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A STUDY OF THE MECHANISM OF HYDROLYSIS OF NITROPHENYL ESTERS CATALYZED BY POLYETHYLENEIMINE CONTAINING BENZYL GROUPS

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Abstract—The mechanism of hydrolysis of *n*-nitrophenyl acetate (NPA), butyrate (NPB), caprylate (NPC), and *o*-methoxycinnamate (NPOMC) catalysed by benzyl-containing polyethyleneimines of linear and branched structures was investigated in aqueous media. The reaction seems to proceed via a general basic mechanism of catalysis and does not involve acylation of the catalyst. Benzyldiethylamine is an analogue of the active centres in polymers with $pK_a = 8{\cdot}35 \pm 0{\cdot}1$, localized in the polymer globules at sites of higher hydrophobity.

The reaction has a three-step mechanism involving binding of the substrate to an active centre (to give Michaelis sorption complex), substrate conversion and desorption of products. For each step, rate constants were determined. The effect of polymer $(K_2/K_m)/K_{II}$ increases from NPA to NPC; in the latter case, it is of order 10^5.

Reactions involving low mol. wt catalysts differ from those in which homogeneous polymeric catalysts participate, in that the molecules of the substrate bind to the macromolecules before their catalytic transformation occurs. This is evident from the non-linear dependences of reaction rates on the substrate concentration [1–3]. In this respect, polymeric catalysts have much in common with enzymes for which substrate binding is the first step in a sequence of catalytic acts. Investigation of soluble polymeric catalysts may help elucidate the role of this step in the whole process, the possible ways of interfering with various steps and compare with similar stages in enzymic reactions. Sorption of substrate analogue on polyethyleneimine (PEI), catalysts for hydrolysis of nitrophenyl esters [2, 3], has been studied [4]. This work has also dealt with the effect upon the sorption and conformational changes in PEI macromolecules due to introduction of benzyl groups (thought to be the sorption centres). It has been shown that sorption is stronger the more hydrophobic is the molecule of the sorbed substance and the higher is the concentration of the benzyl groups in the polymer.

We also aimed at establishing the nature of catalytic centres and the relationship between the sorption properties and the catalyst activity. Moreover, to understand the specific role of a macromolecule, catalytic behaviour of polymeric systems was compared with that of low molecular weight micelles.

EXPERIMENTAL

The starting linear [PEI(L)] and branched [PEI(B)] samples of PEI and the synthesis of the alkylated compounds have been described [4]. Benzyldiethylamine (BDEA), a low mol. wt analogue of the catalytic centre in benzylated PEI (BPEI), was prepared by alkylation of diethylamine with benzyl chloride at 65°. The reaction product was purified by vacuum distillation, the fraction (86°, 10 mm Hg) was collected. *p*-Nitrophenyl-*o*-methoxy cinnamate (NPOMC) employed for identification of the catalytic hydrolysis products was obtained from *o*-methoxycinnamoyl chloride; the latter was prepared by the three-step synthesis from coumarylic acid via its reaction with dimethylsulphate and the treatment of the resulting methyl ester with alkali and thionyl chloride. The purities of the products were estimated by the concn of the products of alkaline hydrolysis. *p*-Nitrophenylcaprilate (NPC) and *p*-nitrophenylbutyrate (NPB), analytical grade reagents, used in the catalytic hydrolysis, were further purified by distillation and passed through an alumina column. Nitrophenylacetate (NPA) was obtained from acetic anhydride and *p*-nitrophenol [2], m.p. = 78°.

Hydrolysis of the above-mentioned *p*-nitrophenyl esters (NPE) was carried out in 0·01 M aqueous solution of Tris–HCl buffer, containing 2·4 vol.% of dimethylsulphoxide, pH 7·2–8·7 at 25°. *p*-Nitrophenyl liberation was recorded by a "Specord" spectrophotometer at 400 nm. Comparative measurements of the rate of catalytic reaction in H_2O and D_2O was carried out in 0·05 M phosphate buffer at pH 7·7 (for H_2O) and 7·3 (for D_2O) [5]. Heavy water (99·9%) was used. Synthesis of substrate analogues, *p*-nitroacetanilides, and sorption conditions have been described [4].

RESULTS

Reaction products

It has been previously shown [4] that alkylation of PEI with benzyl chloride results in secondary

Table 1. The constants of catalytic hydrolysis of NPB on linear and branched BPEI polymers in D_2O and H_2O*

Catalyst	β	$K'_{H_2O} \times 10^3$ sec^{-1}†	$K'_{D_2O} \times 10^3$ sec^{-1}	K'_{H_2O}/K'_{D_2O}
BPEI(L)	0·13	2·95	1·80	1·64
BPEI(L)	0·37	3·40	2·19	1·55
BPEI(B)	0·25	3·42	2·08	1·64

* Reaction conditions: 0·05 M phosphate buffer, pH 7·7 (H_2O), pH = 7·3 (D_2O), [NPB] = $1·42 \times 10^{-4}$ M.
† $K' = V_0/[Cat]$; [Cat] = $0·9 + 1·2 \times 10^{-5}$ M.

