We suggest that this high selectivity for reaction with water instead of alcohols represents a localized solvent effect that arises from the development of a high charge density in a restricted environment in the transition state for formation of the oxocarbenium ion. Space-filling CPK molecular models indicate that a large fraction of the reaction center is surrounded by hydrocarbon, the 6-hydroxymethyl group, and the leaving group, so that its access to solvent is severely restricted. The high selectivity for reaction with water instead of alcohols suggests that the development of this charge is favored when it is surrounded by water molecules, rather than alcohol. The lifetime of the oxocarbenium ion is too short to allow diffusion and a choice between different nucleophiles once it is formed, so that it is very likely to react with water if it is formed in the presence of water. There is a large increase in the rate of hydrolysis of α-D-glucosyl fluoride with an increase in the concentration of water in the mixed solvent system (Figure 6B). In methanol–water mixtures, the rate increases by 50-fold with a 10-fold increase in the concentration of water, from 5.5 to 55 M (Table III); over most of this range, the increase in rate is approximately second-order with respect to the concentration of water (not shown).

These conclusions are supported by the results of a comprehensive study of the reactions of a series of *p*-nitrophenyl β-D-ribofuranoside derivatives by Czarnik and co-workers.[10] The rate of pH-independent hydrolysis in this series of compounds is decreased by 330-fold in the fluorenone ketal, which provides a nonpolar environment near the 1-carbon atom. Slightly smaller decreases are observed with fluorenone derivatives that have amide and ester groups below the 1-carbon atom, but a small rate increase is observed with a carboxylate group in the same position.

The same localized solvation and electrostatic effects, as well as steric effects, may account for the occurrence of bimolecular nucleophilic reactions of anions, but not of amine nucleophiles, with α-D-glucosyl fluoride. There is a large amount of bond breaking and little bond making in the dissociative transition state of these A_ND_N reactions, so that there is a large amount of positive charge development at the reaction center. This charge interacts favorably with an anionic nucleophile, but not with an uncharged amine nucleophile. It is conceivable that the attack of anionic nucleophiles is also assisted by hydrogen bonding to the 6-OH group of α-D-glucosyl fluoride.

Registry No. 1, 2106-10-7; β-D-glucopyranosyl azide, 20379-59-3; 1-*O*-acetyl-β-D-glucopyranose, 135758-71-3; α-D-glucopyranose, 492-62-6; 1,6-anhydro-β-D-glucopyranose, 498-07-7; β-D-glucopyranose, 492-61-5.

General-Acid and General-Base Catalysis of the Cleavage of α-D-Glucopyranosyl Fluoride[1]

Narinder S. Banait and William P. Jencks*

Contribution No. 1728 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254-9110. *Received March 26, 1991*

Abstract: The hydrolysis of α-D-glucopyranosyl fluoride is catalyzed by phosphate and phosphonate buffers in the pH-independent region in water and in deuterium oxide at 30.0 °C and μ = 2.0 M (KCl). General-base catalysis by the dianions accounts for most of the rate increase, but there is also significant general-acid catalysis by the monoanions. The solvent isotope effect for catalysis by phosphate buffers is $k_H/k_D = 1.9$ for the dianion and $k_H/k_D = 2.0$ for the monoanion. The Brønsted slopes are small, with $\beta = 0.06$ for catalysis by general bases and $\alpha = 0.15$ for catalysis by general acids; however, $\alpha = 0.4$ if the rate constant for H_3O^+ is included. Catalysis by L_3O^+ shows a solvent isotope effect of $k_H/k_D = 1.4$; this differs from the value of $k_H/k_D \simeq 0.5$ that is expected for specific-acid catalysis with equilibrium protonation of the substrate. Catalysis by LO^- and the uncatalyzed solvolysis in L_2O show inverse solvent isotope effects of $k_D/k_H = 1.5$ and $k_D/k_H = 1.1$, respectively. No reaction with methanol is observed in the absence of catalysts, but the addition of methanol to α-D-glucosyl fluoride in methanol–water (45:55 by volume) is catalyzed by phosphate buffer, 50% dianion, and gives 1-*O*-methyl-β-glucopyranoside as the product. It is suggested that the general-base catalysis represents a concerted mechanism of nucleophilic attack and proton abstraction that is enforced by the absence of a significant lifetime for the glucosyl cation in the presence of fluoride ion, and that general-acid catalysis occurs by hydrogen bonding to the leaving fluoride ion. Both mechanisms of catalysis are facilitated by an electrostatic interaction between the anionic catalyst and the developing positive charge of the transition state. The significance of these results for the mechanism of catalysis by glycosidases is discussed.

We would like to understand the mechanisms that are utilized by lysozyme and related enzymes to catalyze the cleavage of glycosidic bonds. Possible mechanisms include nucleophilic catalysis and electrostatic stabilization of the carbocation-like transition state by a carboxylate group, ground-state distortion toward the structure of the transition state, and general-acid–base catalysis of proton transfer to or from leaving or entering groups.[2] However, it has generally been believed that the hydrolysis of glycosides occurs by specific-acid catalysis, with complete protonation of the leaving oxygen atom followed by bond breaking to form an unstable oxocarbenium ion intermediate that reacts rapidly with water.[3] The lifetime of the glucosyl oxocarbenium ion has been estimated to be approximately 10^{-12} s, so that the reaction of this cation with water is expected to occur rapidly without assistance by general-base catalysis.[4] The Brønsted β value for general-base catalysis of the addition of water to electrophilic centers decreases with increasing reactivity of the cation, according to the relationship $p_{xy} = -\partial\beta/\partial\sigma = \partial\rho/-\partial pK_{BH^+}$, and general-base catalysis is not observed for the hydration of highly unstable cations.[3,5-7] If there is no general-base catalysis of

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[1] This paper is dedicated to Robert Abeles on the occasion of his 65th birthday. This work was supported in part by grants from the National Institutes of Health (GM 20888) and the National Science Foundation (DMB 8715832).

