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Effect of Added Anions on the Catalyzed Decarboxylation of 6-Nitrobenzisoxazole-3-Carboxylate by Polyethylenimine Derivatives

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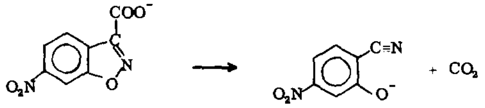
Rates for the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate were measured in the presence of polyethylenimine derivatives in solutions with added chloride, acetate, or azide ions. The values of k_{cat} were not affected by the nature of the added anion, but those of K_m were markedly changed. Kinetic schemes that account for the observed results are described.

Poly(ethylenimine), a highly branched polymer derived from ethylenimine (*1–3*), has been modified by alkylation, acylation, or Schiff base formation (*4*). Derivatives of poly(ethylenimine) containing various functional groups or providing hydrophobic microenvironments have been found to catalyze a variety of reactions (*5–7*). For example, marked catalysis of the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate, has been observed with several derivatives of poly(ethylenimine) containing apolar lauryl groups and fully quaternized amine nitrogens (*5*). The kinetics of this decarboxylation reaction fit equations analogous to those of enzyme kinetics, and thus the mechanism can be characterized by the formation of complexes between substrate and polymer and by corresponding turnover rate constants.



(1)

Since the common derivatives of poly(ethylenimine) are polyelectrolytes with a large number of positive charges on each macromolecule, one might expect substantial interactions with any added small anions. We have examined, therefore, the effects of three different small anions, acetate, chloride, and azide, on the kinetic behaviour of reaction (1) in the presence of these cationic polymers.

MATERIALS AND METHODS

Two derivatives of poly(ethylenimine) were prepared by methods described previously (*5*). Their compositions may be represented as

$$[(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}(C_2H_4N)_m]^{m+}mCl^-; \quad m = 1400. \qquad (I)$$

$$[(C_{12}H_{25})_{0.25m}(C_2H_5)_{1.75m}(C_2H_4N)_m]^{m+}mCl^-; \quad m = 1400. \qquad (II)$$

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In essence, one fourth of the monomer residues in each polymer were alkylated with apolar lauryl groups, and all of the nitrogens in the resultant polymer were quaternized by methyl groups, (I), or by ethyl groups, (II).

The preparation of 6-nitrobenzisoxazole-3-carboxylic acid has been described previously (*5*). Rates of decarboxylation were followed spectrophotometrically (*5*), in 0.01 *M* 2-(*N*-morpholino)ethanesulfonate (MES) buffer containing 0.05 *M* added salt.

RESULTS AND DISCUSSION

Rates of decarboxylation of nitrobenzisoxazole carboxylate in the presence of polymer are markedly sensitive to the nature of added anions. This behavior is strikingly illustrated by a comparison of observed pseudo-first-order rate constants, k_{obs}, in the presence of acetate, chloride, and azide, respectively (Figs. 1 and 2). At 0.05 *M* concentration, azide ions diminish the rates severalfold compared to acetate. Chloride ions have an intermediate effect.

For quantitative comparisons the observations have been analyzed in terms of the simplest mechanistic scheme for a catalyzed decarboxylation, Eq. (2):

$$\mathrm{C} + \mathrm{S} \underset{}{\overset{K_m}{\rightleftharpoons}} \mathrm{C}\cdot\mathrm{S} \xrightarrow{k_{cat}} \mathrm{C} + \text{products}. \tag{2}$$

In this expression, C represents one catalytic domain in the polymer, S, the substrate, K_m, the dissociation constant of the C · S complex, and k_{cat} the first-order rate constant for the catalyzed decarboxylation in the catalytic complex C · S. As has been shown previously (*5*), when the initial concentration, C_0, of catalytic domains exceeds initial substrate concentration, S_0, (i.e., $[C_0] \gg [S_0]$) then the observed rate k_{obs} is the following function of initial polymer concentration $[\mathrm{Pol}]_0$:

$$k_{obs} = \frac{k_{cat}[\mathrm{Pol}]_0}{K_m/n + [\mathrm{Pol}]_0}. \tag{3}$$

In this equation n represents the number of catalytic domains on a single polymer molecule. A plot of the double-reciprocal transform of Eq. (3), that is, of $1/k_{obs}$ versus $1/(\mathrm{Pol})_0$ provides a convenient format for evaluation of k_{cat} and of K_m/n.

Values of these kinetic parameters under various conditions are listed in Table 1. Saturation kinetics were observed in the presence of each of the added ions at high concentrations of polymer. In graphical comparisons (Figs. 1 and 2) only data up to polymer concentrations of 2×10^{-6} *M* are plotted so that the low concentration region can be seen on an expanded scale.

Kinetic constants were also obtained in Tris buffer at pH 7.4. These are only slightly different from those observed previously with different samples of the same types of polymer (*5*). There are also small differences in kinetic constants for the solutions containing 0.05 *M* $\mathrm{Cl^-}$ at pH 7.0 as compared to pH 7.7. These variations may also reflect small differences in ionic strength of the two solutions.

