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Stereoselectivity in Deacylation of Nitrophenyl Acylates by Poly(ethylenimine) Derivatives

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Deacylation of nitrophenyl acetates containing carboxyl substituents [4-acetoxy-3-nitrobenzoic acid (**1**), 3-acetoxy-4-nitrobenzoic acid (**2**), and 2-acetoxy-5-nitrobenzoic acid (**3**)] was studied in the presence of poly(ethylenimine) derivatives. The polymers examined contained lauryl groups ($Lau_{12}PEI$) or both lauryl and imidazolyl groups ($Lau_{12}Im_{10}PEI$). The reaction with active ester proceeds through the attack of primary amino groups of the polymers at the acyl carbons of the substrates. The reaction of $Lau_{12}Im_{10}PEI$ with a hydrophobic ester, *p*-nitrophenyl caproate (NPC), however, has been reported to involve the attack by the imidazolyl group of the polymer. Thus, the anionic (carboxyl-containing) and the hydrophobic esters bind to different domains on $Lau_{12}Im_{10}PEI$. Among the anionic substrates, **3** has uniquely large k_{cat} values compared with **1** or **2**. This is explained in terms of closer proximity between a nucleophile amino group of the polymer and the scissile bond of the substrate in the polymer–substrate complex.

INTRODUCTION

Enzymatic reactions proceed through the formation of catalyst–substrate complexes. Complex formation is also involved in micelle- or polymer-catalyzed reactions (*1–3*). By utilizing their structural cavities cyclodextrin derivatives (*4, 5*) or crown ether compounds (*6*) also form complexes with substrates. In such complexes, proximity between the catalytic group of the catalyst and the reaction site on the substrate is very important to achieve effective catalyses. A good example thereof is the stereoselectivity observed when the structures of substrates and cyclodextrin catalysts are varied (*4, 5*).

Derivatives of poly(ethylenimine) have been studied as catalytic macromolecules exhibiting some of the characteristics of enzymes (synzymes). Various types of organic reactions are catalyzed by these derivatives and several catalytic factors have been elucidated (*7–12*). In the present study, the reactivity of poly(ethylenimine) derivatives with a series of active esters varying in structures has been examined. In particular, kinetic data have been obtained for the deacylation of nitrophenyl acetates containing carboxyl substituents, 4-acetoxy-3-nitrobenzoic acid (**1**), 3-acetoxy-4-nitrobenzoic acid (**2**), and 2-acetoxy-5-nitrobenzoic acid (**3**), by modified poly(ethylenimine)s containing lauryl and imidazolyl groups. Stereoselectivity by the polymer derivatives toward compounds **1–3** and a hydrophobic substrate, *p*-nitrophenyl caproate (NPC), has been found.

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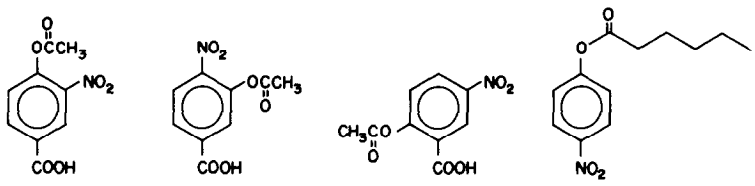


1 2 3 NPC

EXPERIMENTAL PROCEDURES

Materials. Esters **1–3** were prepared according to the literature (*13–15*): **1**, mp 151–152°C [lit. (*13*) 152°C]; **2**, mp 188–190°C [lit. (*14*) 187–188°C]; **3**, 154–156°C [lit. (*15*) 153.5–154.5°C]. NPC was purchased commercially.

Modification of poly(ethylenimine) was carried out according to literature procedures (*7–10*). On an average, the sample of poly(ethylenimine) used contains 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, first against 0.1 M NaCl and then against water.

The poly(ethylenimine) derivatives prepared had the following stoichiometric compositions. $Lau_{12}PEI$: $(C_{12}H_{25})_{0.12m}(C_2H_5N)_{1.0m}(HCl)_{0.54m}$, m = 1400. $Lau_{12}Im_{10}$ PEI: $(C_{12}H_{25})_{0.12m}$(4-imidazolylmethyl)$_{0.10m}(C_2H_5N)_{1.0m}(HCl)_{0.50m}$, m = 1400.

