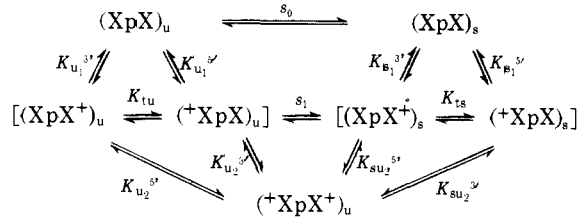
Scheme III



$$K_{s_1}{}^{3'} = \frac{a_H[(XpX)_s]}{[(XpX^+)_s]} \qquad K_{s_1}{}^{5'} = \frac{a_H[(XpX)_s]}{[(^+XpX)_s]}$$

$$K_{su_2}{}^{3'} = \frac{a_H[(^+XpX)_s]}{[(^+XpX^+)_u]} \qquad K_{su_2}{}^{5'} = \frac{a_H[(XpX^+)_s]}{[(^+XpX^+)_u]}$$

and $K_{su_1}{}^{3'} = K_{u_1}{}^{5'} = K_{u_2}{}^{3'} = K_{u_2}{}^{5'}$.

References and Notes

(1) Abbreviations: nucleosides are specified by the usual symbols, A (adenosine), C (cytidine), G (guanosine), or U (uridine). The protonation to the 3′- and 5′-linked nucleoside base in a homodinucleoside monophosphate, XpX, is denoted by $^+$XpX and XpX$^+$, espectively, and to both nucleoside bases by $^+$XpX$^+$; poly A = polyriboadenylic acid; poly C = polyribocytidylic acid; CD = circular dichroism; ORD = optical rotatory dispersion.
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(33) As a referee has pointed out, the reason why GpG was not done before is probably the aggregative nature of GpG. We also believe that earlier difficulties were due to the presence of aggregates in the prepared solution. We found that the preheating at 60–70 °C for 30 min was effective to dissociate the aggregates,[28] and no significant amount of aggregates was re-formed in the resulting solutions during the period of the pK measurements at 25 °C and the thermal denaturation experiments. Without this treatment, our titration and melting data cannot be obtained.
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Catalysis of Decarboxylation of Nitrobenzisoxazolecarboxylic Acid and of Cyanophenylacetic Acid by Modified Polyethylenimines

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Abstract: Modified polyethylenimines have been prepared containing apolar lauryl groups and fully quaternized amine nitrogens. These derivatives catalyze markedly the decarboxylation of nitrobenzisoxazolecarboxylate and of cyanophenylacetate. The kinetics of decarboxylation fit equations analogous to those of enzymic kinetics. The preequilibrium binding constant is in the range of 10^4–10^5 M^{-1} and the catalytic constant, k_2, is 10^{-2}–10^{-3} s^{-1}. In terms of the turnover number, k_2, the reaction at a catalytic site on the polymer is about 1300 times faster than the spontaneous reaction in water.

It has been shown by Kemp and Paul[1] that the decarboxylation of benzisoxazolecarboxylicacids is very markedly accelerated in an aprotic solvent, as contrasted to water. An apolar, non-hydrogen-bonding environment evidently stabilizes the charge-delocalized transition state of the benzisoxazole carboxylate anion and thereby accelerates the rate of decarboxylation. Further application of this concept to aqueous micelles by Bunton and Minch[2] has revealed substantial ac-

celerations of decarboxylation by cationic detergents. Smid et al.[3] have also found that anion-binding poly(vinyl crown ethers) manifest catalytic properties in the same reaction.

We have described previously the very strong binding affinity of modified polyethylenimines[4] for anions and the marked catalytic effects of these polymers in the hydrolysis of activated esters.[5] Since these polymers can also be made to provide tetraalkylnitrogen cations in the macromolecular matrix,[6] as well as apolar domains, it seemed that they should be effective catalysts of decarboxylation of benzisoxazolecarboxylates dissolved in a fully aqueous solvent. We have examined, therefore, the kinetics of catalysis in these systems and analyzed them in mechanistic terms analogous to those used in enzymic catalyses.

Experimental Section

Preparation of Modified Polymers. Quaternized polyethylenimines were prepared by the following procedure. Ten (monomer) millimoles of polyethylenimine 600 (Dow Chemical Co.), mol wt 60 000, in 10 ml of ethanolic stock solution[7] was diluted with 5 ml of hexamethylphosphoramide and 5 ml of dried benzene. The benzene and ethanol were removed in a rotary evaporator at 40 °C. Benzene was added twice more and removed in a rotatory evaporator to extract traces of water azeotropically. The polymer in hexamethylphosphofamide was then transferred to a 25-ml, three-necked flask equipped with a stirrer, gas inlet, and condenser. Ten millimoles of pentamethylpiperidine[8] and 2.5 mmol of dodecyl bromide were added, and the reaction mixture was stirred in an inert atmosphere (preferably Ar) at 45 °C. Progress of the reaction was followed by removal of a small aliquot from the mixture and analysis by gas–liquid chromatography. Alkylation was complete in 2–4 days. For quaternization the reaction mixture was chilled in an ice bath and 17.5 mmol of CH_3I in 2.5 ml of hexamethylphosphoramide was added dropwise over a 5-min period. The mixture was then stirred at room temperature for 48 h.

In alternative preparations, lauryl iodide was also used in place of lauryl bromide. Similarly for quaternization, $(CH_3)_2SO_4$ can be used instead of CH_3I.

