Nucleophiles of High Reactivity in Phosphoryl Transfer Reactions: α-Effect Compounds and Fluoride Ion[1]

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Abstract: The second-order rate constants for reactions of hydrogen peroxide, acetohydroxamate, and fluoride anions with phosphorylated pyridine monoanions (H_2O, 25 °C, ionic strength 1.5) are larger than those of "normal" oxygen nucleophiles of the same pK_a by factors of 600-, 160-, and 30-fold, respectively. The values of $\{-\beta_{lg}\} = 0.70$ and 0.86 for reactions of phosphorylated pyridines with hydrogen peroxide and fluoride anions are smaller than predicted by a plot of $\{-\beta_{lg}\}$ against the pK_a of normal oxygen nucleophiles; however, they fit a plot of $\{-\beta_{lg}\}$ against log k for the reactions of oxygen nucleophiles with a phosphorylated pyridine. Thus, the transition-state structure, as measured by $\{-\beta_{lg}\}$, follows the reactivity, not the basicity of the nucleophiles. This is consistent with a thermodynamic origin for at least some of the rate enhancements for the α-effect nucleophiles and fluoride ions, such that the ratio of the phosphorus to proton basicities is larger for hydrogen peroxide and fluoride ion than for normal oxygen bases; i.e., the rate enhancements for these nucleophiles may reflect an enhanced thermodynamic affinity for the phosphoryl group that influences the stability and structure of the transition state. The observed changes in transition-state structure are not predicted for rate enhancements from hydrogen bonding in the transition state or from weak solvation of the α-effect nucleophile.

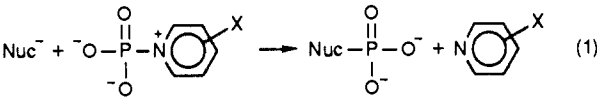
"α-Effect" nucleophiles, which have a heteroatom adjacent to the nucleophilic atom, often react rapidly compared with "normal" oxygen or nitrogen bases of comparable pK_a and have been found to have high reactivity with several phosphorus(IV) substrates.[2-14] The reason for this high reactivity has not been established, although several explanations have been proposed.[2-4]

We present data here that show that the rate constants and structure of the transition state, as measured by β_{lg}, for the reactions of hydrogen peroxide ion, an α-effect nucleophile, and of fluoride ion with phosphorylated pyridines (eq 1) are those expected for "normal" oxygen bases of higher basicity. This is consistent with an enhanced thermodynamic affinity of these ions for the phosphoryl group; that is, the ratio of phosphoryl basicity to proton basicity could be large for hydrogen peroxide and fluoride ions compared with "normal" oxygen bases, with the large phosphorus basicity providing stabilization in the transition state.

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

		phosphorylated		
nucleophile	pK_a	pyridine: $10^2k/M^{-1}\ s^{-1}$	γ-picoline: $10^3k/M^{-1}\ s^{-1}$	4-morpholinopyridine: $10^6k/M^{-1}\ s^{-1}$
HOO^- [b]	11.6[c]	150	480	5900
HO^- [d]	15.74	1.5	4.5	31
k_{HOO^-}/k_{HO^-}		100	110	190
$CH_3C(O)N(H)O^-$ [e]	9.4[c]			500
$CF_3CH_2O^-$ [d]	12.4[c]	0.7	1.8	11
F^- [f]	3.2[c]	0.61	1.59	7.0
$(CH_3)_2AsO_2^-$ [d]	6.16	0.3	0.75	1.6
k_{F^-}/k_{Cac^-}		2.0	2.1	4.4

[a] At 25 °C and ionic strength 1.5 (KCl). [b] The rate constants were obtained with 70% free base for PyrP and PicP and with 70% free base and varying free base and 0.1 M total H_2O_2 for MPP (Figure 1). [c] Reference 20. [d] Reference 36. [e] Rate constant obtained with 90% free base (Figure 1). [f] The rate constants for PyrP and PicP were obtained with 0.05 M CHES buffer, pH 8.3, and the rate constant for MPP was obtained with 0.05 M Tris buffer, pH 7.8 (Figure 1).



(1)

Experimental Section

Materials. γ-Picoline, pyridine, and acetohydroxamic acid were purified by distillation or recrystallization. Aqueous solutions of phosphorylated pyridine (PyrP),[16] γ-picoline (PicP), and 4-morpholinopyridine (MPP) were prepared as described previously.[15,17] 4-Morpholinopyridine was a gift from Dr. Mark Skoog.

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(14) There is a small rate enhancement for reactions of nitrogen α-effect bases at phosphorus with some substrates (ref 11 and 13) and not with others (ref 15).

