Foundation (BSF) for financial assistance. We thank Dr. M. Kapon for his help with computing.

Registry No. Dithizone·I_2, 88995-88-4; ethylenethiourea·2(I_2), 88995-89-5; 2(ethylenethiourea)·3(I_2), 88995-90-8; 2(dithizone)·7(I_2), 81615-07-8; 1-(1-imidazolin-2-yl)-2-thioxoimidazolidinium triiodide-(ethylenethiourea diiodine), 88995-91-9; ethylenethiourea, 96-45-7; dithizone, 60-10-6.

Supplementary Material Available: Tables of atomic parameters, anisotropic temperature factors, and observed and calculated structure factors are given for the five crystal structures reported (72 pages). Ordering information is given on any current masthead page.

Catalysis of Anilide Hydrolysis by Poly(ethylenimine) Derivatives

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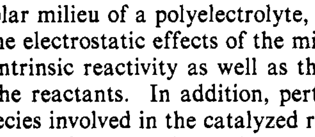
Abstract: Amide hydrolysis in 4-nitro-3-carboxytrifluoroacetanilide (**1**) is catalyzed by poly(ethylenimines). The rate-controlling step is the formation of a tetrahedral intermediate, breakdown of which is catalyzed by the conjugate acid and base forms of the amine groups of the polymers. As estimated from the pH profiles of k_{cat}, the rate of hydroxide attack is 6–180 times greater in the polymer milieu than in bulk water. This enhancement in rate is largely a reflection of the increased local concentration of hydroxide in the vicinity of the cationic sites of the polymers; the hydrophobic local environments of the macromolecule appear to have little effect on the intrinsic reactivity of hydroxide ion. At low pH, water molecule attack on complexed anilide, **1**, is observed, and bound acetate, as a general base, assists therein.

Although micelle- or polymer-catalyzed reactions have been studied extensively,[1-3] successful estimation[4-6] of the intrinsic reactivity of nucleophiles in micellar or polymeric domains relative to that in bulk water has been rare. In part this is because resolution of catalytic effects into contributions from enhanced intrinsic reactivity and from increased effective concentrations of the reactants has seldom been achieved. For reactions taking place in the apolar milieu of a polyelectrolyte, both the hydrophobicity and the electrostatic effects of the microenvironment can affect the intrinsic reactivity as well as the effective concentrations of the reactants. In addition, perturbation in the ionization of species involved in the catalyzed reactions further complicates analysis of the kinetic results.

Many derivatized micelles or polymers have been used as catalysts for ester hydrolysis, and the results have been frequently compared with the actions of proteases. Although amides are the natural substrates of the proteases, their hydrolysis has seldom been investigated in micellar or polymeric systems, due to the much greater stability of amide bonds compared with ester bonds. In this article, kinetic results in the hydrolysis of 4-nitro-3-carboxytrifluoroacetanilide (**1**) catalyzed by various poly(ethylenimine) (PEI) derivatives are reported. As the amide hydrolysis of **1** occurs through the attack of hydroxide ion, the enhanced rates have been analyzed in terms of the intrinsic reactivity and the effective concentration of hydroxide ion in the hydrophobic milieu of the cationic polymers. Contributions of other catalytic factors have also been evaluated.



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Experimental Section

Materials. 4-Nitro-3-carboxytrifluoroacetanilide was prepared from 2-nitro-5-aminobenzoic acid (0.5 g) and trifluoroacetic anhydride (5 mL) by stirring the mixture at room temperature overnight. The crystals that formed were filtered, washed with petroleum ether, and recrystallized from ether-petroleum ether: mp 217–221 °C dec. Anal. Calcd for $C_9H_5F_3N_2O_5$: C, 38.86; H, 1.81; N, 10.07. Found: C, 38.94; H, 1.98, N, 10.09.

Modifications of poly(ethylenimine) (PEI) was carried out according to literature procedures.[7-9] The sample of polymer used, PEI 600 (Dow Chemical Co.), contains, on the average, 1400 monomer residues per macromolecule. Of these, 25% provide primary amino groups, 50% secondary, and 25% tertiary. Lauryl groups become attached preferentially to the primary nitrogens and 4-imidazolylmethyl groups are linked mainly to the secondary nitrogens. The modified polymers were purified by ultrafiltration against 0.1 M NaCl and then against water.

The poly(ethylenimine) derivatives prepared had the following stoichiometric compositions: $Lau_{12}PEI$, $(C_{12}H_{25})_{0.12m}(C_2H_4N)_{1.0m}(Cl)_{0.54m}$; $Lau_{12}Im_{10}PEI$, $(C_{12}H_{25})_{0.12m}$(4-imidazolylmethyl)$_{0.10m}(C_2H_4N)_{1.0m}$-$(Cl)_{0.50m}$; $Lau_{25}PEI$, $(C_{12}H_{25})_{0.25m}(C_2H_4N)_{1.0m}(Cl)_{0.50m}$; $Lau_{25}Me_QPEI$, $(C_{12}H_{25})_{0.25m}(CH_3)_{1.45m}(C_2H_4N)_{1.0m}(Cl^-)_{0.70m}$; m is 1400.

