Catalytic Hydrolysis of Dinitrophenyl Sulfate by Poly(ethylenimine) Derivatives†

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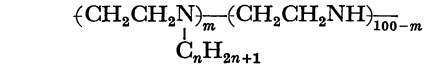
The catalytic hydrolysis of nitrophenyl sulfates by poly(ethylenimine)(PEI) derivatives was investigated at 30 °C in aqueous buffers. PEI derivatives which were made partly hydrophobic by incorporation of higher alkyl groups include alkylated PEI's, quaternized PEI's, and PEI's with imidazole and hydroxamate functions. Nitrophenyl sulfates could not be cleaved by these polymers. The catalytic hydrolysis of 2-hydroxy-5-nitrophenyl sulfate by an PEI with imidazolylmethyl and dodecyl substituents failed to proceed in spite of the contrary report by Kiefer *et al.* 2,4-Dinitrophenyl sulfate was cleaved fairly readily by PEI derivatives. Simple sulfate transfer was observed in the case of alkylated PEI, but the catalytic hydrolysis proceeded according to the Michaelis-Menten kinetics in the case of quaternized PEI. Partly quaternized PEI's showed highest catalytic efficiencies reported to date for the hydrolysis of dinitrophenyl sulfate.

Poly(ethylenimine) and its derivatives have been used widely as catalysts for hydrolysis[1-5] and decarboxylation.[6,7] Poly(ethylenimine)s with hydrophobic alkyl substituents are particularly interesting because their compact conformations in water provide excellent model enzyme systems.[8] Most of the hydrolytic study have been carried out by using phenyl ester substrates, and some functionalized poly(ethylenimine)s were shown to possess extremely high catalytic activities.

The hydrolysis of sulfate monoesters is usually not markedly accelerated by polymer catalysts. A unique exception is the hydrolysis of 2-hydroxy-5-nitrophenyl sulfate catalyzed by an imidazole-containing poly(ethylenimine). This catalytic hydrolysis was reported to be faster by a factor of more than 10^{12} fold than the imidazole-catalyzed hydrolysis and the catalytic efficiency exceeds that of arylsulfatase A (2-hydroxy-5-nitrophenyl sulfate is the specific substrate for this enzyme).[9]

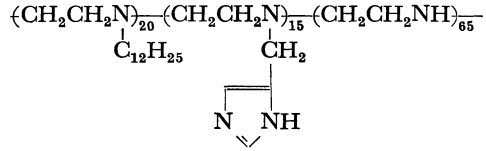
We have found that some cationic micelles which contain the hydroxamate nucleophile cleave a dinitrophenyl sulfate quite efficiently.[10] Therefore, the functionalized poly(ethylenimine) is also expected to be a good catalyst for the hydrolysis of sulfate esters. In this study, we report the catalytic hydrolysis of nitrophenyl sulfates by various poly(ethylenimine) derivatives. The hydrolysis of a phenyl ester by these polymer catalysts was also examined for comparison. The structures of the polymer catalysts and substrates and their abbreviations are given below:

polymer:

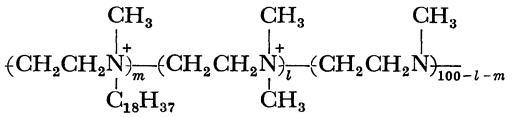


PEI-C_{12} (14): $n=12$, $m=14$

PEI-C_{16} (8) : $n=16$, $m=8$



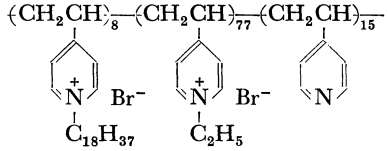
PEI-C_{12} (20)-Im (15)



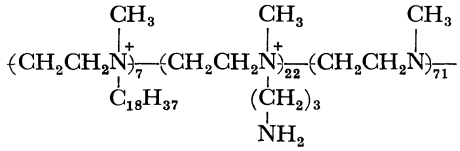
PEI$^+$-C_{18}(9) : $m=9$, $l=0$

PEI$^+$-C_{18}(16): $m=16$, $l=0$

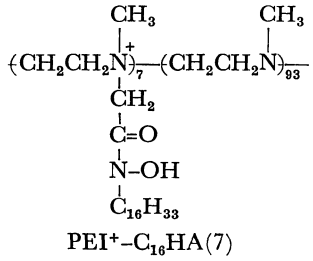
PEI$^+$-C_{18}(12)-C_1(68): $m=12$, $l=68$



PVP$^+$-C_{18}(8)-C_2(77)