[2] (a) Vernon, C. A. *Proc. R. Soc. London* **1967**, *167B*, 389–401. Phillips, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 484–495. (b) Sinnott, M. L. In *The Chemistry of Enzyme Action*; Page, M. I., Ed.; Elsevier: Amsterdam, 1984; pp 389–431. (c) Jencks, W. P. In *Catalysis in Chemistry and Enzymology*; Dover: New York, 1987; pp 226–229.

[3] Cordes, E. H.; Bull, H. G. *Chem. Rev.* **1974**, *74*, 581–603. Fife, T. H. *J. Am. Chem. Soc.* **1965**, *87*, 271–275.

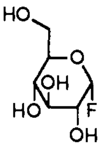
[4] Amyes, T. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7888–7900, 7900–7909.

0002-7863/91/1513-7958$02.50/0

hydration, there will be no general-acid catalysis for the expulsion of water, or of alcohols that resemble water, in the reverse direction.

The reactions of a number of carbohydrate derivatives with nitrogen, oxygen, and fluoride leaving groups have been found to show only specific-acid or specific-base catalysis.[2b,8] On the other hand, there is evidence for both general-acid catalysis and electrostatic stabilization of the rate-limiting transition state in a number of reactions of sugars and related acetals that involve intramolecular catalysis by carboxylic acid and carboxylate groups.[9]

We report here the occurrence of general-base and general-acid catalysis of the hydrolysis of α-D-glucopyranosyl fluoride, **1**, by phosphate and phosphonate buffers in aqueous solution that is facilitated by an electrostatic interaction of the catalyst with the cationic transition state. It is suggested that the general-base catalysis is enforced by the absence of a significant lifetime for the oxocarbenium ion in the presence of fluoride ion and that the general-acid catalysis involves hydrogen bonding to the leaving fluoride ion. These experiments were stimulated, in part, by a report that the rate of disappearance of α-D-glucosyl fluoride is increased in the presence of nucleophilic buffers.[10]



1

Experimental Section

Materials. Reagent-grade potassium chloride, potassium hydroxide, and potassium phosphate were used without further purification. The sources of substituted phosphonates and α-D-glucopyranosyl fluoride are described in the previous paper.[11]

Kinetics. The rate of hydrolysis of α-D-glucosyl fluoride in the presence of added buffers was measured under pseudo-first-order conditions by polarimetry or proton NMR at 30.0 °C and 2.0 M ionic strength, maintained with KCl. The solution contained 0.01 M 1:1 KD_2PO_4–K_2DPO_4 buffer, and the ionic strength of 2.0 M was maintained with sodium perchlorate. Catalysis by H_3O^+, HO^-, and KH_2PO_4–K_2HPO_4 buffers in water was followed by polarimetry. The reactions at pH 0.89, 1.14, and >11 were followed to the end point. Pseudo-first-order rate constants were obtained from semilogarithmic plots of $[\alpha] - [\alpha]_\infty$ against time and the relationship $k_{obs} = \ln 2/t_{1/2}$. The plots were linear for 2–4 half-lives. Other rate constants in water were obtained from the rate, ν, of the initial linear change in optical rotation, $[\alpha]$, of reactions that were followed for 5–10% toward completion. The rate constant k_{obs} was obtained from the equation $k_{obs} = \nu/[S]\Delta\epsilon$, in which [S] is the substrate concentration and $\Delta\epsilon$ is the change in optical rotation upon completion of the reaction. The value of $\Delta\epsilon$ was determined by following the change in $[\alpha]$ upon reaction to completion at pH 0.89 and 1.14. The reaction solutions typically contained 0.012–0.016 M substrate. The kinetics for the pH-independent hydrolysis of α-D-glucosyl fluoride in H_2O are described in the previous paper.[11] The hydrolysis in D_2O was measured by polarimetry.

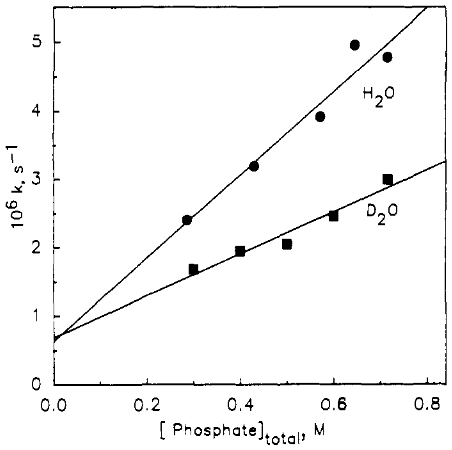


Figure 1. Catalysis of the hydrolysis of α-D-glucosyl fluoride by phosphate buffers, 90% dianion, in water (●) and in D_2O (■) at 30 °C, μ = 2.0 M (KCl). The observed rate constants were corrected for the reaction with Cl^-. The solvent isotope effect is $k_H/k_D = 1.9$.