Buffer Solutions. Since the kinetic behavior of the reactions catalyzed by poly(ethylenimine) derivatives is influenced by the nature and concentrations of anions present, buffer solutions were prepared as follows. For monoamine buffers [3-(cyclohexylamino)-1-propanesulfonic acid (Caps) at pH 9.5–11, tris(hydroxymethyl)aminomethane (Tris) at pH 7.5–9, bis(2-hydroxyethyl)aminotris(hydroxymethyl)methane (Bistris) at pH 5.5–7], the base (0.02 M) was first neutralized in solution with hydrochloric acid or with acetic acid to yield a 1:1 salt, a desired amount of polymer was added, and then the pH of the solution was adjusted with sodium hydroxide. At pH 4–5.5, three other buffer systems were used. When chloride was to be the anion, 0.02 M methoxyamine hydrochloride

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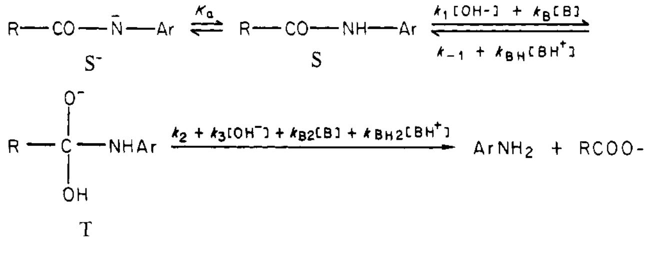
Table I. Values of Parameters Derived from the pH Profiles of k_{cat} for Hydrolysis[a] of 4-Nitro-3-carboxytrifluoroacetanilide (1) Catalyzed by Derivatives of Poly(ethylenimine)

polymer	anion[b]	$\mathrm{p}K_a'$ [c]	$10^4 k_1'/K_c'$ $(=k_1'K_w'/K_a')$, s^{-1}	$k_1'K_w'/10^{-14}$, $M \cdot s^{-1}$	$10^5 k_w'$, s^{-1}	eq[d]
$Lau_{12}PEI$	Cl^-	6.40 (6.5)	5.4	22000	0.7	11
$Lau_{12}PEI$	Ac^-	6.00 (6.1)	4.4	44000	2.7	11
$Lau_{12}Im_{10}PEI$	Cl^-	6.40 (6.5)	4.0	16000	0.9	11
$Lau_{12}Im_{10}PEI$[e]	Ac^-	6.50 (6.4)	4.0[f]		1.4	14
$Lau_{25}PEI$	Cl^-	6.35 (6.4)	3.4	15000		11
$Lau_{25}Me_QPEI$[g]	Cl^-	(6.8)	0.93	1500		12
PEI	Cl^-	6.70 (6.8)	14.0	28000	1.3	11
none	Cl^-	(8.3)	4.9	250		1

[a] Measured at 25 °C and 0.02 ionic strength in the presence of 0.8% (v/v) acetonitrile. [b] Anion of the supporting electrolyte. [c] Kinetically obtained values. The numbers in parentheses were obtained from spectroscopic titrations. [d] Equation used for analysis of the pH profile. [e] $\alpha = 1.8$. [f] The relationship $K_c' = K_w'/K_a'$ does not hold. [g] $k_3'K_w'/k_2' = 3.5 \times 10^{-8}$ M, $k_{-1}'/k_2' = 0.6$. These values, however, are not as reliable as those listed in the table.

Scheme I



solutions were adjusted to the desired pH by addition of sodium hydroxide (buffer a). To provide acetate anion, 0.02 M sodium acetate solutions were acidified to proper pH with acetic acid (buffer b). When mixed anions were needed, 0.02 M acetic acid solutions containing 0.02 M sodium chloride were treated with sodium hydroxide for pH adjustment (buffer c). In buffer c, the concentration of acetate anion was less than 0.02 M. Basic amine buffers (Caps, Tris, Bistris) from commercial sources were used without purification, but methoxyamine hydrochloride was recrystallized from 2-propanol. Water was deionized and redistilled before use in kinetic studies as well as in purification of the polymers. Measurements of pH were performed with an Orion Model 701A pH meter. For hydrochloric acid solutions the pH was calculated as $-\log [H^+]$.

Kinetic Measurements. The rate of the hydrolysis of **1** was measured with a Cary 14 spectrophotometer by following the increase in absorbance at 400 nm due to the appearance of the released aniline (ϵ° at pH 4–11, 1.5×10^4 cm^{-1} M^{-1} at 400 nm, 1.7×10^4 cm^{-1} M^{-1} at 380 nm ($\lambda_{\max}$)). To maintain the concentration of catalytic domain (C_0) much higher than that of substrate (S_0), 1×10^{-5} M amide (**1**) was used in the polymer-catalyzed reactions. Whenever possible, the absorbance readings were measured at "infinite" time for use in the calculation of the pseudo-first-order rate constants (k_0). For reactions with half-lives greater than 5 h ($k_0 < 4 \times 10^{-5}$ s^{-1}), absorbance readings of the products were measured under the identical conditions as those for the kinetics so that k_0 could be calculated therefrom. All of the rate data were collected at 25 °C in the presence of 0.8% (v/v) acetonitrile. The ionic strength (μ) was maintained at 0.02 except in solutions undergoing spontaneous hydrolysis at pH <1.5 where μ was 1.0.