nitrogens being converted to tertiary ones and, in the case of PEI(B), in primary nitrogens being also converted to secondary. So in both cases, when the degree of alkylation is not maximal, the linear polymers contain secondary and tertiary nitrogen atoms, and branched species have primary nitrogens also. That is why, in addition to hydrolysis, NPE may undergo aminolysis by primary and secondary amino groups of PEI. For identification of the products of reaction between NPE and the polymers, the u.v. spectra of the products of alkaline hydrolysis of *p*-nitrophenyl-*o*-methoxycinnamate (NPOMC) (Fig. 1a) and its decomposition products in the presence of PEI(L), the degree of alkylation being 0·17 (Fig. 1b) were taken. The spectrum of spontaneous hydrolysis products show absorption peaks at 272 nm ($\epsilon = 1·9 \times 10^4$ M^{-1} cm^{-1}), 318 nm ($\epsilon = 1·05 \times 10^4$ M^{-1} cm^{-1}), which could be due to the absorption of *o*-methoxycinnamic acid and a peak at 400 nm ($\epsilon = 1·8 \times 10^4$ M^{-1} cm^{-1}) due to *p*-nitrophenolate (Fig. 1a, curve 1). The spectrum of the products of catalytic hydrolysis also has peaks at 272 nm ($\epsilon = 1·85 \times 10^4$ M^{-1} cm^{-1}), 320 nm ($\epsilon = 1·13 \times 10^4$ M^{-1} cm^{-1}), 400 nm ($\epsilon = 1·8 \times 10^4$ M^{-1} cm^{-1}) (Fig. 1b, curve 1). The similarity of the spectral characteristics of the products of alkaline and catalytic hydrolyses of NPOMC means that the latter hydrolysis proceeds towards formation of *o*-methoxycinnamic acid and *p*-nitrophenol, and the upper limit for acylation of PEI is very low. The reaction products are strongly bound to the polymer, as evident from the spectra of the samples (Fig. 1a, b, curves 2–4) taken at different stages during dialysis of the mixture against BPEI(L) ($\beta = 0·17$). In both cases, the dialysis occurs at nearly the same low rate. *p*-Nitrophenol ($\lambda = 320$ nm, pH 4·0) dialyses faster than the acid.

Neither the hydrolysis of nitrophenyl esters of aliphatic acids catalysed by BPEI nor that of NPOMC is accompanied by noticeable aminolysis of the polymers. Thus, catalytic hydrolysis of NPB taken in excess with respect to the catalyst and continued until the substrate was fully consumed, decreases the catalyst activity due to the binding of the products. However, subsequent dialysis allows removal of the products from the reaction, and the initial activity of the catalyst is restored.

The changes in the u.v. spectra of NPOMC with time during catalytic hydrolysis show that the ester converts rather rapidly into acid and phenol (Fig. 2). No intermediate acyl complexes with the catalyst were detected. Probably the active centres in BPEI participate in the reaction as general basic catalysts.

Isotopic effect

A comparison of initial reaction rates in H_2O and D_2O may serve as some indication of a general basic mechanism of catalysis involved in the hydrolysis.

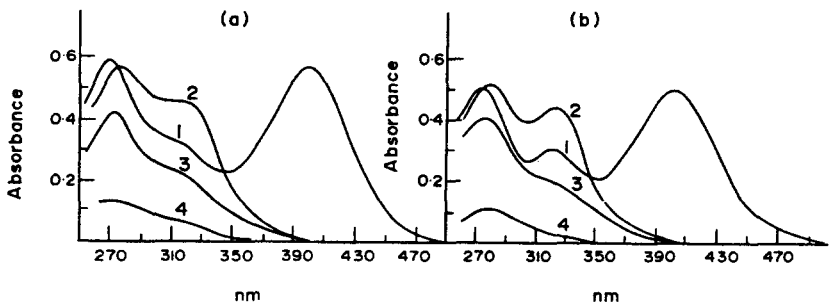


Fig. 1. Changes in the u.v.-spectra of the products of (a) alkaline and (b) catalytic [BPEI(L), $\beta = 0·17$] hydrolyses of NPOMC as it is dialysed from the mixture with BPEI(L) $\beta = 0·17$, $t = 25°$ [NPOMC] (a) = $3·2 \times 10^{-5}$ M, (b) $2·7 \times 10^5$ M [BPEI(L)] = 2×10^{-3} M, 1—pH 8·5, $t = 0$; 2—pH 4·0, $t = 0$; 3—pH 4·0, $t = 24$ hr; 4—pH 4·0, $t = 72$ hr.

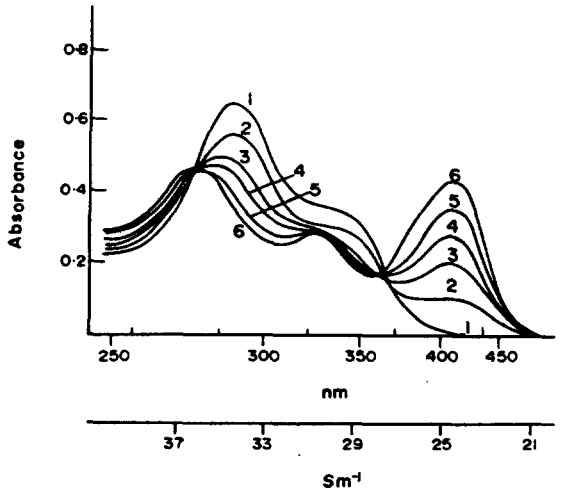


Fig. 2. Changes in the u.v.-spectrum of *p*-nitrophenyl *o*-methoxycinnamate (NPOMC) with time in catalytic hydrolysis [BPEI(B), $\beta = 0{\cdot}25$], [BPEI(B), $\beta = 0{\cdot}25$] = 2×10^{-3} M, pH = 8·5, $t = 25°$, [NPOMC] = $3{\cdot}2 \times 10^{-5}$ M, 1—0 min, 2—1 min, 3—3 min, 4—10 min, 5—20 min, 6—40 min.