Each plot of k_{obs} versus $[\mathrm{Pol}]_0$ in Figs. 1 and 2 shows increasing curvature with increasing polymer concentration. Such saturation behavior in the presence of polymer (or enzyme) catalysts is generally regarded as a manifestation of complex formation

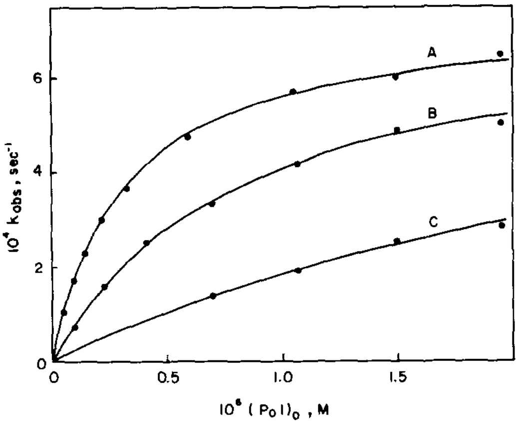


FIG. 1. Variation of rate of decarboxylation of nitrobenzisoxazole carboxylate as a function of concentration of polymer. Methyl-quaternized polymer, (I), in buffer solutions at pH 7.0 with added $NaC_2H_3O_2$ (A), NaCl (B), or NaN_3 (C). The solid lines drawn are based on the kinetic parameters listed in Table 1.

between macromolecule and substrate. Since polyethylenimines are highly charged polymers, one might also be concerned about their contribution to the ionic strength of the solution and the associated weakening of interactions between oppositely charged species. Such ionic strength contributions are difficult to estimate for polyelectrolytes. However, these effects seem not to be operative here, for one would expect parallel curvatures in the presence of acetate, chloride, and azide; and this is clearly not the case (Figs. 1 and 2). Furthermore, the limiting value for k_{obs} at very high polymer concentrations is the same in the presence of azide as with acetate or chloride. These features of the kinetic behavior support the assumption that reactions catalyzed by poly(ethylenimine) derivatives (*5*, *6*) proceed through a complex of polymer and substrate.

A comparison of kinetic constants in Table 1 shows that k_{cat} is not significantly affected by the nature of the specific anion added as part of the supporting electrolyte. There are differences between the polymer quaternized with ethyl groups and that with methyl groups, but for each polymer k_{cat} is essentially fixed. In contrast, K_m/n is

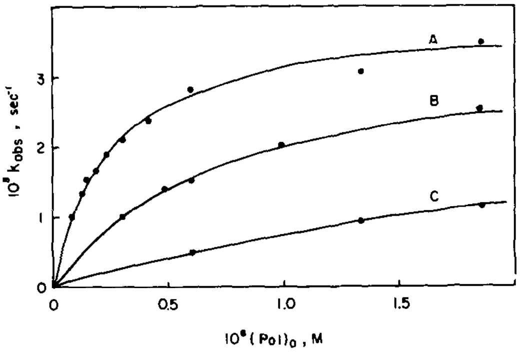


FIG. 2. Variation of rate of decarboxylation of nitrobenzisoxazole carboxylate as a function of concentration of polymer. Ethyl-quaternized polymer, (II), in buffer solutions at pH 7.0 with added $NaC_2H_3O_2$ (A), NaCl (B), or NaN_3 (C). The solid lines drawn are based on the kinetic parameters listed in Table 1.

TABLE 1

KINETIC PARAMETERS FOR THE DECARBOXYLATION OF NITROBENZISOXAZOLE CARBOXYLATE IN VARIOUS BUFFER SOLUTIONS[a]

Polymer	Buffer	k_{cat} (10^{-3} sec^{-1})	K_m/n (10^{-6} M)	nk_{cat}/K_m (10^3 sec^{-1} M^{-1})
Lau$_{25}$-Me$_Q$PEI (I)	0.05 M $NaC_2H_3O_2$ 0.01 M MES, pH 7.0	0.75 ± 0.03	0.33 ± 0.04	2.3 ± 0.2
	0.05 M NaCl 0.01 M MES, pH 7.0	0.75 ± 0.06	0.83 ± 0.17	0.89 ± 0.16
	0.05 M NaN_3 0.01 M MES, pH 7.0	0.81 ± 0.07	3.3 ± 0.4	0.24 ± 0.02
	0.05 M Tris–Cl^- total Tris = 0.0591 M, pH 7.4	0.87 ± 0.05	0.84 ± 0.05	1.04 ± 0.12
Lau$_{25}$-Et$_Q$PEI (II)	0.05 M $NaC_2H_3O_2$ 0.01 M MES, pH 7.0	3.9 ± 0.2	0.25 ± 0.02	15.7 ± 0.9
	0.05 M NaCl 0.01 M MES, pH 7.0	3.5 ± 0.2	0.78 ± 0.06	4.6 ± 0.3
	0.05 M NaN_3 0.01 M MES, pH 7.0	3.5 ± 0.3	3.71 ± 0.3	0.94 ± 0.05
	0.05 M Tris–Cl^- total Tris = 0.0591 M, pH 7.4	3.8 ± 0.5	0.78 ± 0.12	4.9 ± 0.4