The rates of hydrolysis of α-D-glucosyl fluoride in the presence of D_3O^+ and in a 50% H_2O–D_2O mixture at pH 1.00 were followed by polarimetry to the end point at 30.0 °C. The H_2O–D_2O solvent was prepared by weight from 99.9% D_2O, glass-distilled water, 1.0 M HCl, and 0.0347 g of substrate in which the protons of the hydroxyl groups had been exchanged for deuterons. Pseudo-first-order rate constants were obtained from semilogarithmic plots of $[\alpha] - [\alpha]_\infty$ against time, which were linear for 4 half-lives.

Kinetics in the presence of (trichloromethyl)phosphonate and phosphate buffers were measured by proton NMR of the C-1 proton of **1** in D_2O. The reaction solutions typically contained 0.018 g of α-D-glucosyl fluoride that was weighed directly into the NMR tubes. The reactions were initiated by the addition of buffer and KCl in D_2O to give 0.1 M substrate concentration and a final volume of 1.0 mL. The NMR tubes were incubated in a water bath at 30.0 °C. Pseudo-first-order rate constants were obtained by measuring the disappearance of the starting material with time; at least five NMR spectra were obtained during the first half-life. Pseudo-first-order rate constants were calculated from eq 1, in which FG is the fraction of starting material remaining at time t, and $k_{Cl^-} = 1.0 \times 10^{-6}$ M^{-1} s^{-1} is the second-order rate constant for nucleophilic reaction with chloride ion;[11] KCl was used to maintain the ionic strength at 2.0 M.

$$k = \frac{-\ln FG}{t} - k_{Cl^-}[Cl^-] \qquad (1)$$

The kinetics for the disappearance of α-D-glucosyl fluoride in ethylphosphonate mono–dibasic buffers were measured by polarimetry in D_2O at 30.0 °C, and μ = 2.0 M (KCl). The reactions were followed for 5–10% toward completion, and the pseudo-first-order rate constants were obtained as described above.

The products from the reaction of 0.01 M α-D-glucosyl fluoride in water–methanol (55:45 (v/v)) solvent were identified by NMR. The reaction was initiated by adding 0.0202 g of the substrate into the solvent buffered with 0.71 M 1:9 mono–dibasic phosphate. The tube was tightly sealed and incubated in a water bath at 30.0 °C. Aliquots of the solution were removed at intervals, the solvent was lyophilized, and the residue was dissolved in D_2O for analysis by NMR. At the end point, the product distribution of glucose–methyl β-D-glucopyranoside was 5:1.

Results

The kinetics for the hydrolysis of α-D-glucopyranosyl fluoride, **1**, in water in the presence of buffers were measured at 30.0 °C and ionic strength 2.0 M, maintained with KCl. Second-order rate constants were obtained from the slopes of plots of the pseudo-first-order rate constants against total buffer concentration with at least four buffer concentrations, as described in the Experimental Section. Typical data are shown in Figure 1 for experiments in H_2O and D_2O. The dependence on pH of log k for the disappearance of α-D-glucosyl fluoride is shown in Figure 2.

Rate constants for catalysis of the disappearance of α-D-glucosyl fluoride by the monoanions and dianions of phosphate and phosphonate buffers were obtained from plots of the observed catalytic constants at different buffer ratios against the fraction

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(5) Ta-Shma, R.; Jencks, W. P. *J. Am. Chem. Soc.* **1986**, *108*, 8040–8050.

(6) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1396–1401.

(7) Fife, T. H. *Acc. Chem. Res.* **1972**, *5*, 264–272.

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(9) See, for example, Capon, B.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2* **1972**, 2057–2062. Anderson, E.; Fife, T. H. *J. Am. Chem. Soc.* **1973**, *95*, 6437–6441. Fife, T. H.; Przytas, T. J. *J. Am. Chem. Soc.* **1977**, *99*, 6693–6699. Fife, T. H.; Przytas, T. J. *J. Am. Chem. Soc.* **1979**, *101*, 1202–1210. Warshel, A. *Acc. Chem. Res.* **1981**, *14*, 284–290. Kirby, A. J. *Crit. Rev. Biochem.* **1987**, *22*, 283–315. Cherian, X. M.; Van Arman, S. A.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 4490–4498.

(10) Jung, S. M.; Mayer, R. M. *Arch. Biochem. Biophys.* **1981**, *208*, 288–295.

(11) Banait, N. S.; Jencks, W. P., previous paper in this series.

(12) *The Sadtler Handbook of Proton NMR Spectra*; Sadtler Research Laboratories, Inc.: Philadelphia, PA: (a) Spectrum 2865. (b) Spectrum 17066.