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(16) The following abbreviations are used: phosphorylated pyridine, PyrP; phosphorylated γ-picoline, PicP; phosphorylated 4-morpholinopyridine, MPP; 2-(cyclohexylamino)ethanesulfonic acid, CHES; tris(hydroxymethyl)aminomethane, Tris; ethylenediaminetetraacetic acid, EDTA.

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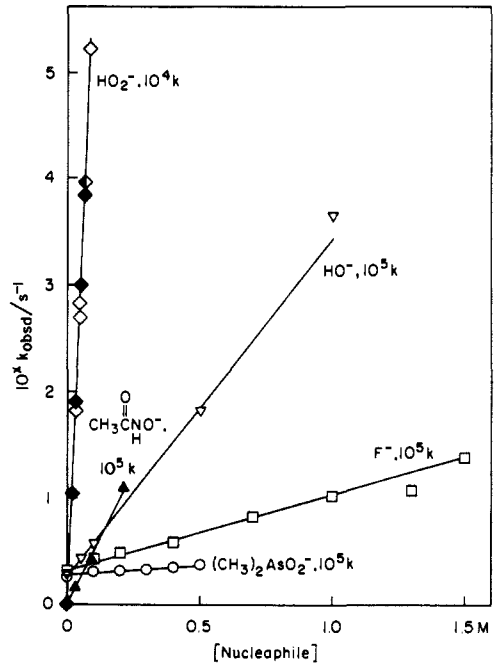

Figure 1. The dependence on nucleophile concentration of the rate constants for reaction of phosphorylated 4-morpholinopyridine monoanion (MPP) with cacodylate ion, fluoride ion, acetohydroxamate ion, hydroxide ion, and hydrogen peroxide ion at 25 °C and ionic strength 1.5 (KCl). The reactions of cacodylate ion were buffered with 0.05 M CHES, pH 8.3; those of fluoride ion were buffered with 0.05 M Tris, pH 7.8; and those of acetohydroxamate and hydroxide ions were buffered by the nucleophile, with 90% free base and varying percent free base, respectively. For reactions of hydrogen peroxide ion, either the fraction anion was varied with 0.1 M hydrogen peroxide (open symbols) or the total concentration of hydrogen peroxide was varied with 70% anion (closed symbols).

Methods. Reactions of 5×10^{-4} M PyrP, 2×10^{-4} M PicP, and 1×10^{-4} M MPP at 25.1 ± 0.1 °C and ionic strength 1.5 (KCl) were followed spectrophotometrically at 260–262, 258, and 305 nm, respectively. The reactions were first order for >3 $t_{1/2}$. Endpoints for the reactions of the phosphorylated pyridines with fluoride ion and of PyrP with hydrogen peroxide ion were determined after ≥10 $t_{1/2}$; endpoints for the reactions of PicP and MPP with hydrogen peroxide ion were determined from the absorbance at 5 $t_{1/2}$, which was corrected to the infinity value by multiplying the observed change in absorbance by 1.031;[18] the endpoint determined by this method gave a calculated change in total absorbance within 1% of that observed at 10 $t_{1/2}$ for reactions of PicP and MPP. The second-order rate constants for fluoride and hydrogen peroxide ions were determined from ≥8 individual rate measurements with 0–1.5 M fluoride ion and 0–0.01 M hydrogen peroxide ion. The pH was determined at the end of each reaction.

The concentration of a stock solution of hydrogen peroxide was determined by titration with potassium permanganate,[19] and values of ϵ_{260} = 17 and 230 M^{-1} cm^{-1} were obtained for HOOH and HOO^-, respectively. The concentration of HOO^- in reaction mixtures was determined prior to the addition of substrate according to the equation: $A_{260} = 17([H_2O_2]_{tot} - [^-OOH]) + 230[^-OOH]$; $[H_2O_2]_{tot}$ was determined from the concentration of the stock solution. The concentrations of hydrogen peroxide anion calculated from the absorbance at 260 nm agreed within ~5% with the values calculated from the total hydrogen peroxide concentration, the amount of hydroxide anion added, and the pK_a of hydrogen peroxide of 11.6.[20] In addition, the observed pH values of reaction mixtures were those expected for the calculated acid/base ratio and pK_a = 11.6. Reactions with hydrogen peroxide anion were carried out in the presence of 1×10^{-4} M EDTA and were followed with a Perkin-Elmer Lambda 4B spectrophotometer, which followed Beer's law up to the observed maximum absorbance of ~3.5 from hydrogen peroxide anion and PicP.

