkylphosphonates with hydroxide ion as the electron-withdrawing ability of the alkyl group increases in the series $CH_3 > CClH_2 > CCl_2H$.[46]

(3) The value of $\beta_{nuc} = 0.54$ for the reaction of pyridines with bis(2,4-dinitrophenyl) phosphate[35] is larger than $\beta_{nuc} = 0.34$ for the reaction with methyl 2,4-dinitrophenyl phosphate,[43] which corresponds to a positive p_{xy}' coefficient.

An increase in associative character of the transition state with increasing substitution on the phosphoryl group is also suggested by changes in isotope effects and in the entropy of activation. The large ^{18}O isotope effect for hydrolysis of aryl-^{18}O-2,4-dinitrophenyl phosphate is consistent with a large amount of bond breaking to the leaving group in a dissociative transition state, while the small ^{18}O isotope effect for the base-catalyzed reactions of the phosphate triester aryl-^{18}O-2,4-dinitrophenyl dibenzyl phosphate is consistent with little or no bond breaking in an associative transition state.[47] The near-zero values of the entropy of activation for hydrolysis of monosubstituted phosphate compounds, such as $\Delta S^{\ddagger} = +6.6$ eu for the hydrolysis of 2,4-dinitrophenyl phosphate, are consistent with a dissociative metaphosphate-like transition state, while the large negative entropy of activation of $\Delta S^{\ddagger} = -25.5$ eu for hydrolysis of bis(2,4-dinitrophenyl) phosphate is consistent with an associative transition state.[35,48]

Estimates of the p_{xy} coefficients for phosphoryl transfer between pyridine and oxygen bases show no significant difference in the presence and absence of bound Mg^{2+}; i.e., there is no indication of a more facile change in transition-state structure with Mg^{2+} bound; this is consistent with the previous observation that Mg^{2+} does not change the structure of the transition state.[5] A value of $p_{xy} = 0.01(2)$ with bound Mg^{2+}, which is similar to the value[9] of $p_{xy} = 0.013$ in the absence of Mg^{2+}, is obtained from the following data: (1) A slope of $p_{xy} = (\partial \beta_{nuc}/\partial pK_{lg}) = 0.01(3)$ for phosphoryl transfer with bound Mg^{2+} is obtained from a correlation of β_{nuc} for reactions of substituted acetate ions with phosphorylated pyridines (Table IV) and the pK_a of the pyridine leaving group (correlation coefficient, 0.98; not shown); and (2) an approximate value of $p_{xy} = \partial \beta_{nuc}/\partial pK_{lg} = 0.01(1)$ is obtained from the values of β_{nuc} = 0.14 and 0.17 for the reverse reaction of pyridines with the Mg^{2+} complexes of AcP (Figure 4; Table IV) and p-nitrophenyl phosphate,[5] respectively, and the values of pK_a = 4.4 and 7.1 for acetate ion and p-nitrophenolate ion. The

---

(43) The value of $\beta_{nuc} = 0.34$ for reactions with methyl 2,4-dinitrophenyl phosphate was obtained from a least-squares fit to a Brønsted-type correlation with the rate constants from ref 24 and the pK_a values for pyridines from ref 6. However, it should be noted that a value of $\beta_{nuc} = 0.56$ has been reported for the reaction of three substituted pyridines with 2,4-dinitrophenyl phosphate monoanion.[34]

(44) Correction of these β_{nuc} values for solvation of the pyridines[41] gives values of $\beta_{nuc}{}^{cor}$ = 0.17, 0.45, and 0.68 for the mono-, di-, and triesters, respectively.

(45) Bromilow, R. H.; Khan, S. A.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **1972**, 911–918.

(46) Aksnes, G.; Songstad, J. *Acta Chem. Scand.* **1965**, *19*, 893–897. Hudson, R. F.; Moss, G. E. *J. Chem. Soc.* **1964**, 1040–1045.

(47) Gorenstein, D. G.; Lee, Y.-G.; Kar, D. *J. Am. Chem. Soc.* **1977**, *99*, 2264–2267.

(48) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 510–513. Osterheld, R. K. *Top. Phosphorus Chem.* **1972**, *7*, 103–254.

values of β_{1g} in Table III show similar changes with varying pK_a of the oxygen nucleophile in the presence and absence of bound Mg^{2+}.