Deionized water, ethylenediamine, and *N*,*N*-diethylethylenediamine were redistilled before being used in kinetic studies.

Kinetic measurements. The rates of the deacylation of compounds **1–3** were measured with a Cary 14 uv–vis spectrophotometer by following the release of the nitrophenol portions of the products. Absorbance readings were taken at 420 or 360 nm, depending on the substrate and pH. Because the reactions of cationic poly(ethylenimine) derivatives with anionic substrates are influenced by the nature and concentrations of anions present (*11*), a total concentration of 0.01 M Cl^- was maintained as follows. Solutions of the polymer derivatives containing 0.01 M Cl^- (inherently present in the polymer preparations) were made and their pH's were adjusted with sodium hydroxide. Solutions (0.01 M) of the HCl salt of Tris or 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol (Bistris) were prepared and the pH was adjusted with sodium hydroxide. A polymer solution was then diluted with a buffer solution of the same pH prior to the kinetic measurement. Reactions of **1–3** with ethylenediamine, *N*,*N*-diethylethylenediamine, Tris, or imidazole were carried out at an ionic strength of 1.0. A solution of the di-HCl salt of an ethylenediamine derivative made at ionic strength 1.0 was added to that of the respective mono-HCl salt make at ionic strength 1.0 until a desired pH was attained. In this way, the ionic strength was maintained at 1.0. pH values were obtained with an Orion Model 701 pH meter. Stock solutions of **1–3** were made in acetonitrile, and the final reaction mixtures for kinetic measurements contained 0.8% (v/v) acetonitrile. For the reactions in the presence of excessive

amounts of the polymers, the concentration of **1–3** initially added was 1×10^{-5} M. In the aminolysis of **1–3** (at 1×10^{-4} M), 0.1–1.0 M Tris, 0.03–0.1 M imidazole, 0.1–0.33 M ethylenediamine, or 0.1–0.33 M *N,N*-diethylethylenediamine were used. All of the rate data were collected at 25 ± 0.1°C.

RESULTS

Rate data for the deacylation of compounds **1–3** in the presence of $Lau_{12}PEI$ or $Lau_{12}Im_{10}PEI$ were analyzed in terms of

$$C + S \underset{K_m}{\rightleftharpoons} C \cdot S \xrightarrow{k_{cat}} \text{products.} \qquad [1]$$

Here, C stands for the reaction site on the polymer and S denotes substrate. Initially added concentration of polymer (C_0) is expressed in terms of monomer residue-molar (resM) concentration (*7*), that of substrate is denoted by S_0. K_m is the dissociation constant of the complex, C · S. When $C_0 \gg S_0$, first-order kinetics are observed with the pseudo-first-order rate constants (k_0) given by (*7*)

$$k_0 = k_{cat}C_0/(K_m + C_0). \qquad [2]$$

When $K_m \gg C_0$,

$$k_0 = k_{cat}C_0/K_m \qquad [3]$$

is applicable and then k_0 is proportional to C_0.

Typical kinetic data for the deacylation of compounds **1–3** in the presence of excess $Lau_{12}Im_{10}PEI$ at pH 7.5 are illustrated in Fig. 1. As shown in this figure, the

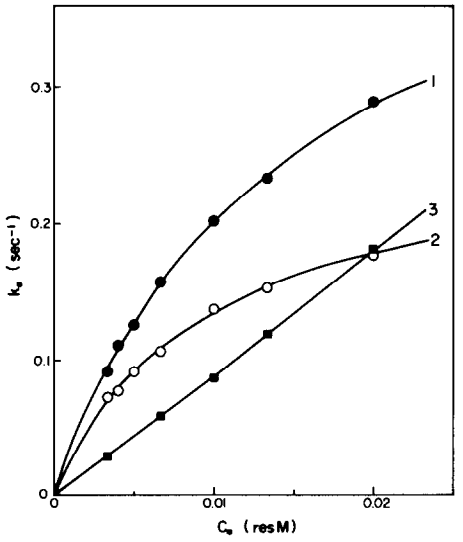


FIG. 1. Plot of k_0 against C_0 for the reactions of substrates **1–3** (1×10^{-5} M) with $Lau_{12}Im_{10}PEI$ at pH 7.5. ●, **1**; ○, **2**; ■, **3**.