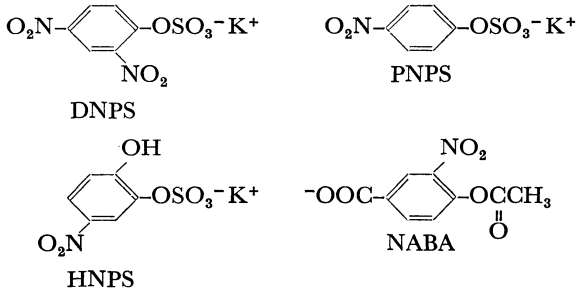


PEI$^+$-C_{18}(7)-NH_2(22)



PEI$^+$-C_{16}HA(7)

substrate:



DNPS PNPS

HNPS NABA

Experimental

Preparation of Substrates. Potassium 2,4-dinitrophenyl sulfate (DNPS) was prepared according to the procedure of

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Sunamoto *et al.*[10,11] Potassium *p*-nitrophenyl sulfate (PNPS) and potassium 2-hydroxy-5-nitrophenyl sulfate (HNPS) were prepared by following the procedures reported by Fendler and Fendler,[12] and Robinson, *et al.*,[13] respectively. The elemental analyses agreed with the calculated values. The preparation of 3-nitro-4-acetoxybenzoic acid (NABA) was reported previously.[14]

Partially Alkylated Poly(ethylenimine). An aqueous solution of commercial poly(ethylenimine) (Tokyo Kasei Co., *MW* 40000—50000, highly branched) was concentrated *in vacuo*, ethanol added to the residue and the solution was again concentrated. This procedure was repeated in order to completely replace water with ethanol. Finally, dry ethanol was added to obtain a 2—5 wt % solution, which was stored over molecular sieve 4A for the later use. The ethanolic solution was concentrated and 10—15 mol % of alkyl iodides (n-$C_{12}H_{25}I$ or n-$C_{16}H_{33}I$) were added. The mixture was heated at 60—70 °C for 24 h under nitrogen, concentrated and poured into excess ether. The yellowish powder was recovered, and washed several times with ether. The polymer was dissolved in water and purified by dialysis. The extent of alkylation was determined by the relative peak area of the main chain methylene proton (at *ca.* 2.7 ppm) and the alkyl proton (at *ca.* 1.3 ppm) in the NMR spectrum (D_2O solution).

Quaternized Poly(ethylenimine).[4] All the primary and secondary amino groups in poly(ethylenimine) were converted to the tertiary amino group by treating with formic acid and formalin. The product polymer was parly quaternized by octadecyl bromide in ethanol. The extent of quaternization was determined by NMR spectroscopy as mentioned above. Further quaternization was performed with methyl bromide in order to obtain PEI^+–C_{18}(16)–C_1(68).

Introduction of Functional Groups. The incorporation of the imidazole group into alkylated poly(ethylenimine) was carried out according to the procedure of Kiefer, *et al.*:[9] PEI–C_{12}(20)–Im(15). The methylated poly(ethylenimine) was allowed to react with *N*-(3-bromopropyl)phthalimide and then with dodecyl bromide and treated with hydrazine hydrate to give PEI^+–C_{18}(7)–NH_2(22).[15] The content of the aminopropyl group was determined from the relative peak area of the aromatic proton prior to the hydrazine treatment and by reaction with 2,4,6-trinitrobenzenesulfonic acid after the hydrazine treatment.[2] The hydroxamate function was introduced by reaction of a polymer with benzyl *N*-hexadecylchloroacetohydroxamate (see below). The hydroxamate content in the polymer was determined from the peak area of the aromatic proton. The benzyl group was removed by treatment with 30% hydrogen bromide in acetic acid. The complete removal was confirmed by NMR spectroscopy. The polymer was purified by reprecipitation after each stage.

Other Materials. The preparation of quaternized poly(vinylpyridine)s was described elsewhere.[16] Commercial hexadecyltrimethylammonium bromide (CTAB) was recrystallized two times from ethanol. For the preparation of benzyl *N*-hexadecylchloroacetohydroxamate, 25.2 g (0.11 mol) of benzyl benzohydroxamate in dry acetone was treated with 12.4 g (0.22 mol) of powdered KOH and 19.5 g (0.055 mol) of hexadecyl iodide. The oily alkylation product was hydrolyzed in a mixture of ethanol and concentrated hydrochloric acid to give *O*-benzyl-*N*-hexadecylhydroxylamine hydrochloride; colorless flakes after two recrystallizations from acetone, yield 16.0 g (75%), mp 77—79 °C. This compound (7.0 g, 0.018 mol) and 4.0 g (0.040 mol) of triethylamine were dissolved in 100 ml of dry ether and 2.1 g (0.018 mol) of chloroacetyl chloride was added dropwise. After the usual work-up, the product was recrystallized from acetone: mp< 30 °C, yield 4.3 g (56%). NMR and IR spectra were consistent with the expected structure.