A value of $p_{xy} = 0.01(6)$ is obtained for the reaction of methyl aryl phosphate diesters with three pyridine nucleophiles, from the slope of plots of β_{1g} against pK_{nuc} (correlation coefficient, 0.98; not shown); the observed value of β_{1g} changes from −1.06 to −0.98 as the pK_a of the pyridine is increased from 5.5 to 10.1.[24] This value of p_{xy} for the reaction of a disubstituted phosphate substrate does not differ significantly from that observed for reactions of monosubstituted phosphate compounds; i.e., there is no evidence for a significant difference in the curvature of the energy surfaces for the two reactions.[42]

Identity Reactions and Characterization of the Transition State. When bond breaking is much more important than bond making the transition state of a reaction is dissociative, as in the transfer of phosphoryl and methoxymethyl[49] groups for example. The amount of dissociative character may be estimated from the difference between the values of β_{nuc} and $-\beta_{1g}$, to the extent that β_{nuc} and $-\beta_{1g}$ are measures of the amount of bond formation and breaking, respectively. This difference corresponds to the β value for the identity reaction, $\beta_i = \partial \log k_{ii}/\partial \mathrm{p}K$, and is given by eq 8,[50-53] which can be derived directly from linear free energy relationships.[54]

$$\beta_i = \beta_{nuc} + \beta_{1g} \tag{8}$$

The value of β_i can also be determined directly, with somewhat more difficulty, by measuring the rate constants of identity reactions, k_{ii}, with simultaneous changes in the nucleophile and leaving group, or by using interpolated rate constants from structure–reactivity correlations.

The values of k_{ii} = 3.8 and 0.01 $M^{-1}\ s^{-1}$ for phosphoryl transfer with 3-methoxypyridine and with 4-morpholinopyridine, respectively (Table V, footnote *d*), give a change in k_{ii} that is described by $\beta_i = \{\log 0.01 - \log 3.8\}/\{9.0 - 5.1\} = -0.66$. This value agrees with the average of the values of β_i = −0.73 and −0.63 that are obtained directly from the values of β_{nuc} = 0.17 and 0.22 for the reactions of substituted pyridines with phosphorylated 3-methoxypyridine and 4-morpholinopyridine, respectively, and β_{1g} = −0.90 and −0.85 for the reactions of phosphorylated pyridines with pyridine nucleophiles that have the same pK_a as 3-methoxypyridine and 4-morpholinopyridine, respectively.[6] These values of β_i differ because of the change in the values of β_{nuc} and β_{1g}.[55] The negative values of β_i are equivalent to the characterization of the transition state as dissociative or loose from the values of β_{nuc} and β_{1g}. The values of β_{nuc} = 0.25 for the reaction of carboxylate nucleophiles with PicP and β_{1g} = −0.96 for the reaction of phosphorylated pyridines with acetate ion, both with bound Mg^{2+} (Tables III and IV), give a similar estimate of β_i = −0.71 for the isoenergetic phosphoryl transfer between the oxygen and nitrogen bases, acetate ion and γ-picoline.[11]

Table V

(A) "Identity" Rate Constants for Phosphoryl Transfer between Substituted Pyridines and between a Substituted Pyridine and Formate Ion[a]

	N/N		N/O		N/O	
	nucleo-phile	leaving group	nucleo-phile	leaving group	nucleo-phile	leaving group
	XPyr	XPyr	formate	picoline	picoline	acetate
pK_i[b]	6.3	6.3	3.3	6.3	6.3	4.4
k_{ii}[c]	0.5[d]		2.8×10^{-3}[e]		6.6×10^{-5}[f]	
$\frac{k_{ii}(N/N)}{k_{ii}(N/O)}$			180		8×10^3	

(B) Rate Constants for Phosphoryl Transfer between a Nitrogen and Oxygen Base and Two Oxygen Bases with the Same Thermodynamic Driving Force[a]

	N/O		O/O	
	nucleophile	leaving group	nucleophile	leaving group
	water	picoline	water	formate
pK_a[b]	−1.3	6.3	−1.3	3.3
k[c]	2.9×10^{-5}[e]		5×10^{-6}[g]	
$\frac{k(N/O)}{k(O/O)}$	6			