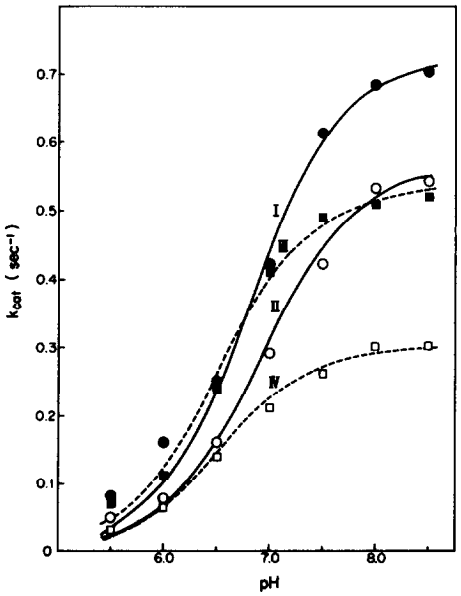


FIG. 2. The pH dependence of k_{cat} for the reaction of **1** (curve I, ●) and **2** (curve II, ○) with $Lau_{12}PEI$ and for that of **1** (curve III, ■) and **2** (curve IV, □) with $Lau_{12}Im_{10}PEI$.

course of k_0 with respect to C_0 reflects saturation behavior for the reaction of **1** and **2** with $Lau_{12}Im_{10}PEI$ or $Lau_{12}PEI$. Analysis of such saturation kinetic data in terms of a linear transform of Eq. [2] (plot of $1/k_0$ against $1/C_0$) produced values of k_{cat}, K_m, and k_{cat}/K_m. In contrast, for the reaction of **3** in the presence of excess of the polymers, k_0 was always proportional to C_0, and analysis of the rate data gave only k_{cat}/K_m. The pH dependences of k_{cat} for **1** and **2** are illustrated in Fig. 2, and those of k_{cat}/K_m in Figs. 3 and 4. The curves drawn in the pH profiles of k_{cat} were constructed according to

$$^{+}HC \cdot S \overset{K_a}{\rightleftharpoons} C \cdot S + H^{+} \xrightarrow{k_{cat}^{lim}} \text{products}, \qquad [4]$$

Totally different kinetic results were obtained when substrate was in excess,

TABLE 1

PARAMETER CALCULATED FROM THE pH PROFILES OF k_{cat}

Substrate	Polymer	pK_a	k_{cat}^{lim} (s^{-1})
1	$Lau_{12}PEI$	6.8	0.72
2	$Lau_{12}PEI$	6.9	0.55
1	$Lau_{12}Im_{10}PEI$	6.5	0.53
2	$Lau_{12}Im_{10}PEI$	6.5	0.30

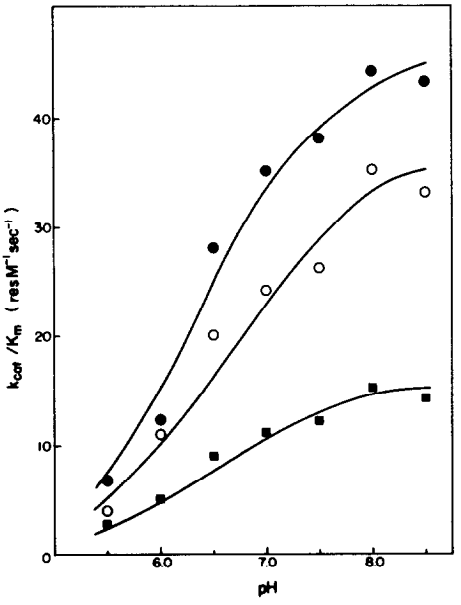


FIG. 3. The pH dependence of k_{cat}/K_m for the reaction of **1** (●), **2** (○), and **3** (■) with $Lau_{12}PEI$.