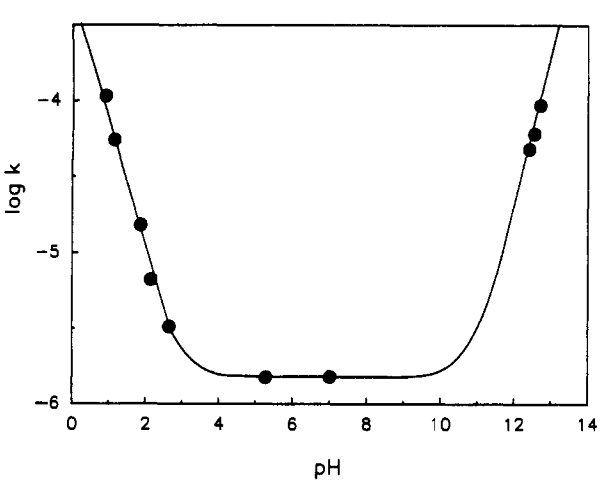

Figure 2. Dependence on pH of the disappearance of α-D-glucosyl fluoride by hydrolysis and cyclization in water at 30 °C. The ionic strength was maintained at 2.0 M with KCl in the acidic and basic regions and with $NaClO_4$ in the pH-independent region.

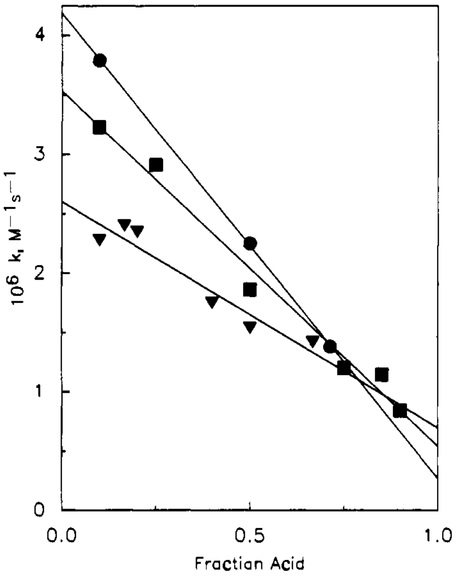

Figure 3. Dependence of the rate of hydrolysis of α-D-glucosyl fluoride catalyzed by phosphate and phosphonate buffers on the fraction of monobasic ethylphosphonate (●), phosphate (■), and (trichloromethyl)phosphonate (▼) in D_2O at 30 °C, μ = 2.0 M (KCl).

of monoanion in the buffer, as shown in Figure 3. The rate constants for catalysis by the monoanions are approximate, but show that there is only a small increase in catalysis with increasing acidity.

The hydrolysis of α-D-glucosyl fluoride in the presence of (trichloromethyl)phosphonate buffer was found to be accompanied by nucleophilic displacement of the fluoride ion by the buffer to give β-D-glucopyranose (1-trichloromethyl)phosphonate. The nucleophilic product accounted for 16% of the product formed and did not vary as the ratio of mono–dianion in the buffer was changed from 2:1 to 1:9. The product was identified by a triplet centered at 5.0 ppm (J = 8 Hz) in the ^1H NMR and a singlet at 5550 Hz in the ^{31}P NMR, with H_3PO_4 as an external standard. A sample of α-glucose-1-phosphate under similar conditions gave a quartet at 5.4 ppm in the ^1H NMR and a singlet at 5330 Hz in the ^{31}P NMR spectrum. The rate constant for the nucleophilic reaction was deducted from the observed rate constant in order to calculate the rate constant for catalysis of hydrolysis. No nucleophilic product was observed by proton or phosphorus NMR spectroscopy after the hydrolysis of α-D-glucosyl fluoride in phosphate or ethylphosphonate buffers.

Table I. Rate Constants for Catalysis of the Hydrolysis of α-D-Glucosyl Fluoride by H^+, HO^-, and Phosphate Buffers in H_2O at 30.0 °C and Ionic Strength of 2.0 M (KCl)[a]

catalyst	buffer ratio acid:base	[catalyst], M	k, M^{-1} s^{-1}
H^+		0.001–0.10	1.0×10^{-3}
HO^-		0.05–0.10	4.5×10^{-4}
phosphate	9:1	0.67–1.67	1.64×10^{-6}
	3:1	0.17–1.00	2.39×10^{-6}
	1:1	0.40–1.00	4.02×10^{-6}
	1:9	0.29–0.71	6.1×10^{-6}

[a] $k(H_2PO_4^-) = 1.1 \times 10^{-6}$ M^{-1} s^{-1}; $k(HPO_4^{2-}) = 6.7 \times 10^{-6}$ M^{-1} s^{-1}.

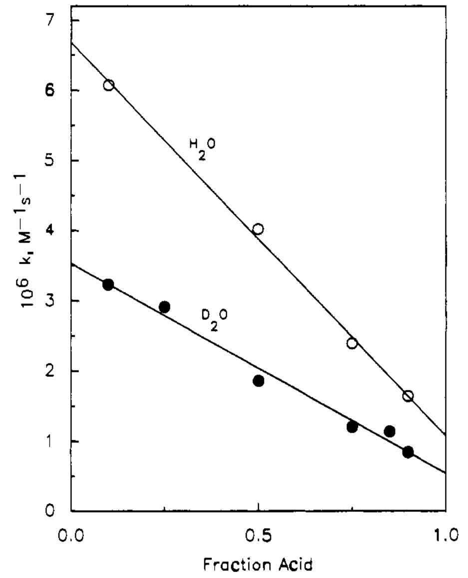

Figure 4. Dependence of the rate of phosphate-catalyzed hydrolysis of α-D-glucosyl fluoride on the fraction of monobasic phosphate in water (○), and in D_2O (●) at 30 °C and μ = 2.0 M (KCl). The solvent isotope effect for catalysis by dibasic phosphate is k_H/k_D = 1.9 and by monobasic phosphate is k_H/k_D = 2.0.