Polymer Recovery. In connection with the elucidation of the hydrolysis mechanism, the polymer catalyst was recovered after reaction with the substrate under the standard conditions. A polymer catalyst (100 mg, 8—16 unit mM) was mixed with 100—500 mg (3.3—16 mM) of DNPS in 100 ml of 0.01 M borate buffer (pH 9.0) and allowed to stand at 30 °C overnight (1 M=1 mol dm^{-3}). The mixture was dialyzed for 24 h using a cellophane tube and then concentrated by ultrafiltration (DIAFLO Ultramembrane UM-2, Amicon Co., Exclusion limit 1000). A saturated aqueous solution of KBr was added and the resulting solution was concentrated by ultrafiltration. The ultrafiltration was repeated after addition of water. The concentrate was dried *in vacuo* and its sulfur content was determined.

Kinetic Measurements. The sulfate esters were dissolved in a 3: 7 mixture of water and acetonitrile and kept in an ice bath. The substrate solution and an aqueous solution of the polymers were added to buffer solutions (borate or Tris) in a quartz cell which had been maintained at 30 °C. Water was added so that the fraction of acetonitrile became 3 v/v %. The ionic strength was adjusted to 0.01 by KCl. The reaction was followed by using the absorbance increase at 360 nm of 2,4-dinitrophenolate anion (ε=12800 M^{-1} cm^{-1}) in the case of DNPS substrate. The λ_{max} at 401 nm was used for PNPS. Hitachi UV-visible spectrophotometers (type 124 or 200) equipped with jacketed cell compartments were used. The pH measurement was done by using TOA Electronics MH-10 glass electrodes and the pH change during the hydrolysis was within 0.03. Excess polymers were used relative to substrates and the reaction obeyed the pseudo first-order rate law for more than 80% completion. The overall rate constant of phenol release was corrected for that of the spontaneous release.

Results

Reaction of Sulfate Esters with PEI Derivatives. Nitrophenyl sulfates, PNPS and HNPS, did not react with poly(ethylenimine) derivatives under the reaction conditions employed. For example, a reaction mixture which contain 1.77×10^{-3} M of PEI–C_{12}(20)–Im(15) and 1.0×10^{-4} M of HNPS was allowed to stand for 20 h at 30 °C (pH 8.8, 0.01 M borate buffer and μ= 0.01). No change was detected in the visible spectrum during this period. Nitrocatechol was not detected in the product by thin layer chromatography using the solvent system similar to that of Ref. 9. Therefore, the pseudo first-order rate constant, $k_{1,obsd}$ should be much smaller than 1×10^{-5} s^{-1} or $k_{2,obsd}$ (apparent second-order rate constant) $\ll5\times10^{-3}$ M^{-1} s^{-1}. This is contrary to the efficient hydrolysis reported by Kiefer *et al.*[9]

On the other hand, DNPS was smoothly cleaved by these polymers. Table 1 summarizes $k_{1,obsd}$ of phenol release from DNPS by various PEI derivatives. In the last column are given $k_{2,obsd}$ values which were derived simply by dividing $k_{1,obsd}$ by the polymer concentration. It is clear from Table 1 that the catalytic groups such as imidazole, hydroxamate or primary amine are not appreciably effective for the sulfate cleavage. However, all of the PEI derivatives are more effective than the conventional cationic micelle (CTAB) or polysoap(PVP^+–C_{18}(8)–C_2(77)). In the subsequent

TABLE 1. CATALYTIC HYDROLYSIS OF DNPS[a)]

Polymer	10^4 [polymer] / M	pH	$10^4 k_{1,obsd}$ / s^{-1}	$k_{1,obsd}$/[polymer] / $M^{-1} s^{-1}$
PEI–C_{12}(14)	20.5	8.86	63.9	3.12
PEI–C_{16}(8)	10.8	9.02	32.0	3.55
PEI–C_{12}(20)–Im(14)	5.19	8.94	31.9	6.14
PEI$^+$–C_{18}(9)	5.06	8.61	80.6	15.9
PEI$^+$–C_{18}(16)	5.14	9.52	130	25.3
PEI$^+$–C_{18}(12)–C_1(68)	1.01	8.88	8.23	8.15
PEI$^+$–C_{18}(7)–NH_2(22)	1.06	8.82	6.79	6.43
PEI$^+$–C_{16}HA(7)	9.71	8.43	31.7	3.26
PVP$^+$–C_{18}(8)–C_2(77)	9.60	9.44	6.4	0.67
CTAB	10.0	8.81	1.90	—

a) [DNPS]$=2.0\times10^{-5}$ M, 3 v/v % EtOH–H_2O, $\mu=$ 0.01 (KCl), 30 °C, 0.01 M borate buffer.

experiments, two types of PEI derivatives, alkylated PEI(PEI–C_{16}(8)) and quaternized PEI(PEI$^+$–C_{18}(16)) were used for more detailed study.