that is, $S_0 > C_0$. The release of 4-hydroxy-3-nitrobenzoic acid from the reaction of **1** (at $S_0 = 1.6 \times 10^{-4}$ M) in the presence of various amounts of the polymers (with $S_0 > C_0$) is illustrated in Fig. 5. In this figure the curves show a rapid initial burst followed by a very slow linear increase. The intercepts on the ordinate of the

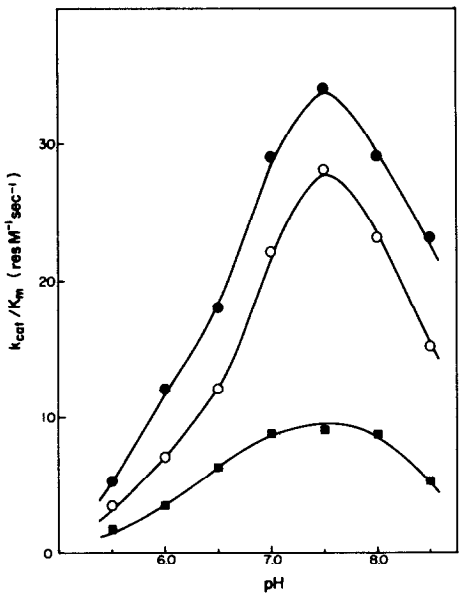


FIG. 4. The pH dependence of k_{cat}/K_m for the reaction of **1** (●), **2** (○), and **3** (■) with $Lau_{12}Im_{10}PEI$.

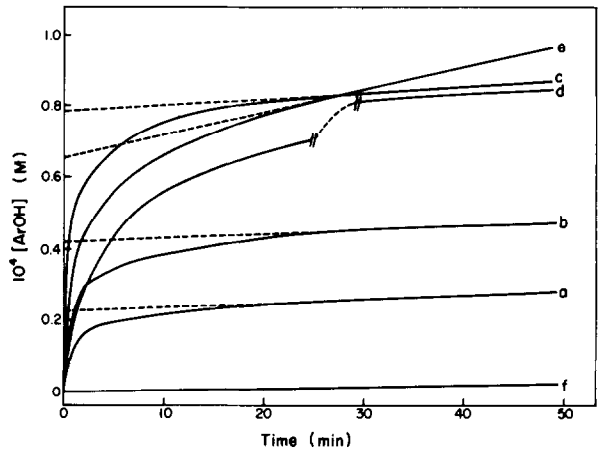


FIG. 5. Release of *4*-hydroxy-*3*-nitrobenzoate during the reaction of the polymers with an excess amount of **1** (1.6×10^{-4} M) at pH 7.5. (a) 1.6×10^{-4} resM $Lau_{12}PEI$; (b) 3.2×10^{-4} resM $Lau_{12}PEI$; (c) 6.4×10^{-4} resM $Lau_{12}PEI$; (d) 6.4×10^{-4} resM $Lau_{12}PEI$ and 0.8×10^{-4} added *4*-hydroxy-*3*-nitrobenzoate; (e) 6.4×10^{-4} resM $Lau_{12}Im_{10}PEI$; (f) no polymer added.

extrapolated linear portions (dotted lines) correspond to 10–15% of the monomer residue-molar concentrations of the polymers. The slope of the linear portion of curve e ($Lau_{12}Im_{10}PEI$) is larger than those of curves a–d ($Lau_{12}PEI$).

Curve d of Fig. 5 was obtained in the presence of added 4-hydroxy-3-nitrobenzoic acid. Compared with curve c, the initial burst of curve d is slower due to the inhibited binding of the phenol. However, the product inhibition cannot account for the extremely slow rate observed after the initial burst in curve c. Instead, this must be due to the inactivation of the primary reaction sites during the reaction.