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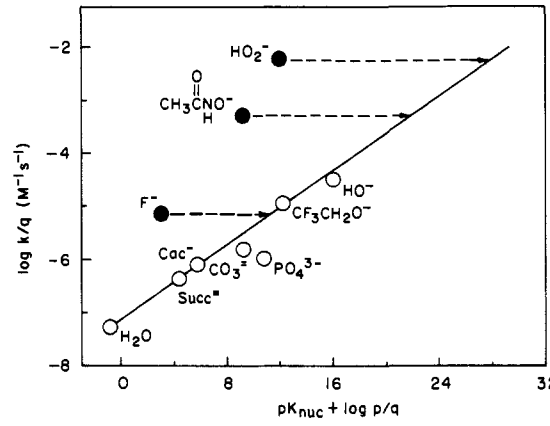

Figure 2. The dependence of log k for reactions of phosphorylated 4-morpholinopyridine (MPP) on the pK_a of the nucleophile with α-effect nucleophiles and fluoride ion (closed symbols) and "normal" oxygen nucleophiles (open symbols; ref 21). The rate constants and pK_a values are statistically corrrected. Abbreviations: Succ$^=$, succinate dianion; Cac$^-$, cacodylate ion.

Results

Figure 1 shows the dependence of the rate constants for the disappearance of phosphorylated 4-morpholinopyridine (MPP)[16] on the concentration of fluoride, acetohydroxamate, and hydrogen peroxide anions; the dependence on the concentration of cacodylate and hydroxide ions is shown for comparison (note the different scales for the y axis in Figure 1). Second-order rate constants obtained from the slopes of these and similar plots with PyrP and PicP are listed in Table I. Varying the fraction of hydrogen peroxide present as the anion did not affect the second-order rate constant for its reaction with MPP (Figure 1) and 0.01–0.02 M HOOH at pH 7.8 gave <10% increase in the rate of disappearance of PyrP (not shown). The formation of fluorophosphate from the reaction of fluoride ion with PyrP and PicP has been observed previously in reaction mixtures that contain the phosphorylated pyridine generated in situ from acetyl phosphate and the pyridine.[12]

Discussion

The Brønsted-type correlation in Figure 2 shows that the second-order rate constants for the reactions of fluoride ion and the α-effect nucleophiles hydrogen peroxide ion and acetohydroxamate ion with phosphorylated pyridines are large relative to those for ordinary oxygen nucleophiles. Fluoride ion, acetohydroxamate ion, and hydrogen peroxide ion deviate positively by factors of 30, 160, and 600, respectively, from a line of slope 0.16 drawn through the values[21] of log k and pK_a for a group of oxygen nucleophiles. The dashed lines show that fluoride, acetohydroxamate, and hydrogen peroxide ions behave like oxygen nucleophiles of pK_a ~ 11, 22, and 28, rather than their actual pK_a values of 3.2, 9.4, and 11.6, respectively, and the rate constant for fluoride is only slightly smaller than that for trifluoroethoxide ion.

The plots of log k against the pK_a of the pyridine leaving group in Figure 3 give slopes of β_{lg} = −0.86 and −0.70 for reactions of phosphorylated pyridines with fluoride ion and hydrogen peroxide ion, respectively. The data for cacodylate and trifluoroethoxide ions that are shown for comparison give slopes of β_{lg} = −0.98 and −0.82, respectively. The rate constants and values of β_{lg} are similar for fluoride and trifluoroethoxide ions despite the 9-unit difference in the pK_a of these ions; the rate constants are larger for hydrogen peroxide ion than for trifluoroethoxide ion, and β_{lg} is less negative for hydrogen peroxide ion despite the similar pK_a values (Figure 3; Table I). The differences in slope that correspond to changes in β_{lg} are shown more clearly by the logarithmic correlation in Figure 4 of the ratio of the rate constants for reactions with hydrogen peroxide and trifluoroethoxide ions (top) and fluoride

(21) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7587–7596. The slope of the line and the size of the deviations in Figure 2 are consistent with the data, but are not exact.

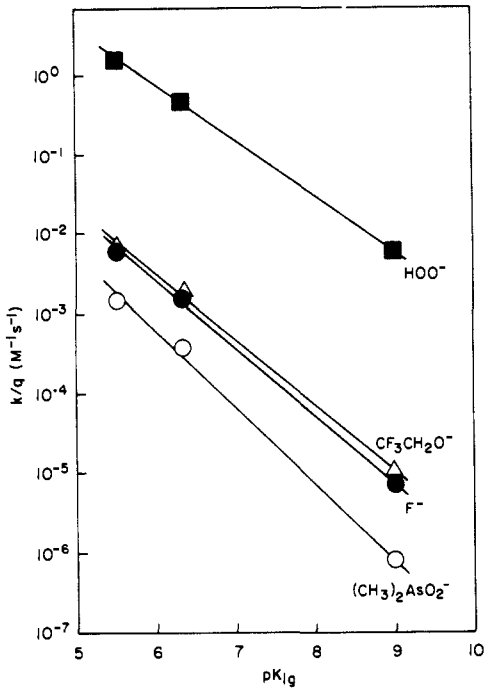

Figure 3. The dependence of log k for reactions of phosphorylated pyridines (Table I) on the pK_a of the pyridine leaving group (ref 21) for hydrogen peroxide ion, fluoride ion, trifluoroethoxide ion, and cacodylate ion.