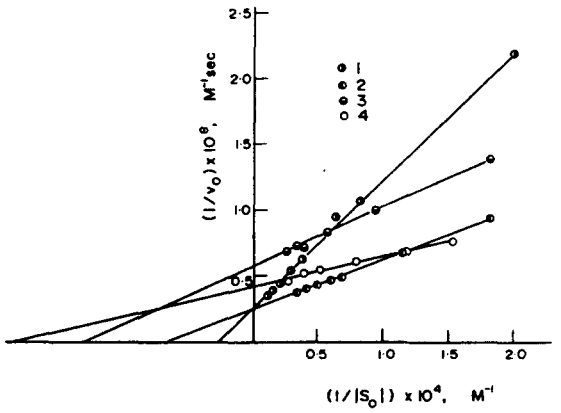


Fig. 3. Lineweaver–Burk plots of V_0 vs $[S_0]$ in catalytic hydrolysis of NPA, NPB, NPC on BPEI(L) and BPEI(B). Tris–HCl 0·01 M, 2% DMSO, pH = 8·3, $t = 25°$.

1—BPEI(B), $\beta = 0{\cdot}25$ + NPA, [Cat] = $8{\cdot}0 \times 10^{-6}$ M.
2—BPEI(L), $\beta = 0{\cdot}37$ + NPB, [Cat] = $2{\cdot}2 \times 10^{-6}$ M.
3—BPEI(B), $\beta = 0{\cdot}25$ + NPB, [Cat] = $7{\cdot}8 \times 10^{-6}$ M.
4—BPEI(L), $\beta = 0{\cdot}37$ + NPC, [Cat] = $4{\cdot}3 \times 10^{-6}$ M.

The values of the isotopic effect do not depend on BPEI structure and are close to the experimental values for reactions occurring via the general basic mechanism of catalysis ($K_{H_2O}/K_{D_2O} = 1{\cdot}8$–$2{\cdot}8$) [6].

The kinetics of hydrolysis

Reactions involving high molecular weight catalysts differ from those catalysed by low molecular weight substances in that in the former initial reaction rates (V_0) reach a maximum and do not grow as substrate concentration (S_0) increases [1–3]. This resembles enzymic catalysis and may be ascribed to adsorption catalytic centres being saturated with the substrate. In polymeric catalysts, these centres may be different from those in low molecular weight catalysts, in that in the former polymer–substrate adsorption complexes are formed. Such V_0 vs S_0 dependences were obtained in the present work. They straighten in the Lineweaver–Burk plots ($1/V_0$ vs $1/S_0$) in Fig. 3. The intersects with abscissa give reciprocal Michaelis constants, $K_m = (K_{-1} + K_2)/K_1$, which are usually close to the constant of the binding of the polymer catalytic centre with the substrate molecule (see the scheme). The Michaelis constants for some of the catalysts and substrates investigated are compared with the sorption constants (on the same polymers) of paranitroanilides, substrate analogues (Table 2), determined by a different method [4].

Parallelism in the change of K_m and K_s as the content of benzyl groups in PEI increases shows that anilides are sorbed where benzyl groups are localized; thereby adsorption centres of the catalysis are formed. This means that cleavage of ester bonds in the substrate is preceded by formation of a polymer–substrate sorption complex.

Table 2. The Michaelis constants (K_m) for hydrolysis of *p*-nitrophenylesters and sorption constants (K_S) of *p*-nitroanilides on linear and branched BPEI*

Catalyst	β	$K_m \times 10^4$ M	$K_s \times 10^4$ M
	p-Nitrophenylacetate		*p*-Nitroacetanilide
BPEI(L)	0·37	2·5	4·8
BPEI(B)	0·17	5·9	9·4
BPEI(B)	0·25	3·3	6·5
	p-Nitrophenylbutyrate		*p*-Nitrobutyranilide
BPEI(L)	0·17	2·7	3·3
BPEI(L)	0·37	0·78	2·7
BPEI(B)	0·12	2·5	2·8†
BPEI(B)	0·17	1·5	1·5†
BPEI(B)	0·25	1·0	0·9†

* K_m determined in 0·01 M Tris–HCl buffer, +2% DMSO, pH 8·3, $t = 25°$.
† K_S determined on BPEI(B) with $\beta = 0{\cdot}1$, 0·17 and 0·3, respectively.

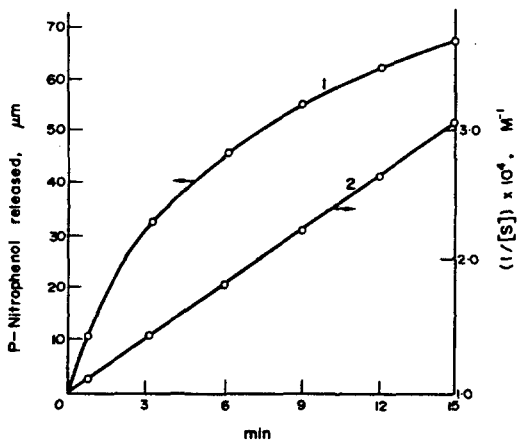


Fig. 4. (1) Formation of *p*-nitrophenol with time in catalytic hydrolysis of NPB [BPEI(L), β = 0·37], 0·01 M Tris–HCl, 2% DMSO, pH 8·35, t = 25°. [Cat] = [NPB] = 10^{-4} M. (2) Kinetic curve (I) in the coordinates of second order equation.

The pattern of the kinetic curves describing the change with time in the quantity of *p*-nitrophenol formed depends on the ratio of concentrations of substrate and catalyst. If the concentrations are equal, the kinetic curve represents a second order reaction (Fig. 4, curves 1 and 2). This may be explained by the fact that the active centres regenerate rather slowly. With an excess of the substrate, a steady state is soon established, as indicated by the curve in Fig. 5a. The prestationary parts of the kinetic curves are linearized in $\ln(1 - P_1/B)$ vs t coordinates (Fig. 5b), where B is the intercept on the ordinate if the stationary part of the kinetic curve is extrapolated to $t = 0$, and P_1 is the quantity of nitrophenol formed (Fig. 5a). The dependences (Fig. 5a, b) may be explained by assuming that, with an excess of the substrate during the initial period, we have a reaction first order with respect to the catalyst. As the substrate is being consumed, and the catalytic centres become unscreened, and the system reaches a steady state, i.e. the rates of formation and desorption of the reaction products are equalized. This is confirmed by the results of dialysis of the hydrolysis products (Fig. 1a, b) and the evidence of the effect of caprylic acid on the kinetics of hydrolysis (Fig. 6a, b). Caprylic acid acts as a competitive inhibitor in the hydrolysis of NPB, i.e. it hinders its binding with the catalyst, which is evident from an increase in K_m as the concentration of the inhibitor grows the constant K_2 remaining the same. The inhibition constant (K_i) is $1{\cdot}9 \times 10^{-3}$ M.