Product Analysis. Excess amounts of DNPS substrate (3—10 fold excesses) were allowed to react with the above-mentioned two PEI derivatives. The time course of the formation of 2,4-dinitrophenol after correction for the spontaneous release is given in Fig. 1. In the case of alkylated PEI's (Fig. 1a and 1b), the reaction slows down at the release corresponding to *ca.* 0.3 equivalent (in monomer unit) of the polymer. In contrast, the reaction proceeds beyond the concentration of the quaternized polymers (Fig. 1c, 1d, and 1e). These results suggest the presence of different mechanisms of the phenol release for the two types of the polymers.

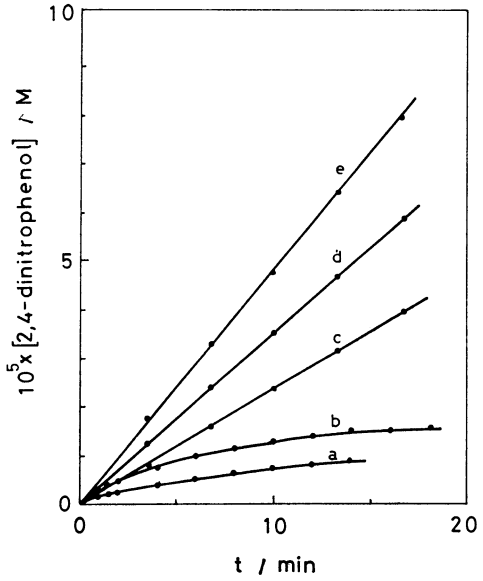


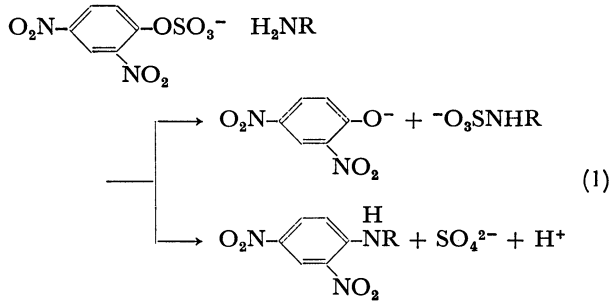
Fig. 1. Time course of dinitrophenol release.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01(KCl), 0.02 M borate, pH 8.4, [DNPS]$=2.4\times10^{-4}$ M. Polymer concentrations: a, [PEI–C_{16}(8)]$=3.95\times10^{-5}$ M; b, [PEI–C_{16}(8)]$=5.92\times10^{-5}$ M; c, [PEI$^+$–C_{18}(16)]$=2.05\times10^{-5}$ M; d, [PEI$^+$–C_{18}(16)]$=4.08\times10^{-5}$ M; e, [PEI$^+$–C_{18}(16)]$=6.16\times10^{-5}$ M.

TABLE 2. SULFUR CONTENT OF PEI CATALYSTS AFTER REACTION WITH DNPS SUBSTRATE

Polymer	Treated with DNPS	Sulfur content /%
PEI–C_{16}(8)	No	—
PEI–C_{16}(8)	Yes	7.06
PEI$^+$–C_{18}(16)	No	0.00
PEI$^+$–C_{18}(16)	Yes	0.35

Table 2 summarizes the sulfur content of the polymers before and after the reaction (see Experimental). Apparently, the sulfate group is fixed to the partially alkylated polymer, PEI–C_{16}(8) but not to the quaternized polymer, PEI$^+$–C_{18}(16). The small sulfur content of the latter polymer is close to the experimental error. The sulfur content of 7% corresponds to the presence of 16 mol % (per monomer unit) of the amidosulfate group according to scheme 1. This value is smaller than the amidosulfate content (30%) estimated from the dinitrophenol release (Eq. 1). Apparently, some of the amidosulfate group was hydrolyzed during the work up of the polymer, as reported by Benkovic and Benkovic in a related system.[17)]

The amino group in the partially alkylated PEI may react with phenyl sulfates *via* sulfate transfer (formation of amidosulfate) or nucleophilic aromatic substitution as shown in Eq. 1. This problem was discussed most recently by Fendler, *et al.*[18)] for the reaction of primary and secondary amines in the micellar system.



(1)

The occurrence of the nucleophilic aromatic substitution would decrease the sulfur content of the polymer.