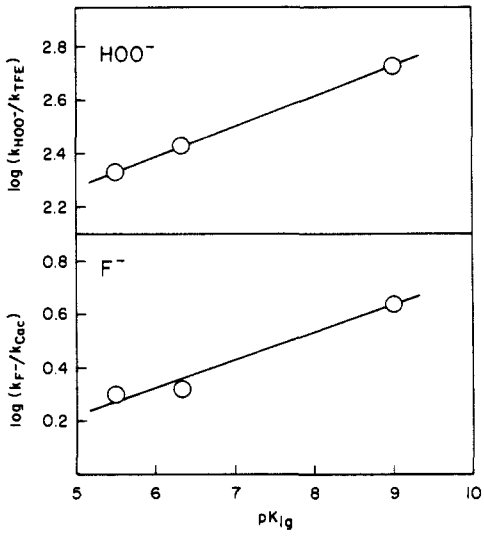

Figure 4. The dependence of the ratio log k_1/k_2 for the second-order reactions of phosphorylated pyridines with hydrogen peroxide and trifluoroethoxide ions (top) and fluoride and cacodylate ions (bottom) on the pK_a of the pyridine leaving group (Table I; ref 21).

and cacodylate ions (bottom) with the pK_a of the pyridine leaving group. If the values of β_{lg} were not different these lines would have a slope of zero.

Figure 5 shows that the values of $\{-\beta_{lg}\}$ for fluoride and hydrogen peroxide ions fall well below the correlation line for $\{-\beta_{lg}\}$ and the pK_a of the "normal" oxygen nucleophiles. The dashed lines in Figure 5 show that the values of $\{-\beta_{lg}\}$ would fall on the line if the pK_a values of fluoride ion and hydrogen peroxide ion were 11 and 22, respectively; these values are comparable to the apparent pK_a values for their nucleophilic reactivity of 11 and 28 in Figure 2.

The correlation in Figure 6 of the reactivity of nucleophiles toward a phosphorylated pyridine with $\{-\beta_{lg}\}$, instead of their pK_a values, shows that fluoride and hydrogen peroxide ions (closed symbols) behave like the "normal" oxygen nucleophiles (open symbols). The line in Figure 6 is a least-squares fit to all of the data.

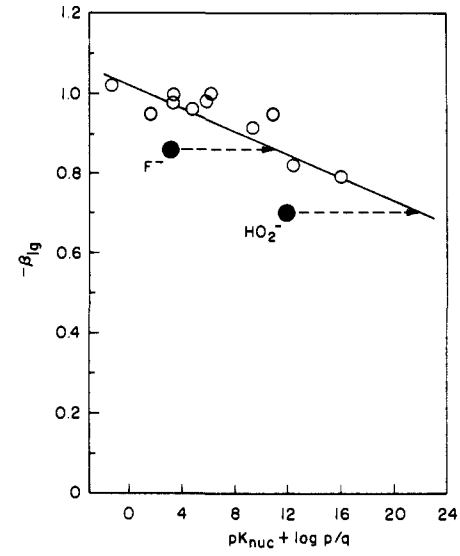

Figure 5. The dependence of $\{-\beta_{lg}\}$ for reactions of phosphorylated pyridine monoanions on the pK_a of the nucleophile for "normal" oxygen nucleophiles (open symbols; ref 21) and for hydrogen peroxide and fluoride ions (closed symbols; Figure 3).