It follows that hydrolysis of NPE on polymeric catalysts involves the following steps:

1. $\text{Cat} + \text{S} \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} \text{Cat S}$

(formation of the catalyst–substrate sorption complex)

2. $\text{Cat S} + \text{H}_2\text{O} \xrightarrow{K_2} \text{Cat } P_2 + P_1$

[substrate hydrolysis with formation of alcohol (P_1) and acid (P_2), the latter being sorbed on catalyst more strongly than P_1].

3. $\text{Cat } P_2 \xrightarrow{K_3} \text{Cat} + P_2$

(diffusion of P_2 to the solution).

The system of differential equations based on this scheme is solved like that for enzymic hydrolysis of esters [7]. The difference is that the step of general base catalysis is assumed to take place instead of the acylation step, and the unscreening of the active centres after desorption of the products and their diffusion from the polymeric coils to the solution is suggested to occur instead of deacylation.

Liberation of P_1 with time is described by

$$P_1 = At + B(1 - \exp(-bt)], \qquad (1)$$

where the coefficients A and B are equal, respectively, to

$$A = \frac{K_2K_3[E_0][S_0]}{K_2[S_0] + K_3(K_m + [S_0])} \qquad (2)$$

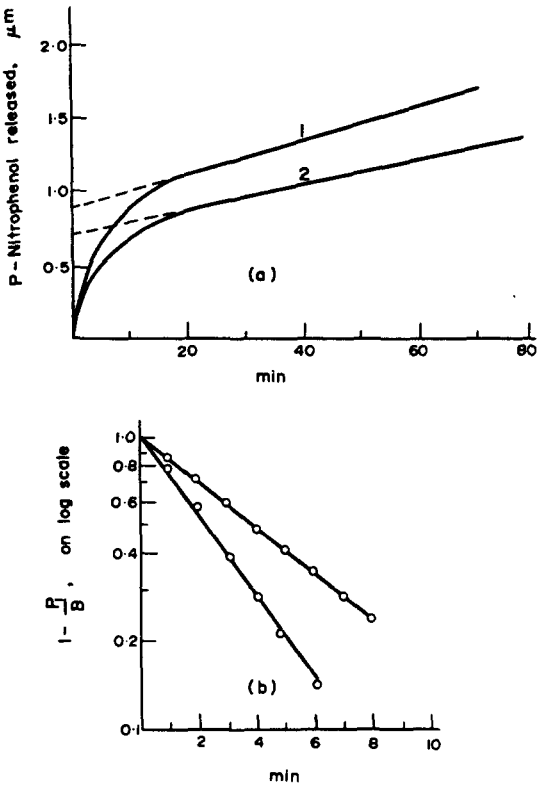


Fig. 5a. Kinetic curves of the catalytic hydrolysis [BPEI(B), β = 0·25] of NPB at $S \gg$ Cat, Phosphate buffer 0·05 M, 2 vol.% DMSO, pH = 7·7, t = 25°.
1, [Cat] = $8{\cdot}7 \times 10^{-6}$ M, [NPB] = $1{\cdot}4 \times 10^{-4}$ M. 2. [Cat] = $6{\cdot}8 \times 10^{6}$ M, [NPB: = $1{\cdot}9 \times 10^{-4}$ M.

Fig. 5b. Fragments of kinetic curves preceding the stationary state as a first order equation.

$$B = \frac{A^2}{[\text{Cat}].K_3^2} \quad (3)$$

$$b = K_3 + \frac{K_2[E_0][S_0]}{K_m + [S_0]}. \quad (4)$$

At the prestationary part of kinetic curve

$$P_1 = B\exp(-bt) \quad (5)$$

and at the stationary part

$$(P_1) = B + At, \quad (6)$$

where $A = V_{st}$ is the rate of reaction during the stationary state, and B is the intercept of the ordinate with the linear part of kinetic curve extrapolated to $t = 0$ (Fig. 5a). At the start, one may assume that

$$V_0 = \frac{K_2[E_0][S_0]}{K_m + [S_0]}. \quad (7)$$

By solving the system of equations (2), (3), (7), we obtain

$$K_3 = \frac{V_{st}(V_0 - V_{st})}{V_0 B} \quad (8)$$

and from (3) we find

$$[\text{Cat}] = V_{st}^2/B\,K_3^2. \quad (9)$$

Thus, using parameters V_0, V_{st} and B found from the kinetic curves, one may calculate the rate constant of desorption, K_3, and the concentration of active centres in the polymers. The latter enables determination of the rate constant, K_2, and Michaelis constant, K_m from the V_0 vs $[S_0]$ dependence. The results of such calculations for BPEI(L) and BPEL(B) substituted to different degrees are shown in Tables 3–5 for three substrates.