Catalysis of the cleavage of α-D-glucosyl fluoride by 0.06 M DO^- in D_2O was found to give the cyclic acetal 1,6-anhydroglucose in 50% yield from intramolecular attack by the anion of the 6-hydroxyl group. The cyclic acetal is stable under the conditions of the reaction and was identified in ^1H NMR spectra by comparison of the chemical shifts of the protons at C-1 and C-5 and the endo proton of C-6 at 5.23, 4.40, and 3.90 ppm, respectively, with reported values for C-1, C-5, and C-6 (endo) protons at 5.17, 4.37, and 3.89 ppm, respectively.[13] Catalysis by hydroxide ion in water was found to give 1,6-anhydroglucose in 40% yield by NMR. The cyclic acetal was not formed in the presence of acid or as a product of the general-base-catalyzed reactions.

The solvent deuterium isotope effect for the pH-independent hydrolysis of α-D-glucosyl fluoride in L_2O was found to be k_{DOD}/k_{HOH} = 1.1; the reactions catalyzed by L^+ and LO^- in L_2O have solvent isotope effects of k_{H^+}/k_{D^+} = 1.4 and k_{OD^-}/k_{OH^-} = 1.5, respectively (Table III). A solvent isotope effect of k_H/k_D = 1.6 for the L^+-catalyzed hydrolysis of α-D-glucosyl fluoride in 1:1 DOD–HOH (mol/mol) was observed in one experiment. The solvent deuterium isotope effects for catalysis by phosphate dianion and monoanion are k_{HOH}/k_{DOD} = 1.9 and 2.0, respectively (Figure 4).

The products from the solvolysis of α-D-glucosyl fluoride in H_2O–MeOH 55:45 (v/v) catalyzed by 0.71 M 1:9 mono–dibasic phosphate buffer were identified by NMR. The stereochemistry of the product from the reaction with methanol was assigned by comparing the shift of the anomeric proton at 4.25 ppm (J = 8.5 Hz) with a reported value of 4.32 ppm (J = 8.5 Hz) for methyl β-D-glucopyranoside.[12a] The resonance for the anomeric proton of methyl α-D-glucopyranoside at 4.83 ppm (J = 2.5 Hz)[12b] was not observed. In the absence of buffer, the yield of methyl β-D-glucopyranoside was <1%, which corresponds to a second-order

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(13) Wollwage, P. C.; Seib, P. A. *J. Chem. Soc. C* **1971**, 3143–3155.

Table II. Rate Constants for Catalysis of the Hydrolysis of α-D-Glucosyl Fluoride by Buffers in D_2O at 30.0 °C and Ionic Strength of 2.0 M (KCl)

buffer (pK_a)[a]	buffer ratio acid:base	[Buffer], M	k, $M^{-1}\,s^{-1}$	k_A, $M^{-1}\,s^{-1}$	k_{A^-}, $M^{-1}\,s^{-1}$
(trichloromethyl)phosphonate (4.28)	2:1	0.48–1.2	1.42×10^{-6}		
	1:1	0.40–1.0	1.54×10^{-6}		
	2:3	0.36–0.91	1.75×10^{-6}	2.6×10^{-6}	7.0×10^{-7}
	1:4	0.31–0.77	2.35×10^{-6}		
	1:5	0.24–0.60	2.40×10^{-6}		
	1:9	0.28–0.71	2.28×10^{-6}		
phosphate (6.46)	9:1	0.67–1.67	0.84×10^{-6}		
	85:15	0.62–1.54	1.14×10^{-6}		
	3:1	0.30–1.00	1.20×10^{-6}	3.5×10^{-6}	5.4×10^{-7}
	1:1	0.30–1.00	1.86×10^{-6}		
	1:3	0.40–0.80	2.91×10^{-6}		
	1:9	0.30–0.71	3.23×10^{-6}		
ethylphosphonate (7.60)	2.2:1	0.51–1.27	1.38×10^{-6}		
	1:1	0.40–1.00	2.25×10^{-6}	4.2×10^{-6}	2.7×10^{-7}
	1:9	0.29–0.72	3.79×10^{-6}		

[a] Funderburk, L. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 6708.

Table III. Second-Order Rate Constants and Isotope Effects for the Solvolysis of α-D-Glucosyl Fluoride in L_2O at 30.0 °C and Ionic Strength 2 M

conditions	[catalyst], M	k, $M^{-1}\,s^{-1}$	k_D/k_H	k_H/k_D
H_2O[a]		2.7×10^{-8}		
D_2O[a]		2.9×10^{-8}	1.1	
HO^-[b,c]	0.06	4.5×10^{-4}		
DO^-[b,c]	0.06	6.9×10^{-4}	1.5	
H_3O^+[b]	0.004–0.100	1.04×10^{-3}		
D_3O^+[b]	0.09	7.2×10^{-4}		1.4
L^+ in 1:1 H_2O–D_2O[b]	0.10	6.4×10^{-4}		1.6

[a] In 0.005 M 1:1 KL_2PO_4–K_2LPO_4 buffer, ionic strength 2.0 M, maintained with sodium perchlorate. [b] At ionic strength 2 M, maintained with KCl. The observed pseudo-first-order rate constants were correcteed for the nucleophilic reaction with Cl^-.[11] [c] Corrected for base-catalyzed cyclization.