For **2** and **3**, the results obtained with excess amounts of substrates were essentially identical with those of Fig. 5, except that the slopes of the linear portions for the reaction of **3** were not much different for the two polymers.[1]

Aminolysis of compounds **1–3** was also studied with ethylenediamine, *N,N*-diethylethylenediamine, Tris, and imidazole. At the pH's employed in the kinetic measurements, the reactive forms of the ethylenediamine derivatives were monocationic. The kinetic results are summarized in Table 2.

DISCUSSION

Reaction Sites

The course of release of the nitrophenol moiety from the compounds **1–3** in the presence of less than stoichiometric amounts of the polymers (Fig. 5) confirms

[1] This difference is attributable to the particularly large reactivity of the primary amino groups of the polymers toward **3** and to the low reactivity (Table 2) of imidazole toward **3** compared with **1** or **2**, as mentioned under Discussion.

TABLE 2

KINETIC RESULTS FOR AMINOLYSIS OF COMPOUNDS **1–3** BY VARIOUS AMINES

Amine	pK_a	pH[a]	Rate constant compared	Relative reactivity of the esters[b]		
				1	**2**	**3**
$Lau_{12}PEI$	6.8	7.5	k_{cat}	1.0 (0.61 s^{-1})[c]	0.69[c]	⩾0.79[c]
$Lau_{12}Im_{10}PEI$	6.5	7.5	k_{cat}	1.0 (0.49 s^{-1})	0.53	⩾0.73
Ethylenediamine	7.52[d], 9.98[d]	7.4	k_2	1.0 (0.36 M^{-1} s^{-1})	0.50	0.20
N,N-Diethyl ethylenediamine	7.07[d], 10.02[d]	6.8	k_2	1.0 (0.092 M^{-1} s^{-1})	0.45	0.23
Tris	8.10[d]	8.3	k_2	1.0 (0.0014 M^{-1} s^{-1})	0.74	0.13
Imidazole	7.05[d]	7.5	k_2	1.0 (1.1 M^{-1} s^{-1})	0.67	0.045

[a] The pH at which the rate constants were obtained.

[b] Numbers in parentheses are the rate constants measured for **1**.

[c] The effective molarities [A. J. Kirby (1980) *Adv. Phys. Org. Chem.* **17**, 183] of the nucleophile amine in C · S complexes are 0.88, 1.2, and ⩾3.5 M for **1–3**, respectively, when k_{cat} is compared with k_2 of ethylenediamine. When the comparison is made with *N,N*-diethylethylenediamine, the effective molarities are 2.8, 4.3, and ⩾9.5 M for **1–3**, respectively. The calculation is performed after the correction of k_{cat} and k_2 for the partial protonation (pK_a for $Lau_{12}PEI$ and pK_{a1} for the ethylenediamines) of the nucleophilic amines at the pH of the rate measurement.

[d] pK_a values are taken from Ref. (*21*).

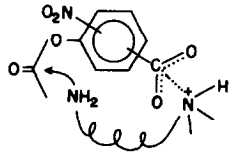
that the primary reaction sites on the polymers are inactivated during the reaction, as had been indicated previously (*16*). Among the nucleophilic groups on the polymers, tertiary amino groups and imidazolyl groups cannot be inactivated by acylation. The amount of the inactivated primary site is about 10–15% of the monomer residues. This is close to the content (13%) of the primary amines of the modified polymers. The contents of the secondary amines are 50% ($Lau_{12}PEI$) or 40% ($Lau_{12}Im_{10}PEI$), but the released nitrophenol does not approach these values. Besides, the secondary amino groups should be less reactive than the primary ones because of steric effects. The initial bursts in Fig. 5, therefore, represent the acetylation of the primary amines of the polymers. The subsequent slow, linear increases can be attributed to the much slower reactions of the secondary or tertiary amines (or to the imidazoles when they are present) and to the spontaneous hydrolysis. In curve e, where imidazole is linked to the polymer, the linear portion is steeper than those of the other curves as a result of the reaction of the imidazolyl residues on the polymer after acetylation of the primary amines is complete. It has been demonstrated previously (*17*) by spectrophotometric observation of the intermediate that catalytic turnover occurs with imidazole moieties on poly(ethylenimine). Furthermore, catalytic activity is manifested by imidazole-containing polymer samples in which all the available amines have been acylated with excess acetic anhydride (*18*).