The nature of active centres

Noting that there are different types of nitrogen atoms in BPEI, it is essential to determine those in

Table 3. The kinetic constants and the polymeric effect in the hydrolysis of *p*-nitrophenylacetate on BPEI(B) and BPEI(L)*

Catalyst	β	$K_m \times 10^4$ (M)	$K_2 \times 10^3$ (sec^{-1})	$K_3 \times 10^4$ (sec^{-1})	K_2/K_m or K_{11} (M^{-1}, sec^{-1})	$\frac{K_2/K_m}{K_{11}}$
BPEI(L)	0·37	2·6	6·3	<0·5	41·5	$2·1 \times 10^2$
BPEI(B)	0·17	5·9	7·4	<0·5	12·6	$0·6 \times 10^2$
BPEI(B)	0·25	3·3	4·5	0·9	13·6	$0·7 \times 10^2$
BPEI(B)	0·35	1·7	7·2	0·6	42·0	$2·1 \times 10^2$
BPEI(B)	0·58	1·3	5·0	<0·5	38·4	$1·9 \times 10^2$
BPEI(B)	0·73	0·9	4·8	<0·5	53·0	$2·7 \times 10^2$
PEI(B)	0				0·12	0·6
Benzyldiethylamine (BDEA)					0·2	

* Experimental conditions: 0·01 M Tris–HCl, 2% vol. DMSO, pH 8·3, $t = 25°$; reagent concentrations (M) BPEI = 10^{-5}–10^{-4}, PEI(L) = PEI(B) = 2×10^{-4}–4×10^{-4}, BDEA = 10^{-3}–4×10^{-3}, NPA = 10^{-4}–2×10^{-3}.

Table 4. The kinetic constants and polymeric effect in the hydrolysis of *p*-nitrophenylbutyrate on BPEI(B) and BPEI(L)*

Catalyst	β	$K_m \times 10^4$ (M)	$K_2 \times 10^3$ (sec^{-1})	$K_3 \times 10^4$ (sec^{-1})	K_2/K_m or K_{11} (M^{-1}, sec^{-1})	$\frac{K_2/K_m}{K_{11}}$
BPEI(L)	0·12	5·2	8·1	3·4	15·5	$1·7 \times 10^2$
BPEI(L)	0·17	2·7	3·7	3·0	13·7	$1·5 \times 10^2$
BPEI(L)	0·37	0·78	3·0	4·5	38·5	$4·3 \times 10^2$
BPEI(B)	0·09	20·0	4·0	8·5	2·0	$0·2 \times 10^2$
BPEI(B)	0·13	3·3	9·0	—	2·7	$3·0 \times 10^2$
BPEI(B)	0·16	1·5	5·4	4·3	37·0	$4·1 \times 10^2$
BPEI(B)	0·17	0·9	5·1	7·2	57	$6·3 \times 10^2$
BPEI(B)	0·25	1·0	4·3	3·3	43	$4·8 \times 10^2$
BPEI(B)	0·35	0·64	3·1	4·5	48	$5·3 \times 10^2$
BPEI(B)	0·66	0·33	2·2	2·5	67	$7·4 \times 10^2$
BPEI(B)	0·58	0·43	2·2	3·5	50	$5·7 \times 10^2$
BPEI(B)	0·73	0·27	3·4	3·5	130	$1·8 \times 10^3$
PEI(L)	0				0·8	9
PEI(B)	0				0·21	2·3
BDEA					0·09	

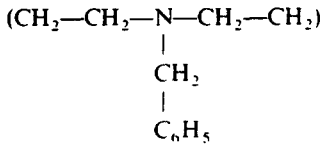
* Experimental conditions are the same as in Table 3, [NPB] = 5×10^{-5}–7×10^{-4} M.

Table 5. The kinetic constants and polymeric effect in the hydrolysis of *p*-nitrophenylcaprylate on BPEI(B) and BPEI(L)*

Catalyst	β	$K_m \times 10^4$ (M)	$K_2 \times 10^3$ (sec^{-1})	$K_3 \times 10^4$ (sec^{-1})	K_2/K_m or K_{II} (M^{-1}, sec^{-1})	$\frac{K_2/K_m}{K_{II}}$
BPEI(L)	0·17	0·45	10	2·5	$2·2 \times 10^2$	4×10^4
BPEI(L)	0·37	0·20	31	<0·5	$2·6 \times 10^3$	$2·8 \times 10^5$
BPEI(B)	0·13	0·48	12		$2·9 \times 10^2$	$4·4 \times 10^4$
BPEI(B)	0·17	0·37	10	<0·5	$2·7 \times 10^2$	$4·9 \times 10^4$
BPEI(B)	0·58	0·24	6·3	<0·5	$2·6 \times 10^2$	$4·8 \times 10^4$
BPEI(B)	0·73	0·06	9·1	<0·5	$1·5 \times 10^3$	$2·8 \times 10^5$
BDEA					5×10^3	

* Experimental conditions are the same as in Table 3, [NPC] = 5×10^{-6}–5×10^{-5} M.

active centres. To this end, we studied the pH dependence of the reaction rate (Fig. 7). The increase in the reaction rate as pH grows is due to a deprotonation of nitrogen atoms in the active centre of BPEI. That the plot has one inflexion point testifies to the monofunctional nature of the catalytic centres. The pK_a estimated from this dependence is 8·35 ± 0·1 and it is the same for the linear and branched BPEI's. That the catalytic centres in BPEI are of the same type is also evident from constant values of K_2 and K_m and from the increase in ratio Cat_0/PEI from pH 7·2 to 8·5 (Table 6) calculated from the data on NPB hydrolysis on two catalysts. The pK_a value obtained from these data for catalytic centres is also 8·35 ± 0·1. The results of potentiometric titration of PEI(L), PEI(B) and benzyl-containing polymers demonstrate that the latter have lower pK_a (by 2 units) than the starting PEI at any degree of neutralization; 15 per cent of the nitrogen atoms have $pK_a > 10$. These results and the fact that the pK_a's of catalytic centres determined from the kinetics of the hydrolysis are 8·35 ± 0·1 show that the benzyl-containing tertiary amino groups, analogues of benzyl-diethylamine, having the structure



are the most probable catalytic centres in BPEI. This conclusion does not contradict the fact that benzyl groups make hydrophobic regions in BPEI macromolecules, i.e. the sites for the substrate molecules to sorb upon.