rate constant for methanol that is <2% of that for water. The 17% yield of methyl β-D-glucopyranoside in the presence of 0.71 M buffer corresponds to an apparent second-order rate constant for methanol that is approximately half of that for water. Phosphorus-31 NMR of the products indicated that glucose-1-phosphate was not formed in the reaction mixture. Rate constants were not determined, but it was noted that the reaction proceeded faster in the presence of buffer. No reaction with methanol was observed in the absence of buffer.[11]

Discussion

The lifetime of the glucosyl oxocarbenium ion in water has not been measured directly; however, there is strong evidence that this cation has a short but significant lifetime in water. A short extrapolation of the rate constants for hydration of a series of substituted oxocarbenium ions gives a rate constant of $\sim 10^{12}\ s^{-1}$ for hydration of the glucosyl oxocarbenium ion;[4] this corresponds to a lifetime of $\sim 10^{-12}$ s. The solvolysis of a series of glucopyranoside derivatives in 1:1 ethanol–trifluoroethanol gives products with both retention and inversion of configuration, which provides evidence that the oxocarbenium ion has a significant lifetime in this solvent. However, the observation of different product ratios with different leaving groups shows that the oxocarbenium ion does not have a sufficient lifetime to become diffusionally equilibrated with the solvent components; this is consistent with the estimated lifetime of $\sim 10^{-12}$ s for this cation. The α- and β-glucosyl fluorides give an increased yield of the trifluoroethyl glycosides with retention in this solvent, which suggests that the fluoride ion assists the attack of trifluoroethanol by hydrogen bonding or general-base catalysis in a solvent-separated ion pair before it diffuses away into the bulk solvent,[14] or that trifluoroethanol stabilizes the rate-limiting transition state by hydrogen bonding to the leaving fluoride anion, so that the probability that trifluoroethanol will be in position to react with the oxocarbenium ion is increased.

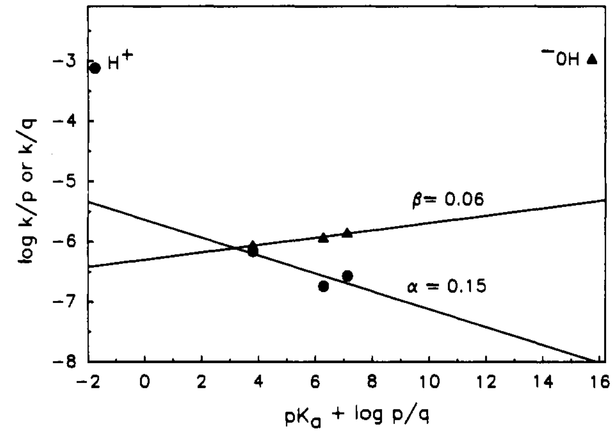
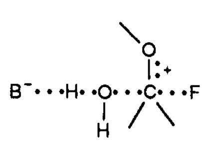


Figure 5. Statistically corrected Brønsted plots for the hydrolysis of α-D-glucosyl fluoride catalyzed by phosphate and phosphonate monoanions and dianions in D_2O at 30 °C, μ = 2.0 M (KCl).

The observed general-base catalysis of the hydrolysis of α-D-glucosyl fluoride is consistent with a concerted mechanism of catalysis in which the anionic base stabilizes the transition state for the attack of water by hydrogen bonding and by an electrostatic interaction with the developing positive charge at the reaction center, **2**. The reaction cannot involve general-base catalysis of the attack of water on the free glucosyl cation because this species reacts rapidly with water in the absence of a catalyst and catalysis would not cause an increase in the rate of disappearance of starting material. Therefore, the observed catalysis must occur either through catalysis of the attack of water on a reversibly formed ion pair or through catalysis of the attack of water on glucosyl fluoride that is concerted with the expulsion of F^-.



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The glucosyl cation is unlikely to have a significant lifetime when it is in contact with a fluoride ion because the rate constant for reaction with water is $\sim 10^{12}\ s^{-1}$ and fluoride ion is a much better nucleophile than water, so that there is not likely to be a significant barrier for collapse of a reversibly formed intimate ion pair. We suggest, therefore, that the intimate ion pair cannot exist as an intermediate species, so that the catalyzed reaction must represent concerted departure of the fluoride ion and attack of water that is facilitated by general-base catalysis. This is consistent with the observed solvent deuterium isotope effect of k_{HOH}/k_{DOD}

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= 1.9–2.0, which shows that there is a significant loss of zero-point energy of the transfered proton in the transition state.

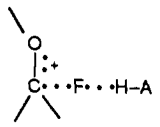
Figure 5 shows that the rate constants for general-base catalysis by buffer bases show only a small dependence on the basicity of the catalyzing base; the line in Figure 5 is drawn with a Brønsted slope of $\beta \approx 0.06$. This small slope indicates that there has been only a small amount of proton transfer to the catalyzing base, so that it is uncertain whether the proton is "in flight" or is strongly hydrogen bonded to the catalyzing base in the transition state.