[a] The value of K_{eq} = 1 for phosphoryl transfer between acetate ion (pK_a = 4.4) and a pyridine of pK_a = 6.6: AcP^{2-} + XPyr = acetate ion + $XPyrP^-$, was determined kinetically (ref 11). The value of K_{eq} = 1 for phosphoryl transfer between a pyridine of pK_a = 6.6 and formate ion (pK_a = 3.3) has been determined[11] from the above equilibrium and the estimated equilibrium constant of K_{eq} = 1 for the reaction AcP^{2-} + formate ion $\rightleftharpoons$ acetate ion + formyl phosphate^{2-}. A statistical correction for the two basic atoms of acetate ion and formate ion gives K_{eq} = 1 for phosphoryl transfer between acetate or formate ion and a substituted pyridine of pK_a = 6.3. [b] Statistically corrected. [c] Second-order rate constants ($M^{-1}\ s^{-1}$; $[H_2O]$ = 55.5 M) at 25 °C. [d] Interpolated from log k_{ii} and pK_i values with k_i = 3.8 and 0.01 $M^{-1}\ s^{-1}$ and pK_i = 5.1 and 9.0, respectively; these values of k_{ii} are taken from plots of log k against pK_{nuc} for phosphoryl transfer to pyridine nucleophiles from phosphorylated 3-methoxypyridine (pK_a = 5.1) and from phosphorylated morpholinopyridine (pK_a = 9.0).[6] [e] From Table I. [f] From Table II. [g] Extrapolated to 25 °C from $k = 2 \times 10^{-6}\ M^{-1}\ s^{-1}$ 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 acetyl phosphate (ref 40), 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*, fourth ed.; Wiley: New York, 1975; p 316.

It was pointed out above that the small values of β_{nuc} and the large values of $-\beta_{1g}$ place the dissociative transition state of phosphoryl-transfer reactions in the upper right quadrant of Figure 6, in which there is relatively little bonding to the nucleophile and the leaving group. It is convenient to describe this dissociative character of the transition state by a "tightness" or "disparity" parameter, τ, which is given by β_i and eq 9; τ gives the distance

$$\tau = \beta_i/\beta_{eq} + 1 \tag{9}$$

from the diagonal in Figure 6 such that values of $\tau = 0$ ($\beta_i/\beta_{eq} = -1$), $\tau = 1$ ($\beta_i = 0$), and $\tau = 2$ ($\beta_i/\beta_{eq} = 1$) place the transition state in the upper right corner, on the diagonal, and in the lower left corner, respectively. The value of τ has been used to characterize the transition states for methyl, hydride, phosphoryl, sulfuryl, and other transfer reactions.[51-53]

It has been suggested that the adherence to eq 8 for phosphoryl- and sulfuryl-transfer reactions is consistent with "first-order Marcus theory", in which small contributions from the quadratic

---

(49) Craze, G. A.; Kirby, A. J.; Osborne, R. J. *J. Chem. Soc., Perkin Trans. 2* **1978**, 357–368. Knier, B. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 6789–6798.

(50) Cohen, A. O.; Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 4249–4256. Bernasconi, C. F. *Pure Appl. Chem.* **1982**, *54*, 2335–2345. Lewis, E. S.; McLaughlin, M. L.; Douglas, T. A. *J. Am. Chem. Soc.* **1985**, *107*, 6668–6673.

(51) Kreevoy, M. M.; Lee, T.-S. H. *J. Am. Chem. Soc.* **1984**, *106*, 2550–2553. Grunwald, E. *J. Am. Chem. Soc.* **1985**, *107*, 4710–4720.

(52) Lewis, E. S.; Hu, D. D. *J. Am. Chem. Soc.* **1984**, *106*, 3292–3296.

(53) Williams, A. *J. Am. Chem. Soc.* **1985**, *107*, 6335–6339.