Results

Spontaneous Reaction. The hydrolysis of trifluoroacetanilides is known to proceed according to Scheme I.[10-14] Except at pH <1.5, the carboxyl group of **1** is predominantly in the anionic state, and its ionization can be disregarded in the kinetic scheme. Only hydroxide ion and the water molecule (in a general-base-assisted reaction) have been found to make a nucleophilic attack at the anilides. These reactions are controlled by the rate constants k_1 and k_B and their reversal by k_{-1} and k_{BH}. B represents the general base. The subsequent breakdown of the tetrahedral intermediate, T, to products is catalyzed by hydroxide ion, general acids, and general bases, for which the respective rate constants are k_3, k_{B2}, and k_{BH2}. At high pH values or in the presence of sufficient amounts of general acids or bases, breakdown of T to products is much faster than return of T to S. Under these circumstances the formation of T is rate controlling, and general base catalysis in this step can lead to an overall rate increase.

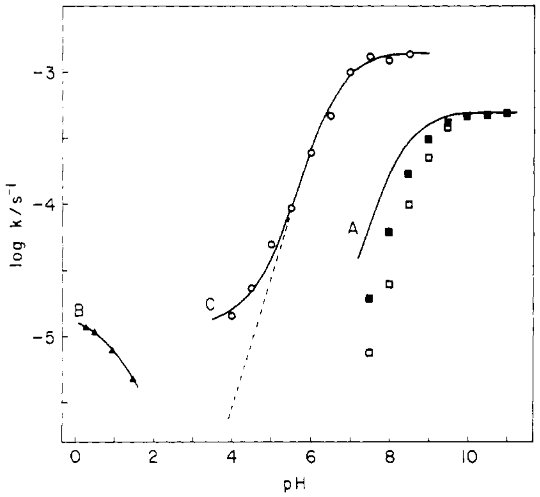


Figure 1. pH dependence of k_0 for the spontaneous hydrolysis of anilide **1** (curves A and B; ■, 0.02 M buffer; □, 0.0025 M buffer) or of k_{cat} for the PEI-catalyzed hydrolysis (curve C). The broken line represents the pH profile expected for the PEI-catalyzed reaction when the water path (k_w' term) is absent. The reactions were carried out with chloride ion as the anion.

In the absence of general acids or bases and if the rate of breakdown of tetrahedral intermediate is much faster than that for its return to starting amide $\{(k_3[OH^-] + k_2) >> k_{-1}\}$, then the pseudo-first-order rate constant, k_0, that governs Scheme I is given by

$$k_0 = \frac{k_1[OH^-]}{1 + K_a/[H^+]} = \frac{k_1 K_w}{K_a + [H^+]} \tag{1}$$

As is evident from this equation, at sufficiently high pH values at which $[H^+] < K_a$, the observed rate k_0 should approach a plateau value of K_1K_w/K_a. In essence what happens with increase in pH is that the reactant S is increasingly converted to unreactive S$^-$ as well as to T (see Scheme I).

Experimental observations of the spontaneous reaction (Figure 1) show that a plateau is indeed reached at high pH. On the other hand, eq 1 is not compatible with the data at pH <9.5. Under these conditions, the breakdown of T, rather than its formation, controls the overall rate. The slower rates at lower buffer concentration reflect buffer catalysis in the breakdown of T. The value of k_1K_w/K_a determined kinetically from the plateau at high pH and that of pK_a measured by spectroscopic titration of anilide

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Scheme II

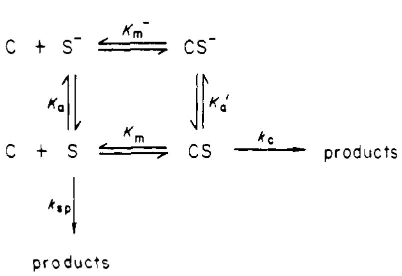


Table II. Values of K_m^{app} and K_m for Hydrolysis[a] of 4-Nitro-3-carboxytrifluoroacetanilide (**1**) Catalyzed by Derivatives of Poly(ethylenimine) at pH 7.5

polymer	anion[b]	$10^4K_m^{app}$ (residue M)[c]	10^3K_m (residue M)[d]
$Lau_{12}PEI$	Cl^-	8.6	10
$Lau_{12}PEI$	Ac^-	3.6	10
$Lau_{12}Im_{10}PEI$	Cl^-	8.3	9.7
$Lau_{12}Im_{10}PEI$	Ac^-	3.3	1.3[e]
$Lau_{25}PEI$	Cl^-	5.1	6.6
$Lau_{25}Me_QPEI$[a]	Cl^-	4.4	2.3
PEI	Cl^-	68.0	43

[a] Measured at 25 °C and 0.02 ionic strength. The data for $Lau_{25}Me_QPEI$ were obtained at pH 9.0. [b] Anion of the supporting electrolyte. [c] Calculated from the linear plots of $1/k_0$ vs. $1/C_0$. (Correlation coefficients are better than 0.98.) [d] Calculated according to eq 4 unless noted otherwise. Whenever possible, the kinetically obtained values of pK_a' were used in calculation. [e] Calculated according to eq 15.

1 at 400 nm are summarized at Table I.

Rates at very acidic pH values, in solutions of hydrochloric acid, are also shown in Figure 1. Kinetic measurements were not made at weakly acidic pH values because the rates were slow and buffer catalysis introduced complications.