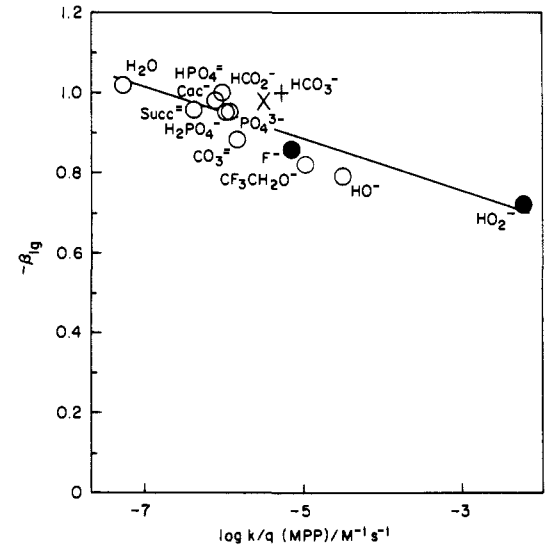

Figure 6. The dependence of $\{-\beta_{lg}\}$ for reactions of phosphorylated pyridine monoanions on log k for reaction of the nucleophile with phosphorylated 4-morpholinopyridine monoanion (MPP) (Figure 3 and ref 21). The line is a least-squares fit to all of the data. The deviations for formate ion (X) and bicarbonate ion (+) are discussed in the text. The rate constants are statistically corrected. Abbrevations: Succ=, succinate dianion; Cac⁻, cacodylate ion.

These data suggest that fluoride and hydrogen peroxide ions behave like oxygen nucleophiles of $pK_a = 11$ and ~25, respectively, in both reactivity and transition state structure.

Thermodynamic Contributions to the α-Effect. The origin, or the explanation, of the "α-effect" is still not clear. Many factors affect nucleophilic reactivity, and it is unlikely that a single factor is responsible for all of the rate enhancements that have been observed for α-effect nucleophiles.[2-4] However, an enhanced affinity of many oxygen and nitrogen α-effect compounds, compared with "normal" bases of comparable pK_a, has been observed for addition to carbonyl compounds, carbocations, and an olefin.[22-28] Correlations of the thermodynamic stability of addition

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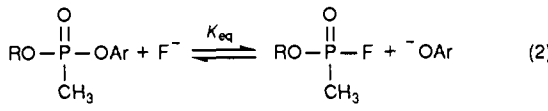
compounds or products with the rate constants for the reactions of these substrates, which include both α-effect and normal nucleophiles, show that in many instances the additional stabilization of the transition state is proportional to the stabilization of the product. This suggests that there is a thermodynamic origin for at least some of the enhanced reactivity of α-effect nucleophiles in Brønsted-type correlations of log k with basicity toward the proton.[23-27]

There is some evidence that the enhanced reactivity of α-effect nucleophiles in methyl transfer from methyl phenyl sulfate is also reflected in changes in the structure of the transition state. Hydrogen peroxide ion and hydrazine react rapidly compared with normal oxygen and nitrogen nucleophiles of similar basicity, and the transition states for reactions with the α-effect nucleophiles exhibit less bond cleavage to the leaving group, as measured by linear free energy relationships, than the transition states for other nucleophiles. There is also less bond cleavage to the leaving group with increasing pK_a and, presumably, with increasing product stability of the non-α-effect nucleophiles.[29,30] This is the change in transition-state structure that is predicted from Hammond effects when there is an increase in the thermodynamic stability of the bond to the nucleophile.[31] Thus, the change in the structure of the transition state for reactions with the α-effect nucleophiles is also consistent with a thermodynamic origin of the kinetic α-effect.

These observations for reactions at carbon suggest that the enhanced reactivity and change in the structure of the transition state for phosphoryl transfer to α-effect nucleophiles also results from an enhanced thermodynamic affinity of α-effect bases, in this case toward the phosphoryl group. The decrease in $\{-\beta_{lg}\}$ with increasing pK_a of normal oxygen nucleophiles for phosphoryl transfer from phosphorylated pyridines is in the direction expected for a decrease in the amount of bond cleavage to the leaving group in the transition state with an increase in the thermodynamic stability of the product (Figure 5).[32] The larger decrease in $\{-\beta_{lg}\}$ for hydrogen peroxide ion than for normal oxygen nucleophiles of comparable pK_a (Figure 5) is consistent with a larger thermodynamic stability of the product of the reaction with the α-effect nucleophile, as expected for a normal Hammond effect.

The reaction of fluoride ion with phosphorylated pyridines also exhibits increased reactivity and a smaller value of $\{-\beta_{lg}\}$ than are expected for a normal oxygen nucleophile of comparable pK_a. The enhanced reactivity and change in transition-state structure with fluoride ion may also be thermodynamic in origin, because there is evidence that fluoride ion has a high affinity for phosphorus,[33] which may be even larger than that of oxygen bases of comparable pK_a:

(1) Values of K_{eq} = 2000 and 300 have been determined for displacement by fluoride ion of *m*-nitrophenolate and phenolate ions, respectively, from aryl isopropyl methylphosphonates (eq 2).[34] An extrapolation of log K_{eq} to that for a substituted phenol



(2)

of pK_a = 3.2, the pK_a of fluoride ion, gives $K_{eq} = 5 \times 10^5$. This suggests that the basicity of fluoride ion toward the phosphonate group is $\sim 5 \times 10^5$ larger than that of a phenolate ion with the same basicity toward the proton.