(54) Equation 8 is derived by the following series of equations, in which the first subscript of the rate constants refers to the nucleophile and the second refers to the leaving group. Equation i has k_{ij} added to and subtracted from the right-hand side. In eq ii, both sides of eq i have been divided by (pK_i − pK_j). The differential form shown in eq iii, which follows by making (pK_i − pK_j) infinitesimally small, gives eq 8.

$$\log k_{ii} - \log k_{jj} = (\log k_{ij} - \log k_{jj}) + (\log k_{ii} - \log k_{ij}) \tag{i}$$

$$\frac{(\log k_{ii} - \log k_{jj})}{(\mathrm{p}K_i - \mathrm{p}K_j)} = \frac{(\log k_{ij} - \log k_{jj})}{(\mathrm{p}K_i - \mathrm{p}K_j)} + \frac{(\log k_{ii} - \log k_{ij})}{(\mathrm{p}K_i - \mathrm{p}K_j)} \tag{ii}$$

$$\beta_i = \partial(\log k_{ii})/\partial \mathrm{p}K_i = \partial(\log k_{ij})/\partial \mathrm{p}K_i + \partial(\log k_{ij})/\partial \mathrm{p}K_j = \beta_{nuc} + \beta_{1g} \tag{iii}$$

(55) The change to more negative values of β_i with decreasing pK_i results from the positive p_{xy} coefficient; the change in the value of β_i and the p_{xy} coefficient suggest that the transition state becomes more dissociative as the pK_a of the nucleophile and leaving group decrease (see Changes in Transition-State Structure and ref 9). It may be noted that the change in β_i and the p_{xy} coefficient are obtained from the same data and are not independent determinations of a change in transition-state structure.

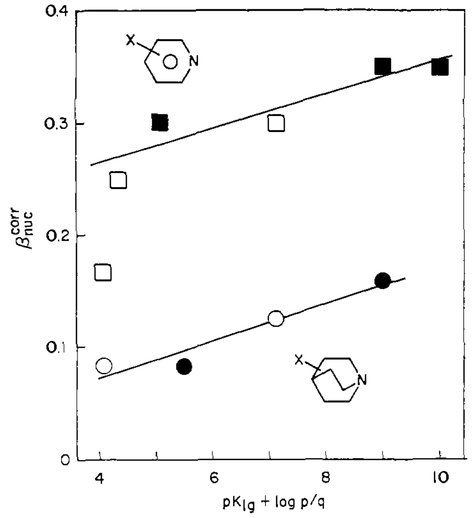


Figure 7. Dependence of β_{nuc} for phosphoryl transfer from oxygen (open symbols) and nitrogen leaving groups (closed symbols) on the pK_a of the leaving group for reactions with quinuclidine nucleophiles (circles) and pyridine nucleophiles (squares). The leaving groups for the reactions with quinuclidines are, in order of increasing pK_a, 2,4-dinitrophenolate ion, pyridine, *p*-nitrophenolate ion, and 4-morpholinopyridine, and those for the reactions with pyridines are 2,4-dinitrophenolate ion, acetate ion, 3-methoxypyridine, *p*-nitrophenolate ion, 4-morpholinopyridine, and ammonia. The values of β_{nuc} are corrected for solvation of the amine[41] with use of the equation $\beta_{nuc}^{cor} = (\beta_{nuc} - \beta_{desolv})/(1 - \beta_{desolv})$, with $\beta_{desolv} = -0.2$; the values of β_{nuc} are from Table IV and ref 5, 6, 13, 35, 41, and 59.

term of the Marcus equation are neglected.[53] However, adherence to this equation is not a test of Marcus theory, because eq 8 can be derived from linear free energy relationships without the use of Marcus theory.[54]

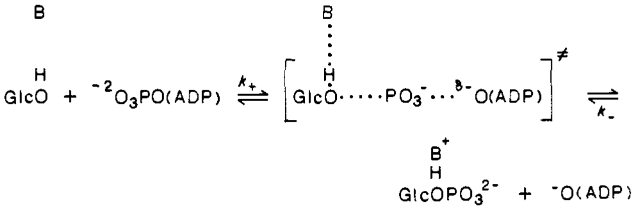
It is also possible to predict rate constants for cross reactions of nucleophiles and leaving groups of similar structure from the rate constants for the two identity reactions with Marcus theory.[52,56] However, this is not a test for Marcus theory because eq 10, which is derived from first-order Marcus theory and is used

$$\log k_{ij} = (\tfrac{1}{2})\ (\log k_{ii} + \log k_{jj}) + (\tfrac{1}{2}) \log K_{ij} \qquad (10)$$

to predict the rate constants for the cross reactions, can be derived without Marcus theory for bases that follow a single linear free energy relationship.[57,58]