Catalyzed Reaction. Kinetics of the hydrolysis of the anilide **1** were measured in the presence of various derivatives of polyethylenimine. In these solutions, Scheme II is an appropriate representation. C represents the catalytic polymer, the K's are the (dissociation) equilibrium constants for the respective reactions, and k_c and k_{sp} are the rates along the breakdown paths shown. When the spontaneous path is negligibly slow compared with the catalytic one, the pseudo-first-order rate constant (k_o) measured under the condition that initial concentration of the catalyst, C_o, is much greater than the initial concentration of substrate, S_o, is[15]

$$k_o = k_{cat}C_o/(K_m^{app} + C_o) \qquad (2)$$

where

$$k_{cat} = k_c/(1 + K_a'/[H^+]) \qquad (3)$$

$$K_m^{app} = K_m(1 + K_a/[H^+])/(1 + K_a'/[H^+]) \qquad (4)$$

To obtain eq 3 and 4, we assume that the ionization of S in CS fits the simple equlibrium expression

$$K_a' = [CS^-][H^+]/[CS] \qquad (5)$$

with $[H^+]$ being the hydronium ion concentration in bulk aqueous solution. An alternative constant, K_c', for the equlibrium between CS and CS^- can be defined as

$$K_c' = [CS^-]/[CS][OH^-]' \qquad (6)$$

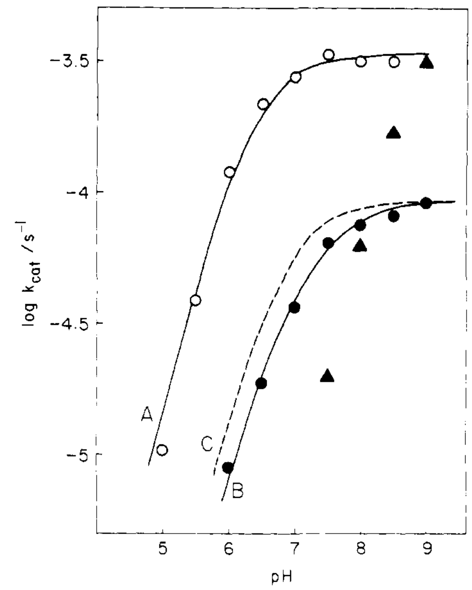
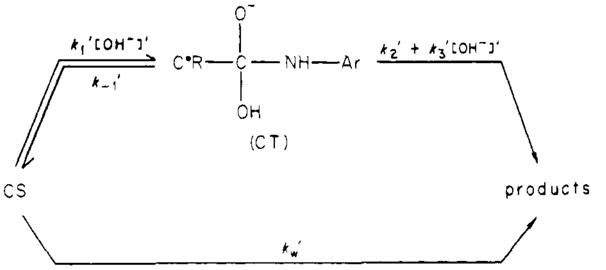


Figure 2. pH dependence of k_{cat} for the $Lau_{25}PEI$- or the $Lau_{25}Me_QPEI$-catalyzed hydrolysis of **1** (curves A and B, respectively). Spontaneous rates with 0.02 M buffer are also indicated (▲). Curve C was constructed for $Lau_{25}Me_QPEI$ by using eq 11. For $Lau_{25}Me_QPEI$ the pH at which k_{cat} is half of the limiting value is 7.2 whereas pK_a' of CS is 6.8.

Scheme III



where $[OH^-]'$ is the hydroxide ion concentration in the polymer domain, a quantity not accessible experimentally. If eq 5 and 6 are combined, we obtain

$$K_a'/K_c' = [H^+][OH^-]' = K_w' \qquad (7)$$

The observed variation of k_o with C_o (measured in monomer residue–molar concentration units) is hyperbolic, as is evident from the linearity of a plot of $1/k_o$ against $1/C_o$ from which the values of k_{cat} and K_m^{app} can be estimated. At pH 7.5 values of K_m^{app}, in terms of the monomer residue–molar concentration (residue M) are summarized in Table II. For the reaction catalyzed by $Lau_{25}Me_QPEI$, K_m^{app} was measured at pH 9 as the rate at pH 7.5 was very slow. For this quaternized polymer, the charge on the surface is almost fixed, and hence K_m at pH 9.0 should be close to that at pH 7.5. Nevertheless, the rate observed at pH 9.0 in the presence of $Lau_{25}Me_QPEI$ was slower than that of the spontaneous reaction (Figure 2). A linear plot of $1/(k_{sp}' - k_o)$ against $1/C_o$, based on a transform of eq 8,[15] was used for the estimation of K_m^{app}.

$$k_{sp}' - k_o = (k_{sp}' - k_{cat})C_o/(K_m^{app} + C_o) \qquad (8)$$

For unmodified PEI, k_{cat} was esimated from a plot $1/k_o$ against $1/C_o$, and its pH dependence is illustrated in Figure 1. For the modified PEI derivatives, however, k_{cat} was taken as the measured k_o at C_o much greater than K_m^{app};[16] the pH profiles of k_{cat} for these polymers are illustrated in Figures 2–4. These profiles are best

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[15] The exact expression for the pseudo-first-order rate constant derived from Scheme II under the condition of $C_o >> S_o$ is $k_o = (k_{cat}C_o + k_{sp}'K_m^{app})/(K_m^{app} + C_o)$. Here, the first term of the numerator arises from the contribution of the polymer-catalyzed path and the second term from that of the spontaneous path. The parameter k_{sp}' $\{=k_{sp}/(1 + K_a/[H^+])\}$ corrects k_{sp} for the ionization of S to S^- and represents the pseudo-first-order rate constant actually observed in the spontaneous reaction.