The validity of such an assumption is also supported by a high catalytic activity of the polymers with a higher alkylation degree.

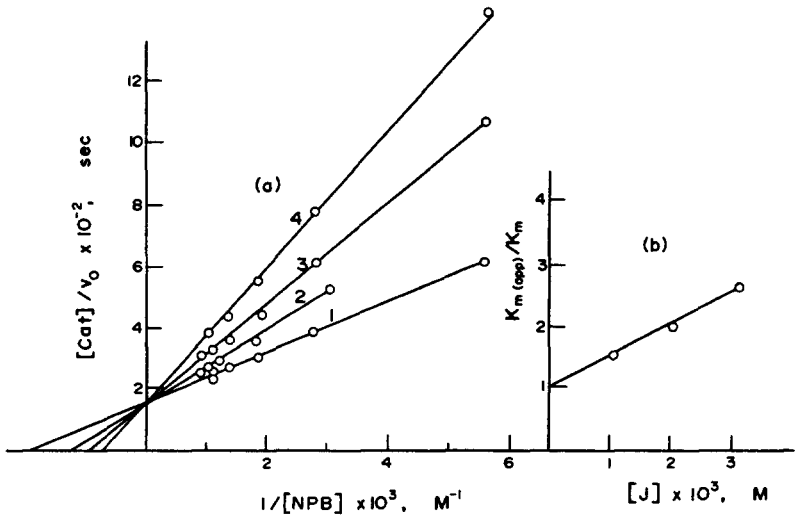


Fig. 6 (a, b). Inhibition of the catalytic hydrolysis of NPB [BPEI(B), β = 0·165] by caprylic acid. 0·01 M Tris-HCl 2% DMSO, t = 25° [BPEI(B), β = 0·165] = 2×10^{-4} M. (a) Lineweaver–Burk plots of V_0 vs $[S_0]$. 1, Without inhibitor; 2, $[I_0] = 1 \times 10^{-3}$ M; 3, $[I_0] = 2 \times 10^{-3}$ M; 4, $[I_0] = 3 \times 10^{-3}$ M; (b) $K_m app/K_m$ vs $[I_0]$.

Table 6. The kinetic constant of catalytic hydrolysis of NPB and the pH-dependent changes in the catalytic centres in BPEI*

Catalyst	β	pH	[Cat_0/BPEI] $\times 10^2$	$K_m \times 10^5$ (M)	$K_2 \times 10^3$ (sec^{-1})	K_2/K_m (M^{-1}, sec^{-1})	$K_3 \times 10^4$ (sec^{-1})
BPEI(L)	0·13	7·8	1·1	6·3	3·5	55	7·9
		8·05	1·7	7·0	3·5	50	6·0
		8·32	2·3	7·7	3·9	51	3·5
		8·5	2·7	7·4	3·6	49	2·6
BPEI(B)	0·73	7·2	2·1	2·8	3·0	110	
		7·55	4·1	2·7	3·5	130	
		7·9	8·9	2·7	3·5	130	
		8·35	17·0	2·7	3·5	130	

* Experimental conditions: 0·01 M Tris-HCl, 2% DMSO, t = 25°. Reagents concentrations (M): [BPEI(L), β = 0·13] = 10^{-4}, [BPEI(B), β = 0·73] = 5×10^{-5}, [NPB] = 5×10^{-5}–7×10^{-4}.

Figure 8 shows that the portion of catalytic centres in BPEI increases with respect to the number of all nitrogen atoms as the degree of benzylation grows. The maximum value of the E_0/BPEI is 0·25. The linear and branched BPEI's have close Cat/BPEI ratios at the same values of β. The size of the radical in the substrate acyl group does not usually affect the concentration of catalytic centres in the polymers, determined from kinetic data. These results, and particularly the increase in the number of active centres in the polymers with increasing β, also demonstrate that such centres are the units of benzyldiethylamine with pK_a equal to 8·35. The number of catalytic centres in macromolecules with a degree of polymerization of 1000 may be more than 200, which testifies to a high efficiency of BPEI as a catalyst for hydrolysis.

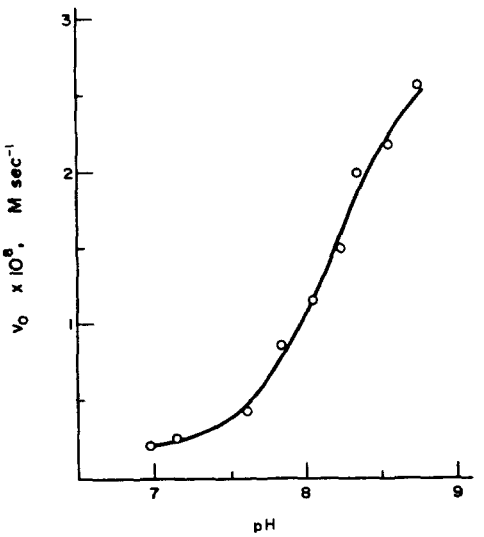


Fig. 7. The rate of catalytic hydrolysis of NPB, vs pH. 0·01 M Tris-HCl, t = 25°. [BPEI(L), β = 0·37] = $6·7 \times 10^{-5}$ M, NPB = $1·7 \times 10^{-4}$ M.