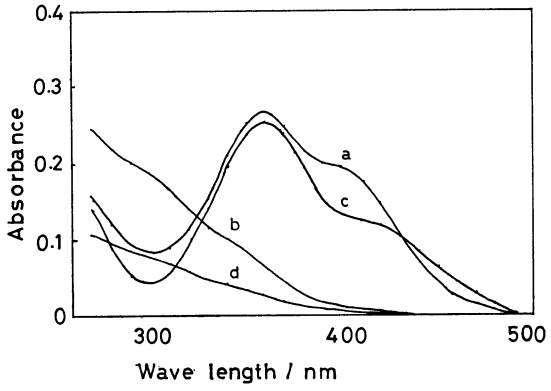


Fig. 2. UV-visible spectra.
a: 1 h after mixing of 1.9×10^{-5} M DNPS and 1.0×10^{-3} unit M PEI–C_{16}(8), pH 8.8, 30 °C. b: The same mixture adjusted to pH 1.6 by hydrochloric acid. c: 1.9×10^{-5} M *N*-methyl-2,4-dinitroaniline, pH 2.1. d: 1.9×10^{-5} M 2,4-dinitrophenol, pH 2.1.

Figure 2 shows a UV-visible spectrum of the reaction mixture after 1 h. (Fig. 2a). Also shown are spectra of the same solution after acidification to pH 1.6 by hydrochloric acid (Fig. 2b) and of *N*-methyl-2,4-dinitroaniline and 2,4-dinitrophenol at pH 2.1 (Fig. 2c and 2d, respectively). The spectrum of the acidified reaction mixture clearly indicates the absence of the dinitroaniline moiety. Therefore, it is concluded that the aromatic substitution path is negligible for alkylated PEI under the present reaction conditions.

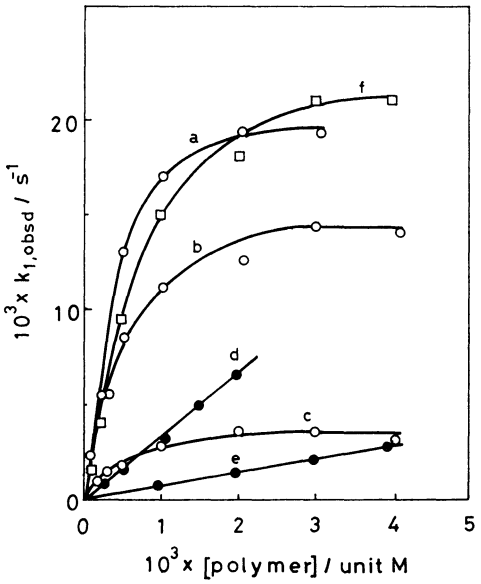


Fig. 3. Dependence of the rate of DNPS cleavage on the concentration of PEI derivatives.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01(KCl), 0.01—0.02 M borate, [DNPS]=2.0×10^{-5} M.
Polymer: PEI$^+$–C_{18}(16)(-○-) a, pH 9.5; b, pH 7.7; c, pH 6.2; PEI–C_{16}(8) (-●-) d, pH 9.0; e, pH 10.1; PEI$^+$–C_{18}(9) (-□-) f, pH 8.5.

Rate Dependence on Polymer Concentration. Figure 3 shows the dependence of $k_{1,obsd}$ of dinitrophenol release on the polymer concentration. In the case of alkylated PEI, $k_{1,obsd}$ increases linearly with the polymer concentration in the range of $(1—40)\times10^{-4}$ M. The results with the quaternized PEI are contrasting in that saturation phenomena are observed. The ease of saturation depends on the pH of the medium.

The saturation kinetics were analyzed according to the Michaelis-Menten kinetics

$$C + S \underset{}{\overset{K_b}{\rightleftharpoons}} CS \xrightarrow{k_{cat}} C + P_1 + P_2 \quad (2)$$

where C, S, P_1, and P_2 denote polymer, substrate, SO_4^{2-} and 2,4-dinitrophenol, respectively, and CS is the polymer-substrate complex.

Since excess polymer is present ($[C]_0 \gg [S]_0$), the following equations obtain.

$$v = k_{1,obsd}[S]_{total} = k_{cat}[CS] \quad (3)$$

and $[S]_{total} = [S] + [CS]$

$$K_b \text{ (binding constant)} = \frac{[CS]}{[C][S]} \quad (4)$$

$$k_{1,obsd} = \frac{k_{cat}\cdot K_b\cdot[C]_0}{K_b[C]_0+1} \quad (5)$$

Therefore

$$\frac{1}{k_{1,obsd}} = \frac{1}{k_{cat}} + \frac{1}{k_{cat}\cdot K_b\cdot[C]_0} \quad (6)$$

The data for the PEI$^+$ system in Fig. 3 were plotted using Eq. 6 and linear relations were obtained with satisfactory correlation coefficients. The K_b and k_{cat} thus derived are given in Table 3. As is clear from the data for PEI$^+$, the binding constant K_b decreases with increasing pH but the intra-complex rate constant k_{cat} increases with increasing pH.