[16] Under these conditions, a 3-fold decrease in C_o resulted in less than a 10% difference in k_o.

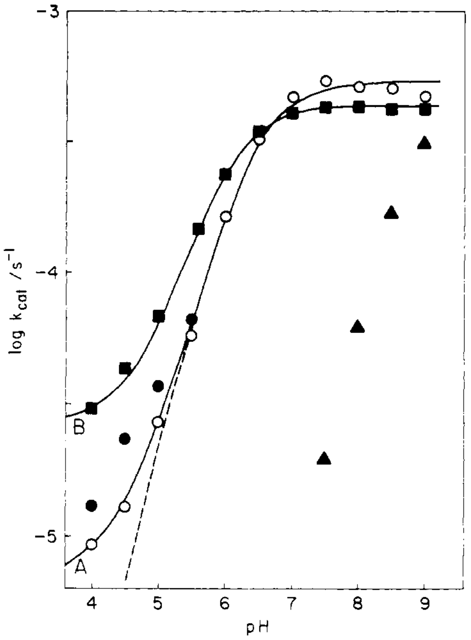

Figure 3. pH dependence of k_{cat} for the Lau$_{12}$PEI-catalyzed hydrolysis of **1** (curve A, chloride as the anion; curve B, acetate as the anion). At pH 4–5.5, buffer a (○), b (■), or c (●) (see Experimental Section) was used. The dotted line is the pH profile expected when the water path (k_w' term) is absent. Spontaneous rates with 0.02 M buffer are also indicated (▲).

analyzed by assuming two paths for the breakdown of CS into products (Scheme III). The hydroxide path is essentially identical with that in Scheme I, except that the general catalysis is not included in Scheme III. The expression for k_c derived from Scheme III is

$$k_c = \frac{k_1'(k_2' + k_3'[OH^-]')[OH^-]'}{k_{-1}' + k_2' + k_3'[OH^-]'} + k_w' \tag{9}$$

When $\{k_2' + k_3'[OH^-]\} >> k_{-1}'$ so that the formation of CT is rate controlling,

$$k_3 = k'_1[OH^-]' + k'_w \tag{10}$$

Combination of eq 3 and 10 leads to

$$k_{cat} = \frac{k_1'/K_c' + k_w'[H^+]/K_a'}{1 + [H^+]/K_a'} \tag{11}$$

The pH profiles of k_{cat} for PEI (curve C of Figure 1), Lau$_{12}$PEI (chloride or acetate counteranion, curves A and B, respectively, of Figure 3), and Lau$_{12}$Im$_{10}$PEI (chloride counteranion, curve A of Figure 4) were analyzed in terms of eq 11. The k_{cat}–pH curve for Lau$_{25}$PEI (curve A of Figure 2) was also analyzed with eq 11 but excluding the k_w' path. Values of k_1'/K_c' ($=k_1'K_a'/K_w'$) and pK_a' derived from the analysis of these pH profiles are summarized in Table I.

The ionization constant K_a' can be also estimated from spectroscopic data, as CS$^-$ absorbs strongly at 400 nm and the absorbance shows sigmoidal dependence on pH. The initial absorbance of a polymer solution containing **1** (1×10^{-5} M) was measured under the condition of $C_0 >> K_m^{app}$, and the pK_a' was taken as the pH at which this absorbance is half that of the limiting value at pH 8–8.5. The pK'_a values obtained in this way agree well with those determined from the kinetic data, as indicated in Table I.

For the Lau$_{25}$Me$_Q$PEI-catalyzed reaction, the curve (C of Figure 2) drawn according to eq 11 (with $k_w' = 0$) with the pK_a' determined from the spectroscopic data deviates greatly from the observed rate data. Similar deviation is also seen with the spontaneous hydrolysis of **1** and is attributable to the shift of rate control to the breakdown of the tetrahedral intermediate.

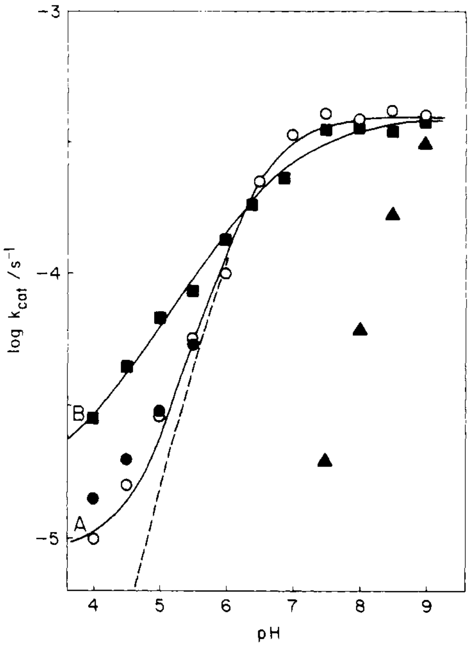

Figure 4. pH dependence of k_{cat} for the Lau$_{12}$Im$_{10}$PEI-catalyzed hydrolysis of **1**. For identification of the symbols, see Figure 3.