[a] Measured under the condition of $[C_0] \gg [S_0]$. In accordance with a previous study (6), the condition of $[A_0] \gg [C_0]$ is also met in the present study. [b] The value of n obtained in a previous study (5) is about 60 for methyl quaternized polymer, (I), and about 110 for ethyl quaternized polymer, (II).

markedly sensitive to the nature of the added anion. The relative magnitudes of K_m/n for polymer (I), quaternized with methyl groups, are in the ratio 1:2.5:10, and those for polymer (II), ethyl quaternized, form the ratio 1:3:15 in the presence of acetate, chloride, and azide, respectively. Since n, the number of catalytic domains on a single polymer molecule is a function of the structure of the macromolecule, it is presumably independent of the nature of the added anion. Thus variations in K_m/n should reflect changes in K_m, the dissociation constant for substrate–polymer complex.

As is apparent in Table 1, k_{cat}/K_m is also markedly sensitive to the nature of the added anion. This parameter corresponds to the second-order rate constant that governs the kinetics at very low concentrations of substrate and polymer ($[S_0] < K_m$, $[C_0] < K_m$). Once again this sensitivity is a reflection of variations in the factor K_m. The simplest interpretation of this variation in K_m is that the added small anion, A, competes with substrate, S, for binding sites on the polymer. This explanation can be expressed quantitatively by kinetic parameters defined in the scheme of Eq. (4):

$$\begin{tikzcd}
C \arrow[r, shift left, "+A"] \arrow[d, shift right, "+S"'] & C \cdot A \arrow[l, shift left, "K_I"] \\
C \cdot S \arrow[u, shift right, "K_1"'] \arrow[r, "k_1"] & C + \text{products.}
\end{tikzcd} \qquad (4)$$

In this equation, K_1 and K_2 are the dissociation constants of the respective complexes, C · S and C · A. The expression for k_{obs} is formally analogous to Eq. (3) under initial concentration conditions such that $[A_0] \gg [C_0] \gg [S_0]$, if we define k_{cat} and K_m by Eqs. (5) and (6):

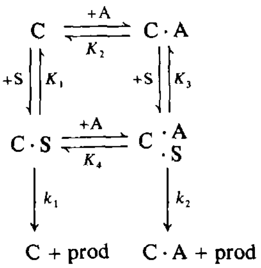
$$k_{\text{cat}} = k_1, \tag{5}$$

$$K_m = K_1 + \frac{K_1}{K_2} A_0. \tag{6}$$

Since K_m is dependent on K_2, which is determined by the nature of the anion A, K_m will vary from acetate to chloride to azide. On the other hand, k_{cat} is not dependent on the specific anion in solution.

The variation in K_m reveals that poly(ethylenimine) binds azide most strongly, chloride next, and acetate most weakly. This order, which parallels polarizabilities of these specific ions, is also that found in binding of ions by proteins (*8*, *9*). Thus chloride is bound more strongly than acetate by serum albumin, and azide is bound more than chloride by hemerythrin. Once again we find a parallelism in the behavior of the synthetic polymer with that isolated from living sources.

The mechanism described in Eq. (4) assumes that if the anion is bound in a catalytic domain, the substrate will not have access to this site. It is also possible, however, to account for the observed kinetics by a somewhat more extensive mechanism, Eq. (7), which allows for binding of both substrate and added anion in a common catalytic domain:



(7)

Here K_1, K_2, K_3, and K_4 represent the dissociation constants for the species shown in the respective preequilibrium steps. Again a rate expression for this mechanism, under conditions of $[A_0] \gg [C_0] \gg [S_0]$, is formally analogous to that of Eq. (3), if one assumes that $k_1 = k_2$. The kinetic parameters are now defined by Eqs. (8) and (9):

$$k_{\text{cat}} = k_1, \tag{8}$$

$$K_m = K_1 + \frac{(K_3 - K_1)\, A_0}{K_4 + A_0}. \tag{9}$$

Once again we see that K_m depends on equilibrium constants involving A, and hence K_m will reflect the nature of the added anion in the solution.

In principle the mechanisms of Eqs. (4) and (7) could be distinguished from each other by measurements of the variation of K_m with concentration A_0 [compare Eqs. (6) and (9)]. In practice, however, this is not feasible, because the ionic strength would also vary with concentration of A_0. If an additional electrolyte is added to maintain ionic strength, the system becomes even more complicated and any interpretation ambiguous.

With either mechanistic interpretation it is apparent that the rates of the catalytic decarboxylation of the anionic nitrobenzisoxazole carboxylate by the cationic poly-(ethylenimine) can be markedly affected by binding of added small anions, particularly highly polarizable ones.

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