Table 3 also contains $k_{2,obsd}$ for PEI–C_{16}(8) determined from the data of Fig. 3. $k_{2,obsd}$ decreases with increasing pH.

pH *Rate Profiles.* Figure 4 illustrates the pH dependence of $k_{1,obsd}$ in the presence of PEI derivatives and a quaternized poly(vinylpyridine). PEI–C_{16}(8) polymer gives a bell-shaped pH dependence with a maximum at *ca.* pH 8. The rate determination was difficult at pH below 7 because of precipitation. A similar pH-rate profile was obtained with an anionic ester substrate NABA (Fig. 5).

On the other hand, when quaternized PEI was used as catalyst, log $k_{1,obsd}$ initially increased linearly with increasing pH and became constant at pH above 8. The log $k_{1,obsd}$ value for NABA (Fig. 5) showed a linear correlation with pH in the pH range studied (pH 7—10). The rate of hydrolysis of NABA by PEI$^+$–C_{18}(16) is 10—100 times slower than that by PEI–C_{16}(8), but the relative effectiveness is reversed in the hydrolysis of DNPS. The reversal suggests that the rate enhancement mechanisms are different between the two systems.

Table 3. Kinetic parameters for the cleavage of DNPS substrate by PEI derivatives[a]

Polymer	pH	Correlation coefficient[b]	$10^3 k_{cat}$ / s^{-1}	K_b / M^{-1}	$k_{cat}\cdot K_b$ or $k_{2,obsd}$ / $M^{-1}\,s^{-1}$
PEI$^+$–C_{18}(16)	9.5	0.991	31.9	761	24.2
	7.7	0.991	22.0	1030	22.7
	6.2	0.997	3.48	2460	8.56
PEI$^+$–C_{18}(9)	8.5	0.997	31.3	745	23.1
PEI–C_{16}(8)	9.0	0.997	—		3.39
	10.1	0.999	—		0.70

a) 30 °C, 0.01—0.02 M borate buffer, μ=0.01(KCl), [DNPS]=2.0×10^{-3} M. b) Correlation coefficients for the plot of Eq. 6 in the case of the Michaelis-Menten kinetics and for the linear relation of Fig. 3, in the case of PEI–C_{16}(8).

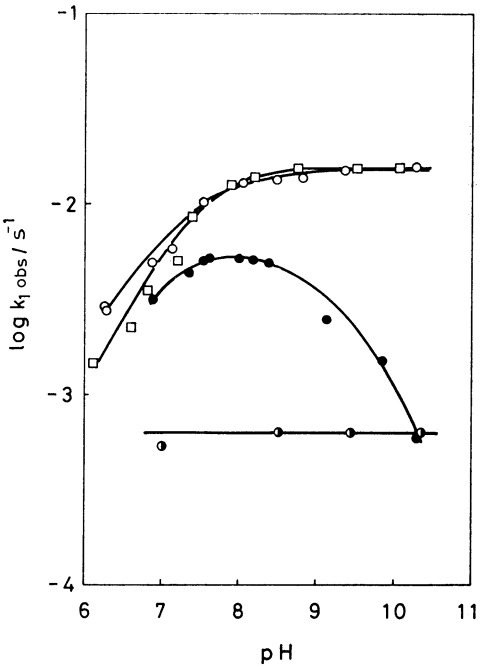


Fig. 4. pH-rate profile for the cleavage of DNPS.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01(KCl), 0.01—0.02 M borate or 0.02 M Tris, [DNPS]=2.2×10^{-5} M, [polymer]=1.0×10^{-3} unit M.
-○-, PEI$^+$–C_{18}(16); -□-, PEI$^+$–C_{18}(9); -●-, PEI–C_{16}(8); -◑-, PVP$^+$–C_{18}(8)–C_2(77).