Therefore, the pH profile for Lau$_{25}$Me$_Q$PEI was analyzed according to eq 12, which is derived from eq 3 and 9 (with $k_w' = 0$),

$$k_{cat} = \frac{k_1'K_w'(1 + k_3'K_w'/k_2'[H^+])/[H^+]}{(1 + k_{-1}'/k_2' + k_3'K_w'/k_2'[H^+])(1 + K_a'/[H^+])} \tag{12}$$

and values of the parameters used in the construction of Curve B of Figure 2 are summarized in Table I.

For the reaction catalyzed by Lau$_{12}$Im$_{10}$PEI with acetate ion added as the anion of the supporting electrolyte, a good fit of the pH profile was not obtained with the equations presented so far. Instead, it was necessary to assume in addition that the ionization of the substrate in the polymer domain is nonideal and can be expressed in terms of eq 13. If eq 13 and 6 are combined and

$$([CS^-]/[CS])^\alpha = K_a'/[H^+] \tag{13}$$

used together with Schemes II and III, we obtain

$$k_{cat} = \frac{k_1'/K_c' + k_w\{[H^+]/K_a'\}^{1/\alpha}}{1 + \{[H^+]/K_a'\}^{1/\alpha}} \tag{14}$$

$$K_m^{app} = K_m(1 + K_a/[H^+])/(1 + \{K_a'/[H^+]\}^{1/\alpha}) \tag{15}$$

Equation 2 holds for k_0. Values for the parameters obtained from analysis of the pH profile according to eq 14 are summarized in Table I.

Discussion

Binding. As in enzymatic reactions, separate estimation of the catalytic constant (k_{cat}) and of the binding constant ($1/K_m$) is necessary for detailed mechanistic analysis of reactions that proceed through the formation of catalyst–substrate (CS) complexes. For many transformations catalyzed by micelles or polymers, weak binding of the substrate to the catalyst or limited solubility of the catalyst often precludes an adequate kinetic analysis. With poly(ethylenimine) derivatives, however, strong binding can be easily achieved because of hydrophobic and electrostatic interactions between the polymer and the substrate.[6,7] Such interactions were also evident between anilide substrate **1** and the modified poly(ethylenimines). The hydrophobic contribution to the binding is reflected in the K_m values, measured at pH 7.5 (Table II), which parallel the hydrophobicity[7] of the polymer surface.[17] That electrostatic interactions are also involved is evident from the observation that the hydrolysis of *p*-nitro-

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[17] Differences in the degree of the protonation of the polymer and the number of monomer residues per catalytic unit[7] are also reflected in K_m.

trifluoroacetanilide is unaffected by PEI derivatives;[18] the carboxylate group in **1** is essential for binding.

Amine Catalysis. Except with Lau$_{25}$Me$_Q$PEI, the catalyzed reactions are consistent with the rate-controlling step being hydroxide attack at the substrate. This feature stands in marked contrast with the mechanism in the spontaneous hydrolysis illustrated to Figure 1 or in the corresponding reactions reported[10,12] for other trifluoroacetanilides. In these spontaneous reactions at pH values near neutrality, the breakdown of the tetrahedral intermediate is the dominant rate-controlling step rather than the formation of the intermediate. Curve A of Figure 1 represents the rates expected when the breakdown of the intermediate is sufficiently catalyzed so that the hydroxide attack at the substrate becomes the sole rate-controlling step.

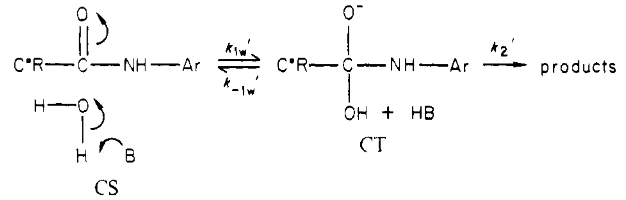
The curves of k_{cat} vs. pH for most of the PEI derivatives are best explained in terms of catalysis in the breakdown of CT and the consequent change in the rate-controlling step $\{(k_2' + k_3'[OH^-]) >> k_{-1}'$, Scheme III$\}$. Accelerated breakdown of CT may be attributed to either enhanced hydroxide assistance ($k_3'[OH^-]'$) or the participation of polymer functional groups (k_2'). Increased assistance by hydroxide ion is less likely, however, in view of the observed rates for Lau$_{25}$Me$_Q$PEI. In this polymer, the increase in the effective hydroxide concentration caused by the electrostatic effects of the positive charge in the polymer matrix should be not much different from that in the other polymers, but the pH profile resembles that of the spontaneous reaction rather than those of other polymers. Besides, a contribution from hydroxide assistance to the breakdown of CT must diminish at low pH's, but changes in rate-controlling step are not seen for most of the polymer-catalyzed reactions over a wide pH range.

Unlike the other polymers, Lau$_{25}$Me$_Q$PEI contains a very limited number of ionizable amine groups. It seems reasonable, therefore, to ascribe the accelerated breakdown of CT observed with the rest of the PEI derivatives to the amines of these polymers. It is well established that both amines and their conjugate acids catalyze the breakdown of tetrahedral intermediates formed in the hydrolysis of trifluoroacetanilides.[10-14] In the spontaneous hydrolysis, the change in rate-controlling step from the breakdown to the formation of the tetrahedral intermediate is achieved with less than 1 M general acid or general base.[10,12,14] Such local effective concentrations of general catalysts in the polymer matrix could be easily provided by the unquaternized PEI derivatives.