Regardless of the contribution of the secondary sites, the esters should react exclusively with the primary amines when the polymer is present in great excess, $C_0 \gg S_0$. Under these conditions, saturation kinetic behavior is observed for hydrolysis of **1** and **2**. The values of K_m at pH 7.5 are 0.016 resM (for **1** and **2**) for

$Lau_{12}PEI$ and 0.014 (**1**) or 0.010 (**2**) resM for $Lau_{12}Im_{10}PEI$. On the other hand, for **3** k_0 is always proportional to C_0 up to the highest concentration of the polymers tried (0.02 resM), that is, there is no indication of saturation. Thus, K_m must be much greater than 0.02 resM for **3**.

The pH profiles of k_{cat} (Fig. 2) reflect pK_a values of 6.5–6.9 (Table 1) for the proton dissociation of the primary reaction sites in the C · S complexes. There are several factors in the polymer environment that could have effects on the intrinsic acidity of the primary NH_3^+ group. Since this substituent is part of an ethyleneimine residue, it must be close to a secondary amine, $-\overset{+}{N}H_2-CH_2-CH_2-NH_3^+$, which can be partially protonated. In the small molecule, ethylenediammine derivative, pK_{a1} values are 7.0–7.5 (Table 2), shifted downward from 10.6 for ethylamine by the strong electrostatic repulsion of the neighboring charged nitrogen. In the polymer, one might expect a further lowering of the pK_a because of the general cationic environment in poly(ethylenimine). On the other hand, the same cationic characteristics cause the polymer to expand and swell (*19*), and hence to increase the distance of the primary NH_3^+ from the framework and reduce electrostatic repulsion. Such an expansion would minimize the electrostatic effect on the primary amines. The presence of lauryl groups on poly(ethylenimine) leads to the formation of hydrophobic clusters (*19*), but amine groups tend to be outside of these domains, particularly when the polymer is swollen. Studies (*20*) of a highly charged poly(ethylenimine) with quaternized nitrogens, which maintain their cationic charge at all pH values, show a pK_a of 8.2 for the attached primary NH_3^+ groups. Thus it seems that the primary amines are affected mostly by the immediately adjacent aminoethyl linkage arm and only secondarily by global influences from the macromolecule. It seems reasonable, therefore, to ascribe the observed pH dependence of k_{cat} (Fig. 2) to the nucleophilic reactivity of primary amines on the polymer.

The cationic charges on secondary and tertiary nitrogens of poly(ethylenimine) probably participate in binding of the anionic substrate, and thereby they facilitate interaction of the primary amine nucleophile with the electrophilic site on the ester [see (**A**)].



A

The pH profiles (Fig. 2) for the two reactions with the imidazole-containing polymer, $Lau_{12}Im_{10}PEI$, have midpoints (pH 6.5) only slightly lower than those (pH 6.9) of the polymer, $Lau_{12}PEI$, without imidazole. If the imidazole moiety were playing a dominant role in the hydrolyses, one would expect a kinetic pK_a substantially below the observed values (Fig. 2) of 6.5–7. The small molecule, 4-aminomethylimidazole analog of that attached to the poly(ethylenimine) has a pK_a of 4.71 (*21*). Proton NMR titrations (*9*) of imidazole-containing poly(eth-

ylenimine)s reveal a pK_a of 4.5–5. Again the dominant effect on the pK_a of the adduct to the polymer comes from the immediate neighbor with the cationic charge, $-\overset{+}{N}H_2-CH_2-$imidazole.