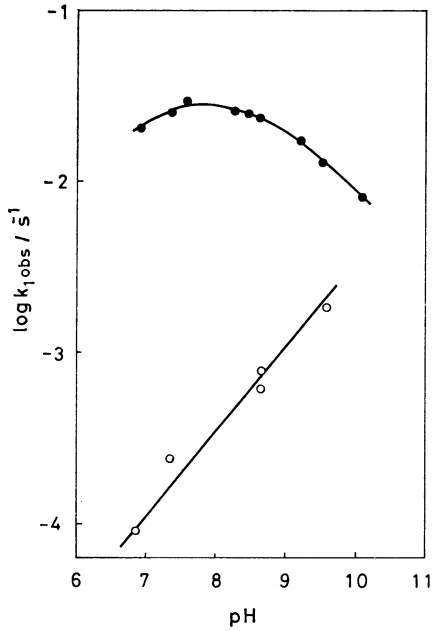
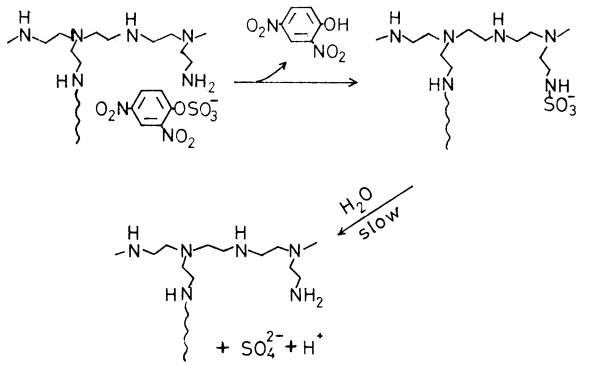


Fig. 5. pH rate profile for the hydrolysis of NABA.
30 °C, 3 v/v % EtOH–H_2O, μ=0.01(KCl), 0.01—0.02 M borate or 0.02 M Tris, [NABA]=2.9×10^{-5} M, [polymer]=1.0×10^{-3} M.
-○-, PEI$^+$–C_{18}(16); -●-, PEI–C_{16}(8).

Discussion

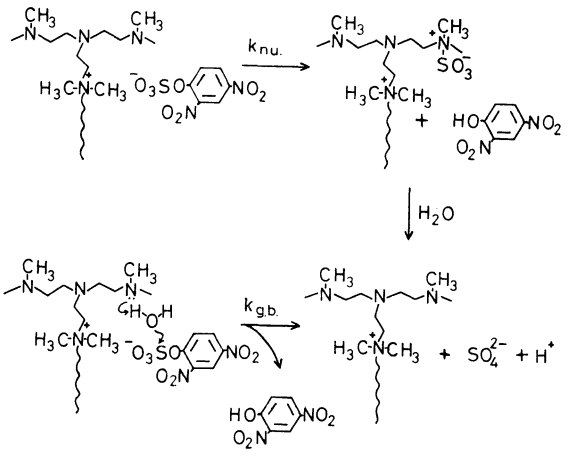
Reaction Scheme. The reaction scheme of alkylated PEI is obviously different from that of quaternized PEI. The most probable mechanisms for these PEI derivatives are shown in Scheme 1. In the case of alkylated PEI, the sulfate group is transfered from DNPS substrate to the nucleophilic amino group. The nucleophilic aromatic substitution was not detected (see above). The amidosulfate group is accumulated with the progress of the reaction, and, as shown in Fig. 1, the remaining amino group becomes less reactive. The zwitterionic adduct, if formed by the nucleophilic attack of tertiary amines toward nitrophenyl sulfates, should be hydrolytically unstable.[19] The accumulation of the amidosulfate group thus indicates the preferential sulfate transfer to the primary and/or secondary amines. This is contrary to the fact that tertiary amines are in general more nucleophilic toward nitrophenyl sulfates than primary and secondary amines.[17,18] The lack of sulfate transfer to the tertiary amino group in PEI may be attributed to severe steric hindrance. The nucleophilic reactivity of sterically-hindered tertiary amines (small molecules) toward sulfate esters is in fact very small.[17] The general-base catalysis by the tertiary amino group, which is sterically less demanding, could not be detected because it is probably less efficient than simple sulfate transfer to the primary and secondary amino groups.



Scheme 1.

The initial rate of sulfate transfer was first order with respect to substrate and polymer. There was found no evidence for substrate binding. However, the bell-shaped pH rate profile of Fig. 4 indicates the cooperative action of substrate attraction by the positively-charged polymer and of the effective neutral nucleophile. Therefore, the imcomplete reaction of the polymer-bound amino group is probably caused by neutralization of the positive charge of alkylated PEI by the negative charge of the amidosulfate group formed. The enhanced catalytic action of partly protonated poly(vinylpyridine) and poly(vinylimidazole) has been known for a long time.[20,21]

On the other hand, the partly quaternized PEI does not contain the primary and secondary amino groups and acts as true catalyst. This is readily seen from the turnover of the polymer catalyst (Fig. 1) and the absence of the intermediate accumulation (Table 2). The catalysis follows the Michaelis-Menten kinetics. The substrate binding is ascribed to both of the hydrophobic and electrostatic attractions between the polymer and substrate. The contribution of the electrostatic interaction is apparent in the fact that K_b decreases with increasing pH.



Scheme 2.