Hydroxide Reaction. The degree of acceleration of hydrolysis of **1** in the presence of the PEI derivatives varies with pH. Thus, the rate is enhanced by over a 100-fold around pH 7, but much less so at higher pHs. Leveling off of the pseudo-first-order rate constant at high pH is due to the ionization of **1** to form the unreactive amide species S$^-$. Thus, the rates of hydroxide attack at **1** can be better compared by examining $k_1'K_w'/10^{-14}$ rather than $k_1'K_w'/K_a'$, the limiting k_o at high pH. The intrinsic reactivity of hydroxide ion toward un-ionized **1** is reflected in k_1', and the effective hydroxide concentration in $K_w'/10^{-14}$. In terms of $k_1'K_w'/10^{-14}$, summarized in Table I, the rate of the hydroxide reaction is enhanced by 6–180 times in the polymer domain.

The two terms, intrinsic reactivity and effective concentration, are not easily separated from each other. Nevertheless, if it is assumed that the ionization of water is enhanced to the same extent as that of **1** in the cationic polymer matrix, K_c' ($=K_a'/K_w'$) would not depend greatly on the environment. Then, k_1'/K_c' could reflect the relative intrinsic reactivity of hydroxide ion toward **1** in various polymer environments. The values of k_1'/K_c' for Lau$_{12}$PEI, Lau$_{12}$Im$_{10}$PEI, and Lau$_{25}$PEI are almost identical with those of k_1/K_c for the spontaneous reaction. Compared with the spontaneous reaction, the k_1'/K_c' is only about 5 times smaller for the Lau$_{25}$Me$_Q$PEI, the most hydrophobic polymer examined, and only about 3 times greater for PEI, the least hydrophobic polymer. Although the exact location in the polymer matrix where the attack of hydroxide ion at **1** takes place is unknown, the binding behavior indicates that the substrate is attracted to the polymer by both hydrophobic and electrostatic interactions. Thus, it is reasonable to assume[19] that the hydrolysis occurs at the periphery of the hydrophobic domain near the positively charged loci of the polymer. The similar k_1'/K_c' values indicate, therefore, that the hydroxide ion is situated at the perimeter of the hydrophobic domain, and hence its reactivity toward anilide **1** is not sensitive to the apolar nature of the adjoining microenvironment.

Scheme IV



The intrinsic reactivity of other anionic reagents in cationic hydrophobic environments has been estimated in a few instances. For example, our previous studies[5] of the reaction of azide ion in the presence of a hydrophobic PEI derivative revealed that the reactivity of the anion depends on the type of reaction. Thus, reactivity was greatly enhanced toward an aromatic halide (S$_{NAr}$) where delocalization of negative charge occurs in the transition state. It is well-known that hydrophobic media facilitate such charge delocalization.[20] On the other hand, the reactivity of azide ion toward an ester substrate under the identical conditions was not affected significantly.[5] Similar observations were also reported later with micelles.[21]

The electrostatic effects exerted within the cationic polymers are also manifested in the decrease in pK_a of bound **1** by 1.5–2 units. This decrease can be compared with changes of 2–3 units in pK_a of some enzymatic functional groups due to electrostatic interaction between two oppositely charged atoms.[22] For pyridine groups pK_a has been found to decrease by 2.5–3 units when they are attached covalently to Lau$_{12}$PEI;[19] both electrostatic interaction and the hydrophobicity shift the equlibrium toward the uncharged form of the heterocycle. Electrostatic effects of PEI polymers also decrease the pK_a of the –COOH group of **1**. Consequently k_{cat} values in the present study, even at the lowest pH (about 4), refer to the reaction of **1** with the carboxyl group in the anionic –COO$^-$ state.

Water Reaction. For the PEI-, Lau$_{12}$PEI-, or Lau$_{12}$Im$_{10}$PEI-catalyzed reaction, k_{cat} approaches a limiting value when the pH is lowered. This trend can be explained by a rate-controlling water attack at CS (Scheme IV). Breakdown of CT is catalyzed by the protonated amines of the polymers, and hence the formation of CT becomes rate controlling. At low pHs, where the hydroxide attack at CS becomes very slow, CT can be formed predominantly through the general-base-assisted attack of a water molecule at CS. That a general base can assist in water attack at CS is supported by the observations at pH ≤ 5 of anion effects on k_{cat}. As is illustrated in Figures 3 and 4, the rate of the water path is enhanced when the acetate concentration is raised. This is consistent with the binding of acetate ion in the polymer matrix and its subsequent assistance in the attack of a water molecule at the bound substrate.

The mechanism of Scheme IV is analogous to that of the imidazole-catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide, in which the imidazole-assisted attack of water at the anilide becomes rate controlling when the breakdown of the intermediate is sufficiently catalyzed[12] to become very fast. The results for the spontaneous hydrolysis of **1** in hydrochloric acid solutions (curve B of Figure 1) are consistent with the formation of T by the attack of water at **1** and subsequent acid catalysis in the breakdown of T. However, the data are too limited to make this conclusion a firm one, and the ionization of the carboxyl group of **1** further

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complicates the mechanistic interpretation.