Comparison of the Transition States for Reactions with Oxygen and with Nitrogen Bases. There is a surprisingly good correlation of β_{nuc}^{cor} for phosphoryl transfer to substituted quinuclidines from both phosphate esters and phosphorylated pyridines with the pK_a of the oxygen or nitrogen leaving group,[41] as shown by the open and closed circles, respectively, in Figure 7. The data are consistent with a single line of slope $p_{xy} = 0.016$ (eq 6). The analogous correlation for phosphoryl transfer to substituted pyridines from phosphate esters, acetyl phosphate, phosphorylated pyridines, and phosphoramidate, shown by the squares in Figure 7, is consistent with a single line for five of the six reactions, with a slope of $p_{xy} = 0.015$; the deviant point is for 2,4-dinitrophenyl phosphate.[5,6,13,35,59,60] Thus, the extent of bonding to a nitrogen nucleophile, as measured by β_{nuc}, is the same for phosphoryl transfer from an oxygen or a nitrogen leaving group of a given pK_a. The values of p_{xy} from the slopes in Figure 7 are the same, within error, as the values of 0.01 – 0.02 described above. The difference of 0.2 in the values of β_{nuc} for substituted pyridines and quinuclidines may arise from greater steric hindrance with the quinuclidines.[61]

Scheme II



Comparison of Rates of Phosphoryl Transfer with Oxygen and Nitrogen Bases. In order to compare kinetic factors for a group-transfer reaction with different classes of bases, the rate constants for reactions with the same thermodynamic driving force can be compared. For phosphoryl transfer between two pyridines, the identity, or "intrinsic" rate constants, k_{ii}, for reactions with no thermodynamic driving force ($K_{eq} = 1$) are $k_{ii} = 3.8$ M^{-1} s^{-1} for pyridines of pK_a = 5.1 and $k_{ii} = 0.01$ M^{-1} s^{-1} for pyridines of pK_a = 9.0 (Table V, footnote *d*). This difference in the values of k_{ii} and other variables make it difficult to reach generalizations that describe the rates of phosphoryl transfer with different classes of bases.

The intrinsic rate constant for the identity phosphoryl-transfer reaction between two substituted pyridines of pK_a = 6.3 is 180-fold larger than the intrinsic rate constant for transfer between this pyridine and formate ion [Table VA; $k_{ii}(N/N)/k_{ii}(N/O)$]. However, the rate constant for hydrolysis of a phosphorylated pyridine with pK_{lg} = 6.3 is only ~6-fold larger than that for hydrolysis of formyl phosphate (Table VB) so that the transfer between the nitrogen and oxygen bases, the pyridine and water, is not much faster than the transfer between the two oxygen bases, formate ion and water, which has the same thermodynamic driving force.

The similar values of $\beta_i = -0.67$ and -0.71 that were noted above for phosphoryl transfer between two pyridine bases and between pyridine and oxygen bases (with bound Mg^{2+}), respectively, suggest that the ratio $k_{ii}(N/N)/k_{ii}(N/O)$ is not very sensitive to changes in the pK_a of the pyridine and oxygen bases. However, the ratio $k_{ii}(N/N)/k_{ii}(N/O)$ is ~40-fold larger if acetate ion is used in the comparison instead of formate ion (Table VA). This suggests that steric and solvation factors, which affect both the stability of acetyl phosphate and the rate constants for phosphoryl transfer to acetate ion,[11] may affect this ratio.

The contributions of these and other factors to the ratios in Table V preclude a unique explanation for the differences in the ratios. Electrostatic interactions between charged groups as well as p–d orbital overlap in the ground state and the transition state that are different for nitrogen and oxygen bases could affect the ratios. A rate enhancement from "synergism" between identical attacking and leaving atoms[62] may influence phosphoryl transfer between two oxygen bases or between two nitrogen bases, and solvation of the nucleophilic atom can stabilize the transition state with an oxygen base, but not with a nitrogen base, because the nitrogen base has only one lone electron pair. This is expected to slow phosphoryl transfer with nitrogen bases compared with

---

(56) Lewis, E. S.; McLaughlin, M. L.; Douglas, T. A. *J. Am. Chem. Soc.* **1985**, *107*, 6668–6673.