(2) A high affinity for phosphorus is also suggested by the rapid reaction of fluoride ion with the phosphate triester 2-(2,4-dinitrophenoxy)-2-oxodioxaphosphorinane, with a rate constant equal to that expected for an oxygen nucleophile of $pK_a \sim 10$, and by the slow reaction with H_2O of the fluoro product, which undergoes hydrolysis at least 10^3-fold more slowly than the starting material, a 2,4-dinitrophenyl ester with a leaving group of pK_a = 4.1.[7,20,35]

Although the fast reaction of fluoride ion might be attributed to decreased steric repulsion relative to the oxygen nucleophiles, this would not explain the decrease in the value of $\{-\beta_{lg}\}$. Formate ion reacts 20-fold faster than acetate ion, presumably as a result of less steric repulsion.[36] However, the value of $\{-\beta_{lg}\} = 0.98$ for formate ion is that expected for a nucleophile with its pK_a of 3.8 (Figure 5),[21,37] so that the rate constant for formate ion falls above the correlation line of $\{-\beta_{lg}\}$ and reactivity, as shown by X in Figure 6. It is possible that the reactivity of fluoride ion would be still larger if it were not so strongly solvated.

The extent of nucleophilic involvement, as measured by β_{nuc} for reactions of carboxylate ion nucleophiles, and the amount of the increased reactivity for hydrogen peroxide and fluoride ions are both greater for reactions of MPP than for the more reactive phosphoryl donors PicP and PyrP. The values of β_{nuc} = 0.29, 0.25, and 0.25 for reactions of carboxylate nucleophiles with the Mg^{2+} complexes of MPP, PicP, and PyrP, respectively, correspond to a positive p_{xy} coefficient and suggest that there is more bond formation in the transition state for the relatively unreactive MPP.[21,38] The ratios of the rate constants for reaction with hydrogen peroxide and hydroxide ions are 190, 110, and 100 for MPP, PicP, and PyrP, respectively (Table I), and the corresponding ratios for reaction with fluoride and cacodylate ions are 4.4, 2.1, and 2.0 (Table I). The larger increases in the reactivity of hydrogen peroxide and fluoride ions with MPP also correspond to a positive p_{xy} coefficient and are consistent with a thermodynamic effect that gives more stabilization of the transition state with more bond formation. Because a number of different factors can affect the stability and structure of a transition state, such a correlation might be expected to hold only for reactions of a series of similar compounds. There is also a correlation between the amount of the enhanced reactivity of nitrogen α-effect nucleophiles and the value of β_{nuc} for reactions at sp^2 carbon centers.[39] This correlation may represent, in part, a thermodynamic α-effect because large kinetic α-effects and large values of β_{nuc} are observed in addition–elimination reactions in which bond formation to the nucleophile is complete in the rate-limiting transition state of bond cleavage.[40]

Are Hydrogen Bonding and Solvation Responsible for the α-Effect? The decreased extent of bond cleavage in the transition

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state for reactions of phosphorylated pyridines with hydrogen peroxide ion, compared with normal oxygen nucleophiles of the same pK_a, would not be expected if the rate enhancement were caused by either hydrogen bonding in the transition state or weak solvation of the hydrogen peroxide ion.

Transition-state stabilization by intramolecular hydrogen bonding has been proposed as an explanation of the α-effect in several systems.[2,3,6,41,42] This may be responsible for the 30- and 50-fold faster reactions of hydrogen peroxide ion than of methyl hydroperoxide ion with *p*-nitrophenyl sulfate and *p*-nitrophenyl methylphosphonate ions, respectively.[9,42] However, the similar rate enhancements for reactions of hydrogen peroxide and methyl hydroperoxide ions with carboxylic acid esters provide no evidence for assistance by hydrogen bonding,[22] and the enhanced reactivity of hydrogen peroxide ion in acid-catalyzed addition to aldehydes is unlikely to result from hydrogen bonding.[24] Finally, the 40-fold faster reaction of bicarbonate than of acetate ion with phosphorylated pyridines presumably involves intramolecular solvation with hydrogen bonding,[36] yet its value of $\{-\beta_{lg}\} = 1.00$ is not smaller than expected for an oxygen nucleophile of $pK_a = 3.8$ (Figure 5);[21] in fact the value of $\{-\beta_{lg}\}$ for bicarbonate deviates positively in the correlation of $\{-\beta_{lg}\}$ and reactivity (Figure 6, +), while hydrogen peroxide ion follows the correlation. Bicarbonate shows the behavior that is expected for an additional interaction, such as hydrogen bonding, that increases log *k* but does not change the structure of the transition state.