Anion Effects. The effects of substitution of acetate for chloride on the kinetic behavior of the catalyzed reactions were examined with $\mathrm{Lau_{12}PEI}$ and $\mathrm{Lau_{12}Im_{10}PEI}$.[23] As for the catalytic constants, that of the hydroxide path (k_1'/K_c') was not affected significantly. The effects of acetate ion on k_w' have been attributed to the general base assistance by the bound acetate ion (see previous section).

In regard to the binding of **1**, K_m for $\mathrm{Lau_{12}Im_{10}PEI}$ is lowered significantly when chloride is replaced by acetate, but binding to $\mathrm{Lau_{12}PEI}$ is unaffected.[24] The different behavior of the two polymers is also seen in the ionization of the bound substrate. In $\mathrm{Lau_{12}PEI}$, $\mathrm{p}K_a'$ is affected significantly. In $\mathrm{Lau_{12}Im_{10}PEI}$, however, $\mathrm{p}K_a'$ is not changed appreciably, but the ionization behavior of CS becomes abnormal ($\alpha = 1.8$) when chloride is substituted by acetate. These specific anion effects cannot be easily explained until more details are available on their disposition in the matrix of the poly(ethylenimines).

Conclusion

It is evident that poly(ethylenimine) polymers can accelerate hydrolysis in an activated amide such as **1**. The effects observed are largely due to increased local concentrations of hydroxide ion and of substrate. The intrinsic reactivity of the $\mathrm{OH^-}$ ion, however, does not appear to be changed, evidently because it is situated at the periphery of the hydrophobic domain rather than within the apolar cluster. It is possible, therefore, that if nonprotic, polar substituents could be built into the apolar cluster, a desolvated $\mathrm{OH^-}$ ion could more readily approximate the substrate to form the tetrahedral intermediate, T. This interpretation suggests alternative modifications of poly(ethylenimines) that might generate more effective catalysts of amide hydrolysis.

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[23] Even when acetate ion (0.02 M) was used as the anion of the supporting electrolyte, a small amount of chloride ion (up to 5 mM) was present in the reaction mixture because of its inherent presence in the polymer preparations. However, the pH profile of k_{cat} illustrated (curve B of Figure 2 or curve B of Figure 3) was obtained in solutions with a fixed amount of chloride ion.

[24] Anion effects have been also studied in the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate catalyzed by $\mathrm{Lau_{25}Me_QPEI}$ or $\mathrm{Lau_{25}Et_QPEI}$ (Suh, J.; Klotz, I. M. *Bioorg. Chem.* **1979**, *8*, 283). In this reaction, k_{cat} was not affected, but binding of the substrate was facilitated by the anion of the supporting electrolyte, in the order azide < chloride < acetate.

α-Nitrodiarylmethyl Cations[1]

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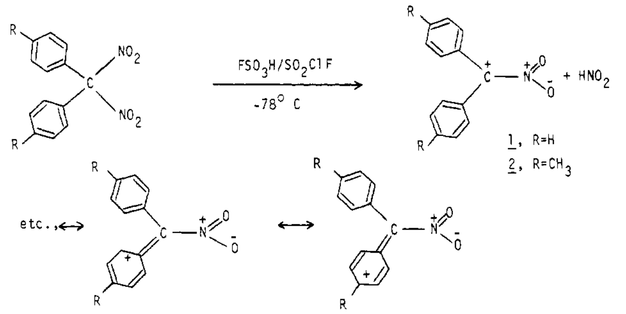
Abstract: The α-nitrodiphenylmethyl and the α-nitrodi-*p*-tolylmethyl cations **1** and **2**, the first α-nitro-substituted carbocations under long-lived conditions, have been prepared by the ionization of the corresponding α,α-dinitrodiarylmethanes in $\mathrm{FSO_3H/SO_2ClF}$ at −78 °C. ^{1}H, ^{13}C, and ^{15}N spectroscopic data are in accord with the assigned structures. The ^{13}C NMR chemical shift data of **1** are in good agreement with those observed for α-(trifluoromethyl)diphenylmethyl cation **3** and α-benzoyldiphenylmethyl cation **5**, indicating the similar electron-withdrawing nature of nitro, trifluoromethyl, and acyl groups adjacent to a carbocationic center. Ions **1** and **2** give their respective protonated ketones upon raising the temperature. Attempts to prepare the 9-nitro-9-fluorenyl cation from 9,9-dinitrofluorene gave instead directly the protonated fluorenone.

Scheme I



Recently considerable effort has been expended[2-7] to understand the effects of strongly electron-withdrawing substituents on solvolytic reactions leading to electron-deficient intermediates. Substituents that have been experimentally[2-7] and theoretically[8] pursued include $\mathrm{CF_3}$, CN, $-\mathrm{C({=}O)}-$, $\mathrm{NO_2}$ and others. However, thus far the effect of a nitro group α to a carbocationic center under long-lived stable ion conditions has not been explored. More recently under stable ion conditions we have shown[9] that α-cya-

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[1] Considered Stable Carbocation. 250. Arvanaghi, M.; Prakash, G. K. S.; Krishnamurthy, U. V.; Narang, S. C.; Olah, G. A. "Abstracts of Papers", 186th National Meeting of the American Chemical Society, Washington, DC, Aug 1983; American Chemical Society: Washington, DC, 1983; ORGN 97. Abstract No. 97. For part 249 see: Olah, G. A.; Prakash, G. K. S.; Saunders, M. *Acc. Chem. Res.* **1983**, *16*, 440.

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