(57) Lewis, E. S. *J. Phys. Chem.* **1986**, *90*, 3756–3759.

(58) Equations iv and v follow directly from linear free energy relationships. Addition of these equations and division by 2 gives eq vi, which can be rearranged to eq 10 with use of eq 5.

$$\log k_{ij} = \log k_{ii} + \beta_{lg}(pK_j - pK_i) \qquad \text{(iv)}$$

$$\log k_{ij} = \log k_{jj} + \beta_{nuc}(pK_i - pK_j) \qquad \text{(v)}$$

$$\log k_{ij} = (\tfrac{1}{2})(\log k_{ii} + \log k_{jj}) + (\tfrac{1}{2})(\beta_{nuc} - \beta_{lg})(pK_i - pK_j) \qquad \text{(vi)}$$

(59) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1965**, *87*, 3199–3209.

(60) The value of β_{nuc}^{cor} for the 2,4-dinitrophenyl phosphate reaction was omitted from the correlation because its negative deviation could result from a steric interaction with the ortho nitro group.

(61) Le Noble, W. P.; Asano, T. J. *J. Am. Chem. Soc.* **1975**, *97*, 1778–1782. Le Noble, W. P.; Miller, A. R. *J. Org. Chem.* **1979**, *44*, 889–891.

(62) Bunnett, J. F. *J. Am. Chem. Soc.* **1957**, *79*, 5969–5974. Pearson, R. G.; Songstad, J. *J. Org. Chem.* **1967**, *32*, 2899–2900; *J. Am. Chem. Soc.* **1967**, *89*, 1827–1836. Hupe, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 451–464.

oxygen bases,[41,63] which is opposite to the observed trend (Table V).

Implications for Enzyme-Catalyzed Phosphoryl-Transfer Reactions. The small values of β_{nuc} for phosphoryl transfer to oxygen nucleophiles suggest that in the transition state there is little bonding to the nucleophile and, therefore, little increase in charge and acidity of an attacking alcohol or water. Therefore, it is not immediately obvious that general-base catalysis should be important for catalysis of phosphoryl transfer by enzymes. For example, a base of $\mathrm{p}K_{\mathrm{a}} = 7$ at the active site is not expected to give substantial proton removal in the transition state for phosphorylation of a substrate such as glucose (Scheme II, k_+), because the $\mathrm{p}K_{\mathrm{a}}$ value of the attacking OH group in the transition state will be similar to the high $\mathrm{p}K_{\mathrm{a}}$ in the ground state.

The function of the base is clearer in the context of the overall reaction, which involves net proton transfer as well as phosphoryl transfer; a proton is removed from the attacking glucose and is added to the leaving oxygen atom in the reverse reaction. Reaction of glucose anion is possible, in principle, but the concentration of this species is small at neutral pH and the rate of its expulsion in the reverse reaction is very slow in the absence of protonation. There is evidence for basic groups on enzymes that are positioned to accept and donate a proton in the two directions.[64]

The advantage from general-acid–base catalysis is shown most clearly by the advantage from general-acid catalysis in the reverse reaction (Scheme II, k_-). The large negative values of β_{lg} for phosphoryl transfer from oxygen leaving groups (see Transition-State Structure) suggest that there is a large amount of bond cleavage in the transition state and a large development of negative charge on the oxygen atom of the leaving anion. Stabilization of this negative charge by proton transfer from a general acid, or by metal ions, can provide catalysis.[65] Examples in nonenzymic reactions include the following: the hydrolysis of phosphate monoester monoanions with poor leaving groups, which is much faster than that of the corresponding dianions because the proton is transferred to the oxygen leaving group in the transition state;[66] catalysis of ~20-fold by Mg^{2+} of the reaction of acetyl phosphate dianion with pyridines through stabilization of the large negative charge on the acetate leaving group;[11] catalysis of ~10^5-fold by Zn^{2+} for the hydrolysis of pyridine-2-carbaldoximyl phosphate dianion,[38] which is equivalent to an increase in the acidity of the leaving group by 10^4; and catalysis by Cu^{2+} of the hydrolysis of 2-(4(5)-imidazolyl)phenyl phosphate by a similar factor of $>10^4$-fold.[67] The species $\mathrm{RO(H^+)PO_3}^{2-}$ is an unfavorable substrate for the reverse reaction, if it exists at all, because it is a high-energy species that may not be present at a sufficient concentration to diffuse to the active site and account for the observed rate of catalysis.