The pH profiles for k_{cat}/K_m (Figs. 3 and 4) reflect more factors than those for k_{cat}. Both binding of the substrate (measured by $1/K_m$) and reaction within the C · S complex (measured by k_{cat}) contribute to the experimental parameter, k_{cat}/K_m. As the pH is raised, the cationic $\equiv NH^+$ groups dissociate, thereby decreasing the overall charge of the polymer and increasing the number of nucleophilic $\equiv N:$ groups. The decrease in overall charge weakens binding of the substrate (increases K_m), the increase in concentration of nucleophiles increases k_{cat}. These opposing effects on k_{cat}/K_m are most evident in the pH profiles for $Lau_{12}Im_{10}PEI$ (Fig. 4). For each polymer, the shape of the pH profile is the same for the different substrates **1–3**, as would be expected if the mechanism of hydrolysis is the same for each compound.

Stereoselectivity

Previous studies (*9, 12, 18*) of the reactions of LauImPEI polymers with NPC have demonstrated turnover kinetics when $S_0 \gg C_0$, as would be expected if the imidazole group is the nucleophile cleaving the active ester. One would presume the hydrophobic substrate, NPC, should be bound to the polymer at hydrophobic sites, whereas the anionic substrates (**1–3**) should interact electrostatically with cationic sites. This distinction is evident in the predominant attack by the imidazolyl groups when NPC is the substrate and by the primary amino groups when **1–3** are cleaved. In small molecules and reactivity of imidazole against **1** or **2** is intrinsically not less than that of ethylenediamine (Table 2). Thus, the imidazolyl residues of $Lau_{12}Im_{10}PEI$ must have little access to the carbonyl carbon of the bound anionic substrates. Clearly the imidazolyl residues are located in the hydrophobic region of the polymer, where they can readily attack a hydrophobic substrate.

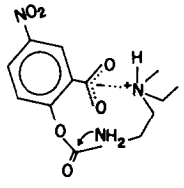
Among the anionic substrates (**1–3**), **3** has the largest values of K_m and k_{cat}. The large value of the dissociation constant, K_m, can be understood in terms of the features depicted in **A**. Ester **3** contains an acetoxy group located at a position *ortho* to the carboxylate group. Steric blocking by this bulky *ortho* substituent should reduce the electrostatic interaction in the C · S complex and hence lead to the large K_m value.

The k_{cat} values for the reaction with **1** or **2** were estimated from the saturation kinetic data. On the other hand, no deviation from the linear dependence of k_0 on C_0 was observed for **3** up to the highest C_0 employed (0.02 resM) and exact values of k_{cat} could not be obtained. However, an estimate could be made by the following analysis. For the reaction of **3** illustrated in Fig. 1 ($k_0 = 0.18\ s^{-1}$ at $C_0 = 0.02$ resM), k_{cat} would be $0.36\ s^{-1}$ if K_m were 0.02 resM. However, K_m must be much greater than 0.02 resM and, consequently, k_{cat} should be much greater than $0.36\ s^{-1}$.

The relative k_{cat} values for **1–3** estimated in these ways are summarized in Table 2. Toward ethylenediamine derivatives, **3** is four to five times less reactive than **1**.

However, toward the primary amines of C · S complexes the reactivity of **3** (k_{cat}) is much greater than that of **1**. Such differences are not seen between the rate data of **1** and **2**.

The scheme depicted in **A** seems to rationalize the particularly large k_{cat} values observed for **3**. Examination of space-filling models reveals that the interaction with a single monomer residue of the polymer as illustrated by **B** is possible only for **3**. For **1** or **2**, primary amino groups must be located much farther from the binding ammonium nitrogen to be able to attack at the scissile carbonyl group. When the length between the nucleophile nitrogen and the anchor nitrogen is increased, the nucleophilic attack should be much less efficient.



B

A major goal in the study of synthetic polymer catalysts has been to achieve properties characteristic of enzyme reactions: formation of strong substrate–macromolecule complexes, large rate enhancements, and stereospecificity in reactivity. Complex formation and large rate accelerations have been described previously with various poly(ethylenimine) derivatives (*3*). The differences observed here in the behavior of the polymer toward isomeric compounds **1–3** and toward NPC reveal additional features in which the synthetic macromolecule behaves catalytically like an enzyme.

ACKNOWLEDGMENTS

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