It has also been suggested that the rate enhancement of α-effect nucleophiles could reflect easier desolvation of these nucleophiles than of normal nucleophiles. Strong solvation can cause a decreased reactivity of a nucleophile if β_{nuc} is small, but it has no effect if $\beta = 1.0$;[43] conversely, a nucleophile that is more weakly solvated than other nucleophiles will be more reactive for a given observed pK_a if β_{nuc} is small. The reactive, desolvated species of a strongly solvated nucleophile has a higher pK_a and reactivity than the observed pK_a and reactivity: if only 1% is desolvated the observed log *k* and pK_a will be 2 units smaller than log *k* and the pK_a of the desolvated species. Therefore, the observed value of $-\beta_{lg}$ for the strongly solvated molecule will be the relatively small value that is expected for the more basic, desolvated species and, conversely, the value of $-\beta_{lg}$ will be relatively large for a nucleophile that does not require desolvation for reaction, for a given observed pK_a. This is the opposite of what is observed for α-effect nucleophiles.

A solvation mechanism also predicts that when β is <1.0 there should be an α-effect for proton abstraction when the proton transfer is direct and not through an intervening water molecule; in contrast, an α-effect that arises from an enhanced affinity for carbon and phosphorus compared with protons should give no rate enhancement for proton transfer. Hupe and Wu have shown that hydrogen peroxide ion, acetohydroxamate ion, and a series of oximates behave like normal oxygen bases for the deprotonation of 4-(*p*-nitrophenoxy)-2-butanone to form the carbanion at C-3; i.e., deviations for the α-effect bases in a plot of log *k* against the pK_a of the base are ≤2-fold.[44] Similarly, Bruice and coworkers have shown that there is no α-effect for nitrogen bases such as hydrazine and hydroxylamine in the deprotonation of nitroethane.[45] There is evidence that proton abstraction from carbon acids is direct,[46] even for normal carbon acids such as hydrogen cyanide and thiazolium ions.[47] These results do not support a desolvation mechanism for α-effect compounds.

Is There an α-Effect in the Gas Phase? The affinity of hydrogen peroxide ion is 10^6-fold greater toward a *tert*-butyl cation than toward a proton in the gas phase, whereas the affinities of methoxide and phenoxide ions toward the methyl cation are only 10^3-fold greater than that toward a proton.[23b] Methoxide and phenoxide ions have a higher and lower gas phase proton affinity, respectively, than hydrogen peroxide ion,[48] so that the greater affinity of hydrogen peroxide for an alkyl cation suggests that there is a thermodynamic α-effect in the gas phase.[23b]

The following data have been cited as providing evidence against a kinetic α-effect in the gas phase and have led to the suggestion that solvation is responsible for the kinetic α-effect; however, there are uncertainties in the interpretation for each of these pieces of evidence.

(1) Hydroxide and hydrogen peroxide ions react with methyl formate in the gas phase to give the same ratios of proton transfer, carbonyl addition, and methyl transfer. Because the product distribution is the same for the normal and α-effect nucleophiles and because no α-effect was expected for proton transfer, it was suggested that the α-effect does not occur in the gas phase.[49] However, the kinetic α-effect in solution is small or absent when the Brønsted β value, which measures the dependence of reactivity on the pK_a of the base, is small. Thus, an α-effect would not be expected in these gas-phase reactions if the values of β are small; these β values have not been determined in the gas phase.[2,38,50,51a] Furthermore, the comparable rates of removal of a proton from the methyl and the carboxylic acid groups of acetic acid by hydroxide and fluoride ions in flowing afterglow experiments[51b] suggest that the low selectivity in the reaction with methyl formate may not provide a measure of relative nucleophilicity.

(2) Calculations of reaction energy profiles suggest that the barrier for methyl transfer from CH_3Cl to hydroxide ion in the gas phase is smaller than for transfer to hydrogen peroxide ion by only ~3 kcal/mol, despite the ~16 kcal/mol greater basicity of hydroxide ion.[52] This difference could provide evidence against a significant α-effect in the gas phase only if β_{nuc} for methyl transfer in the gas phase is very small. If β_{nuc} is 0.3 or larger for normal nucleophiles, then the rate constant for HOO^- will show a positive deviation from a line of slope 0.3 through the rate constant for HO^- and there will be an α-effect. However, if the value of β_{nuc} for normal nucleophiles is 0.2 then an α-effect for the reaction would not be expected because reactions with small values of β_{nuc} give little or no kinetic α-effect, as noted above.[2,39,50]