The intra-complex catalysis will involve the general-base and/or nucleophilic action of the tertiary amino group (Scheme 2). The turnover could result from the nucleophilic attack of the tertiary amino group which produces the unstable zwitterionic adduct. However, the tertiary amino group in PEI's does not appear to be sufficiently nucleophilic because of steric hindrance, as discussed above. Then, the general-base action may become the preferred pathway. The nucleophilic and general-base mechanisms are usually discriminated by the kinetic solvent isotope effect. However, this technique cannot be applied to the present system, since the side chain aggregation (hence the characteristics of the catalytic domain) would be quite different in H_2O and in D_2O. This effect renders the interpretation of the isotope effect very difficult.

A third possibility is the hydroxide-catalyzed cleavage of the bound substrate. However, this mechanism is not likely, as extensively quaternized PEI, PEI^+–$C_{18}(12)$–$C_1(68)$ is much less effective. The activity of the CTAB micelle and the quaternized poly(vinylpyridine) is also much smaller (Table 1). If the hydroxide-catalyzed hydrolysis is the major catalytic pathway, these systems should be at least equally effective, because of their high densities of the positive charge.

These results are contrasting with those of the NABA hydrolysis. The linear relation of Fig. 5 suggests that the phenyl ester is cleaved by the hydroxide ion which is concentrated in the domain of PEI^+–$C_{18}(16)$. The nucleophilic cleavage of NABA is reflected in a greater efficiency of PEI–$C_{16}(8)$ relative to that of PEI^+–$C_{18}(16)$, as also shown in Fig. 4.

Efficiency of Sulfate Cleavage. Table 4 summarizes the kinetic parameters reported for the cleavage of DNPS in the past. Sulfate esters seem to be less reactive with simple anionic nucleophiles. For instance, either A_1 or general-acid mechanism was suggested to occur for the cyclodextrin-catalyzed hydrolysis.[22] The hydroxamate ion in the cationic micelle is not particularly effective for the sulfate cleavage in spite of its enormous reactivity toward phenyl esters. However, sulfate transfer to the zwitterionic hydroxamate proceeds smoothly in the hydrophobic microenvironment.[10] It appears that basic groups act on sulfate esters particularly effectively (either as nucleophiles or as general bases) when they are fixed at positions close to the quaternary ammonium group in the hydrophobic microenvironment.

A comparison of the rate constants between the piperidine–CTAB system and PEI^+–$C_{18}(16)$ is interesting. Both of the catalytic systems contain the amino group, the dense positive charge and the hydrophobic domain. However, the PEI^+ system is 10^2—10^3 times more efficient in the sulfate transfer than the CTAB system. The difference is even greater when the fact that clean turnover is observed for PEI^+–$C_{18}(16)$ is taken into account. The quaternized PEI is the most effective catalyst and the apparent catalytic rate constant is more than 10^7 times greater than that of the spontaneous hydrolysis.

Finally it is noted that the nitrophenyl sulfates HNPS and PNPS were not cleaved by PEI derivatives under the same condition as used for DNPS hydrolysis: 30 °C, pH 8—10. Therefore $k_{2,obsd}$ should be less than 10^{-3} $M^{-1}\,s^{-1}$. These results are totally different from that reported by Kiefer *et al.*[9] These authors reported the apparent catalytic rate constant of 18 $M^{-1}\,s^{-1}$ which is more than 10^{12} larger than that of imidazole. The discrepancy between the two sets of experiment amount to the order of at least 10^4 in the rate constant. The composition of the imidazole-containing PEI prepared

Table 4. Catalytic efficiencies in the hydrolysis of DNPS

Catalyst or nucleophile	$\frac{k_{cat}\cdot K_b \text{ or } k_2}{M^{-1}\,s^{-1}}$	Remarks	Reference
PEI^+–$C_{18}(16)$	24.2	Saturation kinetics, 30 °C K_b=700—2500 M^{-1}	this study
PEI–$C_{16}(8)$	3.39	30 °C	this study
Piperidine–CTAB	0.098	39.0 °C	23
C_{12}–Im^+–HA[a]–CTAB	11	30 °C	10
β-Cyclodextrin	0.073	Saturation kinetics, 37.3 °C K_b=41.7 M^{-1}	22
Oxime-I[b]	0.67	Saturation kinetics, 40 °C $K_b=6.6\times10^4$ M^{-1}	11
DAP[c]–benzene	0.019	24.5 °C	24
None	1.3×10^{-6}		

a) (1-Dodecylimidazolium)-*N*-benzylacetohydroxamate. b) 10-Hydroxy-11-hydroxyimino[20]paracyclophane. c) Dodecylammonium propionate.

by us, PEI–C_{12}(20)–Im(15) is the same as that of Kiefer *et al.*, except that our polymer contain 20% instead of 10% dodecyl group. In fact, the imidazole-containing PEI showed a rather normal behavior in the cleavage of DNPS (Table 1).

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