DISCUSSION

Analysis of the data in Tables 3–5 shows that the bimolecular rate constant (K_2/K_m) of the hydrolysis catalysed by the polymers increases from substrate to substrate in the following order: NPA; NPB; NPC. On the other hand, the bimolecular rate constant of the reaction catalysed by a low-molecular weight analogue of the catalytic centre in polymer, benzyldiethylamine, decreases in the same order. Thus the so-called "polymeric effect", e.g. the ratio of bimolecular rate constants (K_2/K_m) to K_{II} increases still further in the same series of substrates and exceeds 2×10^5 for NPC. High reaction rates of the NPE hydrolysis growing from NPA to NPC but not exceeding 10^3 in the latter case have also been observed in the micelle-catalysed reactions involving cetyltrimethylammonium bromide and N-tetradecanoyl-L-histidine [8]. Thus certain stages of hydrolysis of NPE with different hydrophobicity catalysed by micelles and polymers are in

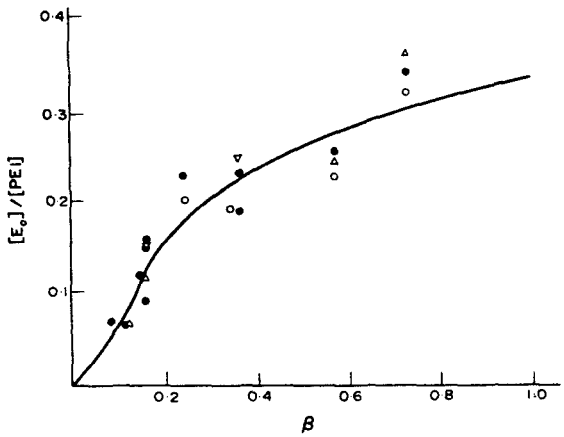


Fig. 8. Fraction of catalytic centre Cat/BPEI in BPEI(L) and BPEI(B) vs degree of benzylation. Catalytic system: BPEI(B) ○—NPA, ⊗—NPB, △—NPC; BPEI(L) ●—NPA, ⊕—NPB, ▽—NPC.

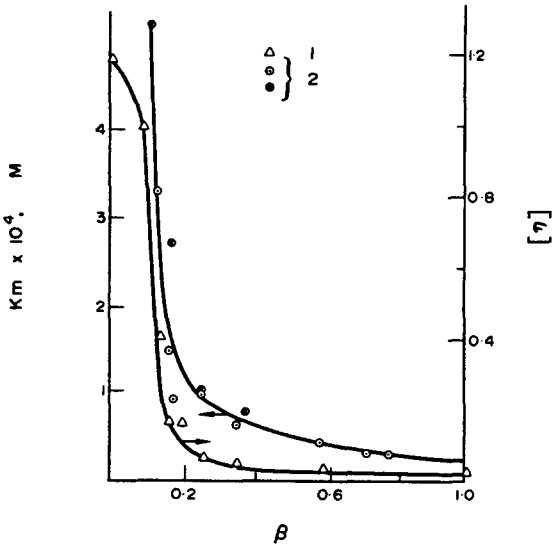


Fig. 9. (1) Intrinsic viscosity of BPEI(B) and Michaelis constant, K_m in NPB hydrolysis (2) vs the fraction of benzyl groups in the polymers. Conditions of hydrolysis are as in Table 3. ⊙ BPEI(B) and ⊗ BPEI(L) refer to (2).

some features similar and in some different from the catalysis by low-molecular weight amines [9]. On the other hand, the localization of active centres in the polymer coil is more favourable for the reaction rate as compared to that in the micelle. Since it is individual amino groups that play the role of active centres in both BPEI and micelles and there are no data on several functional groups being conjugated as is the case in enzymic catalysis, the differences observed in the activity of polymeric and micellar catalysts should be ascribed to different topologies of the catalyst-substrate sorption complex. This should be caused by different structures of a polymeric coil and a micelle, in particular by different mutual localization of the sorption and catalytic centres.

In BPEI, the increase in the number of catalytic centres (Fig. 8) as β grows is more sharp in the region where the macromolecular coils undergo conformational changes and decrease in size, which is indicated by the decrease in intrinsic viscosity (Fig. 9, curve 1). The Michaelis constant decreases in parallel with the decrease in the size of macromolecules (Fig. 9, curve 2). This reflects the relationship between the growing hydrophobicity of the macromolecule of polymeric catalyst and its ability to complex with the substrate. The role of benzyl radicals in such catalysts is two-fold: they participate in the formation of hydrophobic regions sorbing the substrates and the amino groups acting as general bases in the catalysis. Hence the sorption and catalysis centres should be in close proximity. Therefore one may assume that in BPEI the substrate is bound rather "efficiently". The "efficiency" of substrate sorption can be estimated from the increment of free energy of activation of catalytic hydrolysis in a series of substrates with different hydrophobicity of the acyl residue [10]. Transfer of a hydrocarbon molecule from a hydrophilic to a hydrophobic medium results in an energy gain of 0·8 kcal per CH_2 group [11]. When the sorbed molecule in an active centre generated has a favourable orientation, the energy liberated at the sorption step should evidently decrease the free energy of the transition complex. In a sequence of substrate molecules of increasing hydrophobicity, this decrease should regularly grow. Table 7 shows the values of increment of free energy of activation ($\Delta\Delta F^*$) of hydrolysis of the substrate in the NPA–NPC sequence, catalysed by micelles, polymers and α-chymotrypsin.

Table 7. The values of $\Delta\Delta F^*$ in the hydrolysis of nitrophenyl esters in the NPA–NPC sequence catalysed by BPEI†, *m*-brombenzaldoxime + cetyltrimethylammonium bromide and α-chymotrypsin‡

Catalyst	β	$\Delta\Delta F^*$
BPEI(L)	0·16	0·28
BPEI(L)	0·37	0·4
BPEI(B)	0·58	0·28
BPEI(B)	0·73	0·33
m-Brombenzaldoxime + cetyltrimethylammonium bromide		0·2†
α-Chymotrypsin		0·55†

† In the catalysis by BPEI the values of ΔF^* were calculated from bimolecular hydrolysis rate constant (K_2/K_m) in equation:

$$K_2/K_m = kT/h \exp - \frac{\Delta F^*}{RT}.$$

Conditions of hydrolysis: pH = 8·35, t = 25°, 0·01 M, Tris–HCl, 2% DMSO, $[Cat_0] = 1\text{–}5 \times 10^{-6}$, $[S_0] = 5 \times 10^{-6}$–2×10^{-3} M.