The large positive deviation of hydroxide ion from the Brønsted correlation represents, at least in part, a different mechanism of specific base catalysis. This occurs through intramolecular attack by the anion of the 6-hydroxyl group to give 1,6-anhydroglucose and by nucleophilic attack of hydroxide ion at the 1-position to displace fluoride ion directly.[11]

The absence of catalysis by uncharged bases shows that an electrostatic interaction between the negative charge on the catalyst and the developing positive charge on the substrate provides significant stabilization to the transition state. General-base catalysis is observed with the bulky phosphate and phosphonate anions because these compounds do not readily react by nucleophilic substitution; acetate anion is less bulky and reacts as a nucleophile.[11] The importance of the electrostatic interaction is also evident in the reaction with methanol in methanol–water mixtures. Methanol reacts with glucosyl fluoride in the presence of phosphate buffer, an anionic catalyst, but shows no detectable reaction in the absence of catalyst.[11]

A kinetically equivalent mechanism of specific-base catalysis by attack of hydroxide ion with general-acid catalysis of the expulsion of fluoride ion by the protonated base is unlikely because specific-base catalysis gives a high yield of cyclic product from intramolecular attack of the 6-hydroxyl group and none of this product was observed in the general-base-catalyzed reaction. Furthermore, the Brønsted coefficient for general-acid catalysis by this mechanism is given by $\alpha = 1.0 - \beta$, which corresponds to a value of $\alpha = 1.0 - 0.06 = 0.94$ for general-acid catalysis of the expulsion of fluoride ion. The attack of water is assisted by general-acid catalysis with a small Brønsted coefficient of $\alpha = 0.15$, as described below, and it might be expected from Hammond effect considerations that attack by hydroxide ion, a much stronger nucleophilic reagent, would require the same or less assistance by protonation of the leaving group, and would give a Brønsted coefficient of $\alpha \leq 0.15$ rather than 0.94.

The observed general-acid catalysis of the hydrolysis of α-1-glucosyl fluoride by phosphate and phosphonate monoanions provides the first example of general-acid catalysis of the expulsion of fluoride ion of which we are aware. The catalysis is attributed to hydrogen bonding of the buffer acid with the leaving fluoride ion that facilitates the expulsion of this leaving grroup (**3**). This interpretation is consistent with the small dependence of the general-acid-catalyzed reaction on the acidity of the catalyzing acid shown in the Brønsted correlation of Figure 5; the line in the figure is drawn with a slope of $\alpha \simeq 0.15$. However, the slope is $\alpha = 0.4$ if the point for catalysis by H^+ is included. The solvent deuterium isotope effect of $k_{HOH}/k_{DOD} = 2.0$ indicates that there is significant stretching of the H–O bond of the acid catalyst in the transition state, but there is almost certainly not complete transfer of the proton to fluoride ion because HF is a stronger acid than the buffer catalysts. Fluoride ion is known to be a strong hydrogen bond acceptor that forms a stable complex, HF_2^-, with HF in aqueous solution.[15] Catalysis of the solvolysis of α-D-glucosyl fluoride by H_3O^+ and D_3O^+ in H_2O and D_2O gives a solvent isotope effect of $k_H/k_D = 1.4$. This is much larger than the calculated isotope effect for complete transfer of a hydron from L_3O^+ to F^- to give L_2O and LF of $k_H/k_D = (0.69)^3/0.61 = 0.54$, from the fractionation factors of $\phi = 0.69$ for L_3O^+ and 0.61 for LF.[16]



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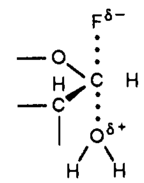
An electrostatic interaction is also significant for the general-acid-catalyzed reaction, because no catalysis was observed with acetic acid–acetate buffers. However, it is unlikely that the reaction proceeds through a cyclic transition state in which the phosphate or phosphonate monoanion forms a hydrogen bond with the leaving fluoride ion and removes a proton from the attacking water molecule. Space-filling CPK molecule models suggest that the oxygen atom of the attacking water molecule would not be in the correct position for reaction if it is hydrogen bonded to a catalyst that is also hydrogen bonded to the leaving fluoride ion on the other side of the glucose ring in the transition state.

The observed general-acid catalysis might also be interpreted as specific-acid catalysis, with complete protonation of the leaving fluoride in a rapid equilibrium step, accompanied by general-base catalysis of the attack of water. This mechanism is unlikely because it requires a value of $\beta = 1 - \alpha = 1.0 - 0.15 = 0.85$ for general-base catalysis. General-base catalysis by buffer bases of the attack of water to expel F^- follows $\beta = 0.06$, and the value of β for the attack of water to expel HF, which is a much better leaving group, would be expected to be the same or smaller, not much larger, compared with β for catalysis of the expulsion of F^-.

The uncatalyzed solvolysis of α-D-glucosyl fluoride in H_2O and D_2O shows a small inverse solvent isotope effect of $k_D/k_H = 1.1$. This is consistent with the conclusion that the uncatalyzed solvolysis of α-D-glucosyl fluoride occurs through a dissociative mechanism to give the glucosyl oxocarbenium ion, without nucleophilic assistance by solvent.[11] A small inverse solvent isotope effect is expected for a late transition state with a large development of negative charge on fluoride; the solvent deuterium isotope effect for the transfer of fluoride ion from H_2O to D_2O is approximately 1.12.[17] A transition state for the attack of water with complete formation of the bond to carbon would contain two hydrons that resemble those in L_3O^+ and would be expected to result in an isotope effect of $k_{DOD}/k_{HOH} = (0.69)^2 = 0.48$, while a transition state with partial formation of the O–C bond, **4**, would be expected to show an isotope effect in the range of $k_D/k_H = 0.48–1.0$. The observed isotope effect of $k_D/k_H = 1.1$ gives no indication of any proton transfer or bond formation from the attack of water on carbon in the transition state for solvolysis. The solvolysis of 2-(p-nitrophenoxy)tetrahydropyran in water and in 50% aqueous dioxane exhibits solvent isotope effects of $k_D/k_H = 0.9$ and 1.1, respectively, and is known to proceed through an intermediate oxocarbenium ion.[18]