Scheme II shows the proton predominantly on glucose rather than on a general base of $\mathrm{p}K_{\mathrm{a}} \sim 7$ in the transition state, as expected from the character of the transition state for the nonenzymic reactions. However, it is conceivable that the enzyme selects a somewhat more associative transition state, with a larger entropic barrier to reaction, because the enzyme can overcome much of the entropic barrier by holding the substrates precisely in place.[68] There could then be more bond formation to the substrate in a more associative transition state and, therefore, a larger advantage from proton transfer to a general base at the active site. Exact fixation of the substrates in the active site may also permit concerted phosphoryl transfer from ATP to the hydroxyl group of mevalonate 5-pyrophosphate in the reaction catalyzed by mevalonate 5-pyrophosphate decarboxylase, even though the substrate is a tertiary alcohol.[9,69]

Registry No. PyrP, 24265-37-0; PicP, 26322-04-3; MPP, 26322-06-5; $\mathrm{CF_3CO_2^-}$, 14477-72-6; $\mathrm{CNCH_2CO_2^-}$, 23297-32-7; $\mathrm{ClCH_2CO_2^-}$, 14526-03-5; $\mathrm{CH_3OCH_2CO_2^-}$, 20758-58-1; $\mathrm{HOCH_2CO_2^-}$, 666-14-8; $\mathrm{HCO_2^-}$, 71-47-6; $\mathrm{CH_3CO_2^-}$, 71-50-1; $\mathrm{CH_3CH_2CO_2^-}$, 72-03-7; $\mathrm{(-CH_2CO_2^-)_2}$, 56-14-4; $\mathrm{(CH_3)_2AsO_2^-}$, 15132-04-4; $\mathrm{CH_3C(O)OPO_3^{2-}}$, 19926-71-7; HOH, 7732-18-5; $\mathrm{H_2PO_4^-}$, 14066-20-7; $\mathrm{HPO_4^{2-}}$, 14066-19-4; $\mathrm{CO_3^{2-}}$, 3812-32-6; $\mathrm{HCO_3^-}$, 71-52-3; $\mathrm{PO_3^{3-}}$, 14901-63-4; $\mathrm{CF_3CH_2O^-}$, 24265-37-1; $\mathrm{HO^-}$, 14280-30-9; Mg, 7439-95-4; 3-cyanopyridine, 100-54-9; nicotinamide, 98-92-0; pyridine, 110-86-1; γ-picoline, 108-89-4; 4-morpholinopyridine, 2767-91-1.

---

(63) 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.

(64) Anderson, C. M.; Stenkamp, R. E.; McDonald, R. C.; Steitz, T. A. *J. Mol. Biol.* **1978**, *123*, 207–219. Evans, P. R.; Hudson, P. J. *Nature* **1979**, *279*, 500–504. Redina, A. R.; Cleland, W. W. *Biochemistry* **1984**, *23*, 5157–5168. Hellinga, H. W.; Evans, P. R. *Nature* **1987**, *327*, 437–439.

(65) Cooperman, B. S. *Methods Enzymol.* **1982**, *87*, 526–548.

(66) Butcher, W. W.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2420–2424. Bunton, C. A.; Llewellyn, D. R.; Oldham, K. G.; Vernon, C. A. *J. Chem. Soc.* **1958**, 3574–3587.

(67) Benkovic, S. J.; Dunikoski, L. K., Jr. *J. Am. Chem. Soc.* **1971**, *93*, 1526–1527.

(68) Haake, P.; Allen, G. W. *Bioorg. Chem.* **1980**, *9*, 325–341. Dietze, P.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 340–344.

(69) Reference 2, p 873.