‡ For the hydrolysis catalysed by micelles and α-chymotrypsin the values of $\Delta\Delta F^*$ were taken from [12].

It can be seen from Table 7 that the lowest increment of $\Delta\Delta F^*$ corresponding to the lowest contribution of the sorption step to the catalytic transformation of substrate, i.e. the lowest "efficiency" of complexation, is observed in micellar catalysis, and the highest in enzymic catalysis. Polymeric catalysts occupy an intermediate position between the two groups, as far as substrate binding is concerned.

As is seen from Table 6, the rate constant of desorption of reaction products, K_3, is by an order lower than the catalytic rate constant, K_2. This drawback of the catalysts investigated may be due to a high density of polymeric globules [Fig. 9, (1)]. A decrease of pH of the medium should cause an increase in the density of the charge on the macromolecules which thereby acquire a looser conformation. This partially removes the diffusion restrictions as a result of which the value

of K_3 becomes several times higher (Table 6). The relation existing between K_3 and the conformation of BPEI may be exemplified by a polymer of a degree of alkylation of 0·09, which has a high value of $[\eta]$ [Fig. 9, (1)], and a relatively large K_m and K_3 (Table 4).

It should be stated in conclusion that the reasons for higher activity of polymeric catalysts with respect to low molecular weight catalysts are mainly the sorption properties of the hydrophobic active centres in the former (sorption effects accelerate the reaction 10^3–10^5 times) and also, perhaps, the effect of the polymer functional groups upon the substrate reactivity.

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Résumé—On a étudié le mécanisme d'hydrolyse de l'acétate (NPA), du butyrate (NPB), du caprylate (NPC), de l'*o*-méthoxycinnamate (NPOMC) et du phosphate (NPP) de n-nitrophényle, catalysée par des polyéthylèneimines contenant des groupes benzyles à structures linéaire et ramifiée. La réaction procède suivant un mécanisme général de catalyse et ne provoque pas de réaction d'acylation du catalyseur. La benzyldiéthylamine est un analogue des centres actifs du polymère avec un $pK_a = 8{,}35 \pm 0{,}1$, localisés dans les globules de polymère aux sites de grande hydrophobicité.

La réaction comporte un mécanisme en trois étapes impliquant la fixation du substrat sur un centre actif (pour donner un complexe d'adsorption de Michaelis), la conversion du substrat et la désorption des produits de réaction. La dernière étape est le goulot d'étranglement de la réaction. On a déterminé les constantes de vitesse pour chaque étape. L'effet du polymère $(K_2/K_m/K_{II})/K_{II}$ augmente de NPA à NPC; pour ce dernier cas, il est égal à $2{,}8 \times 10^{-5}$. Pour un substrat NPP chargé, l'effet du polymère est égal à $4{,}8 \times 10^6$.

Sommario—In mezzi acquosi si è indagato il meccanismo dell'idrolisi dell'acetato (NPA), butirrato (NPB), caprilato (NPC), *o*-metossicinnamato (NPOMC) e fosfato (NPP) dell'*n*-nitrofenile, catalizzata da polietilenimine contenenti benzile e a struttura lineare e ramificata. La reazione procede con un meccanismo di catalisi base generale e non implica l'acilazione del catalizzatore. La benzildietilammina è un analogo dei centri attivi nei polimeri con $pK_a = 8{,}35 \pm 0{,}1$, localizzati nei globuli dei polimeri in luoghi di elevata idrofobicità.

La reazione si svolge secondo un meccanismo in tre fasi e comprende l'attacco del sostrato ad un centro attivo (per formare un complesso di adsorbimento–assorbimento Michaelis), la conversione del sostrato e il deadsorbimento–assorbimento dei prodotti. L'ultima fase limita la velocità. Per ogri fase si sono determinate le costanti di velocità. L'effetto del polimero $(K_2/K_m)/K_{II}$ aumenta da NPA a NPC; nell'ultimo caso è di $2{,}8 \times 10^5$. Per sostrato NPP caricato, l'effetto polimero è di $4{,}8 \times 10^6$.

Zusammenfassung—Untersucht wurde der Mechanismus der Hydrolyse wässriger Lösungen von n-Nitrophenylacetat (NPA), -butyrat (NPB), -caprylat (NPC), -*o*-methoxycinnamat (NPOMC) und -phosphat (NPP), die durch lineares und verzweigtes Polyäthylenimin, das Benzylgruppen enthält, katalysiert wird. Die Reaktion verläuft ganz allgemein nach einem basisch katalysierten Mechanismus, wobei keine Acylierung des Katalysators auftritt. Benzyldiäthylamin mit einem Wert für $pK_a = 8{,}35 \pm 0{,}1$ ist ein Analogon für die aktiven Zentren im Polymeren, die in den globulären Polymerteilchen an Stellen größerer Hydrophoby anzutreffen sind.

Die Reaktion läuft nach einem Dreistufenmechanismus ab mit der Bindung des Substrates an die aktiven Zentren (unter Ausbildung eines Michaelis-Adsorptions-Komplexes), Umsetzung des Substrates und Desorption des Reaktionsproduktes. Der letztere Reaktionsschritt ist geschwindigkeitsbestimmend. Die Wirksamkeit des Polymeren $(K_2/K_m)/K_{II}$ nimmt zu von NPA bis zu NPC und beträgt für den letzteren Fall $2{,}8 \times 10^{-5}$. Für zugesetztes NPP als Substrat beträgt der Polymereffekt $4{,}8 \times 10^{6}$.