(3) Ab initio calculations for reactions of methyl fluoride in the gas phase suggest that the geometry of the transition state varies with reactivity in the same way with both α-effect and normal nucleophiles.[53] However, this does not provide evidence against the α-effect in the gas phase. Figure 6 shows that α-effect and normal nucleophiles follow a single correlation of $\{-\beta_{lg}\}$ with reactivity for phosphoryl transfer in water and similar behavior has been observed for methyl transfer in methanol (see above).[29] Ab initio calculations also suggest that the transition state energy and overall energy for reaction with methyl fluoride follow a single correlation for both α-effect and normal nucleophiles.[54] Such

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a correlation is consistent with a thermodynamic origin for a kinetic α-effect in the gas phase and with analogous correlations of rates and equilibria for α-effect and normal nucleophiles for reactions in solution (see above). It cannot be emphasized too strongly that the α-effect represents unusual reactivity *relative to* the proton basicity of a nucleophile.[2-4]

Origin of the α-Effect. Greater basicity toward carbon or phosphorus than toward the proton for α-effect nucleophiles compared with normal nucleophiles can account for at least some of the observed kinetic α-effects, as described above.[22-28] There may be a differential stabilization that arises from the largely covalent character of bonds to carbon and phosphorus compared with the more ionic character of bonds to hydrogen. The heteroatom substituent of an α-effect base may cause a smaller decrease in the ability of the base to participate in a covalent bond for a given decrease in charge on the basic atom, compared with an electron-withdrawing substituent on a normal base.[55] This suggestion is supported by ab initio calculations that give a larger difference in Mulliken charge and a smaller difference in HOMO energy for hydrogen peroxide ion relative to methoxide ion than for formate ion relative to methoxide ion.[56,57] However, the α-effect compounds and normal bases: hydroxide, methoxide, hydrogen peroxide, and hypofluorite ions, follow a single correlation of Mulliken charge and HOMO energy, while formate ion deviates from this correlation. This suggests that it may be formate ion that is unusual, rather than the α-effect bases.

Electron-density difference maps from X-ray structural analysis show that formation of a covalent bond between two oxygen or nitrogen atoms results in a decrease in the electron density between the two atoms, while the electron density between carbon and oxygen or nitrogen atoms is increased upon bond formation.[58] This indicates that the electronic configuration of the α-effect bases is different from that of the normal bases. It is conceivable that this difference is related to the origins of the α-effect.

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Multiple Alkylation and Mapping of the Active Site of α-Chymotrypsin by Carbonium Ions Generated with Active-Site-Directed Enzyme-Activated Nitrosoamide Substrates

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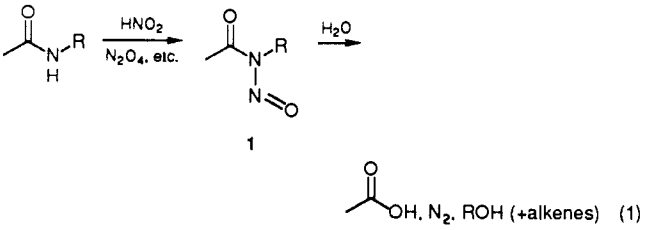
Abstract: The inhibition of α-chymotrypsin with ^{13}C-enriched alanine- and phenylalanine-based *N*-nitrosoamides, as active-site-directed and enzyme-activated inhibitors, results in the alkylation (benzylation) of side chains and also of the amide linkages of the protein backbone (both at O and N). ^{13}C NMR spectra of the denatured inhibited enzyme (in Gdn·HCl) indicate that alkylation has occurred at O, N, S, and C sites. ^{13}C NMR spectra of the amino acid mixtures from fully hydrolyzed inhibited enzymes show that the pattern of alkylation is strikingly different for inhibitions by the alanine- and phenylalanine-based inhibitors. In the case of the phenylalanine-based inhibitor, approximately equally intense signals are observed at 52.32, 51.31, 36.78, and 32.91 ppm, while with the alanine-based inhibitor, a major signal appears at 52.35 ppm, with minor signals appearing at 36.83 and 32.9 ppm. Chromatographic and NMR evidence is presented to indicate that the 52.32-52.35-ppm signal stems from *N*-benzylglycine. The chemical shift data suggest that the 51.31-ppm signal stems from *N*-benzylserine and the 36.78-36.83-ppm signal from *S*-benzylcysteine. Mechanisms are presented to account for the formation of those products.

N-Nitrosoamides,[1] lactams,[2] and sultams[3] are hydrolyzed far more rapidly than the corresponding amido precursors, and as "active" amides and peptides they can serve as substrates for hydrolytic enzymes (eq 1);[4] they are readily prepared by nitrosation



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