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These results suggest that, although bimolecular general-base catalysis for the attack of water on a glucosyl oxocarbenium ion and, therefore, general-acid catalysis for the formation of this ion in the reverse direction, are not likely to be significant, general-acid and -base catalysis of these reactions can be significant for a

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concerted displacement reaction that is enforced by the absence of a significant lifetime of the oxocarbenium ion under the conditions of the experiment. It is likely that substitution reactions on sugars by carboxylate groups do occur by concerted displacement, through a dissociative, oxocarbenium-like transition state, because the cation will not have a significant lifetime in the presence of the carboxylate ion. The concerted displacement reaction of acetate ion with α-D-glucosyl fluoride was described in the preceding paper.[11] General-acid–base catalysis of such reactions will be even more significant if it is brought about by acidic and basic groups that are held in the correct position for reaction in intramolecular and enzyme-catalyzed reactions.[8,9]

Registry No. 1, 2106-10-7; methyl β-D-glucopyranoside, 709-50-2; glucose, 50-99-7; 1,6-anhydroglucose, 498-07-7; β-D-glucopyranose 1-(trichloromethyl)phosphonate, 135646-42-3.

Calixcrowns as Selective Potassium Cation Carriers in Supported Liquid Membranes

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Abstract: Calixcrown derivatives transport potassium cations selectively through supported liquid membranes (Accurel/*o*-nitrophenyl *n*-octyl ether, NPOE). These lipophilic carriers give stable fluxes (>4 weeks) for the membrane system described. Bridging with a tetraethylene glycol unit at the lower rim is essential since simple calix[4]arenes do not transport potassium cations. The potassium/sodium cation selectivity of these carriers was compared with the selectivity of other potassium-selective carriers like valinomycin and dibenzo-18-crown-6 in both single-cation and mixed-cation experiments. In the competition experiments, the order of decreasing K^+/Na^+ selectivity was as follows: valinomycin > 1,3-dimethoxy-*p-tert*-butylcalix[4]arene crown-5 > dibenzo-18-crown-6 > 1,3-dihydroxy-*p-tert*-butylcalix[4]arene crown-5. The transport selectivity is not simply related to the transport rates in single-cation experiments or to association constants. Although the K^+/Na^+ extraction selectivity is high, the K^+/Na^+ transport selectivity of valinomycin and 1,3-dimethoxy-*p-tert*-butylcalix[4]arene crown-5 (**5**) is low due to a high association constant for K^+ in NPOE combined with a low diffusion constant. A mathematical model has been developed that predicts the observed K^+/Na^+ selectivities in competition experiments from the results of single-cation experiments.

Liquid membranes containing selective carriers may give higher fluxes and selectivities than conventional semipermeable porous polymeric membranes. Since bulk liquid membranes[1] require a large quantity of carrier solution in proportion to the interfacial area where phase transfer can take place, supported liquid membranes (liquid-immobilized membranes) have been developed. They consist of a carrier solution immobilized in a thin microporous support ($d \approx 100$ μm) that separates the two aqueous phases. These supported liquid membranes are of interest both for possible technological applications (hollow fibers) and for fundamental studies of the transport process.

Previously, we described the mechanism of single-cation transport of guanidinium and potassium salts through supported liquid membranes by crown ethers.[2–4] The experimental fluxes could be described by a mathematical model based on diffusion-limited transport. Since the membrane volume of a supported liquid membrane is relatively small compared to the volume of the aqueous phases, the carriers must be very lipophilic in order to avoid substantial partitioning to the aqueous phases. We have found that the membrane stability can be improved by using carriers modified with hydrophobic alkyl or aryl groups[2,3] or carriers attached to a polysiloxane backbone.[4]

So far we have only studied single-cation transport. However, membranes have been developed for separations, and consequently we have studied selective transport by macrocyclic carriers through supported liquid membranes. As a model system we have chosen the selective potassium/sodium cation transport by calixcrown derivatives. 1,3-Dimethoxy-*p-tert*-butylcalix[4]arene crown-5 (**5**) shows a high K^+/Na^+ selectivity in both extraction ($CHCl_3/H_2O$, $K_{K^+} = 3.0 \times 10^8$ M^{-1} and $K_{Na^+} = 1.1 \times 10^5$ M^{-1})[5] and in ISFET measurements (the potentiometric selectivity coefficient $K_{K^+/Na^+} = 1600$).[6] Moreover, these compounds are very lipophilic and hence may be applicable as carriers in supported liquid membranes.

So far calixarene derivatives have only been studied as carriers in bulk liquid membranes to transport alkali cations (in particular cesium cations)[7–11] or UO_2 cations,[12] except for one application in which UO_2 cations are transported by calix[6]arenes through a polymer/liquid crystal composite membrane.[12] To the best of our knowledge, we now report the first application of calixarenes as carriers in supported liquid membranes. A mathematical model

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