For purification of the modified polymer, 5 ml of acetic acid was added to the laurylated quaternized polyethylenimine in the reaction vessel and the entire contents poured into 350 ml of hot 20% aqueous ethanol. This solution was ultrafiltered in an Amicon Diaflo ultrafiltration apparatus, using a PM-30 membrane, first with 5 l. of 20% aqueous ethanol containing 0.1 M NaCl, then with 6 l. of aqueous 0.1 M NaCl, and finally with 6 l. of distilled water. The final aqueous solution, after concentration, was lyophilized.

With the mole ratios used in the preparation described, one obtains a polyethylenimine with 25% of the monomer residues laurylated and all the nitrogens quaternized: $[(C_2H_4H)_m(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}]Cl$ (m = 1400). The molecular weight of the modified polymer is 160 000 without the compensatory anions, 210 000 with chlorides. With less lauryl halide one can obtain a modified polymer with fewer lauryl groups. Substitution of C_2H_5I for the methylating reagent leads to the quaternized ethyl polymer. With such variations in procedure the following derivatives were also prepared.

$[(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_5)_{1.75m}]Cl$, m = 1400
$[(C_2H_4N)_m(C_{12}H_{25})_{0.1m}(CH_3)_{1.9m}]Cl$, m = 1400
$[(C_2H_4N)_m(C_{12}H_{25})_{0.25m}]Cl$, m = 1400

The stoichiometric formulas were confirmed by proton magnetic resonance spectra and by microanalyses (within 10% error).

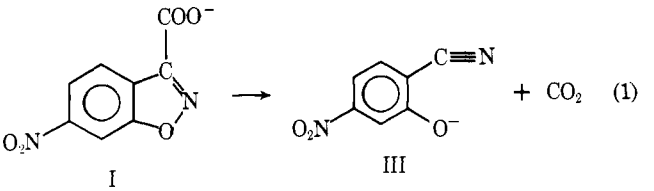
Preparation of Substrates. 6-Nitrobenzisoxazole-3-carboxylic acid (I) was prepared in the following steps. Methyl 6-nitrobenzisoxazole-3-carboxylate (Ia) was obtained from methyl 2,4-dinitrophenylacetate,[9] isoamyl nitrite, and sodium methoxide following the procedure of Borsche.[10] Hydrolysis of Ia in 80% sulfuric acid[11] yielded I: mp 186–188 °C dec (lit.[11] mp 189–190 °C). The sodium salt of I was obtained by neutralization with NaOH and the aqueous solution was kept in the frozen state. In this state the sodium salt was stable for at least 4 months, as judged by the absorbance of its dilute solutions in the region above 400 nm.

2-Cyano-2-phenylacetic acid (II) was prepared by methods in the literature:[12] mp 91–92 °C (lit.[13] mp 92 °C). This acid was also neutralized with NaOH and the aqueous solution of the sodium salt of II was maintained in the frozen state. It too was stable for at least 4 months, as judged by the ultraviolet spectrum of diluted stock solutions.

2-Cyano-5-nitrophenol (III) was obtained by alkaline hydrolysis of Ia: mp 160 °C (lit.[9] 160 °C).

All other materials were purchased from standard commercial sources. The hexamethylphosphoramide was dried over 13X molecular sieves (obtained from Fisher) for 24 h and then distilled over sodium (5 g/l.) at 67 °C (0.1 mm).

Measurement of Rates of Decarboxylation. The reaction studied with the benzisoxazole is



I III

Its rate can be followed by measurement of the increase in absorbance due to the release of 2-cyano-5-nitrophenol. Preliminary spectroscopic studies showed that the absorption peak of the cyanonitrophenol at pH 7.4 was shifted from 400 to 425 nm in the presence of quaternized polymer. The two spectra, in water and with added polymer, respectively, exhibited an isosbestic point at 408 nm, and, hence, the absorbance for kinetic assays was followed at this wavelength. With cyanophenylacetic acid (II) rates of decarboxylation were followed by absorbance measurements at 225 nm. A Tris–hydrochloric acid buffer, of 0.05 ionic strength, adjusted to pH 7.4 was used as the aqueous solvent. Rates were measured at 25 °C in a Cary Model 14 recording spectrophotometer. Reactions were initiated by addition of small aliquots of the substrate stock solution (warmed to 4 °C) to 3 ml of buffer solution containing the polymer. Organic solvents were rigorously excluded since they have such marked effects on the rates of decarboxylation.[1,13,15]

Results

Rates of decarboxylation were used to analyze the kinetics following the general format established by Michaelis and Menten.[14] If C represents one catalytic site on the polymer, and S the carboxylate substrate, then the following scheme may be formulated.

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SC \xrightarrow{k_2} \text{products} + C \qquad (2)$$

For certain sets of the experiments, the initial concentration of catalytic sites C_0 was kept in great excess over that of substrate, S_0. Under these conditions, $C_0 \gg S_0$, the decarboxylation follows pseudo-first-order kinetics, and a corresponding rate constant, k_{obsd}, may be extracted from the observed rates. Also under these conditions, the steady-state rate expression for the mechanism of eq 2 becomes

$$k_{obsd} = k_2 C_0/(K_M + C_0) \qquad (3)$$

where $K_M = (k_{-1} + k_2)/k_1$. If it is assumed that the n catalytic sites on one molecule of polymer, P, behave independently of each other, then

$$k_{obsd} = nk_2 P_0/(K_M + nP_0) \qquad (4)$$

where P_0 is the initial concentration of polymer. A linear transform of eq 4 is

$$\frac{1}{k_{obsd}} = \frac{K_M/n}{k_2}\frac{1}{P_0} + \frac{1}{k_2} \qquad (5)$$

Alternatively, experiments were also carried out under conditions of excess of substrate, that is $S_0 \gg C_0$. Under these circumstances initial velocities V_0 are measured and the steady-state expression for the scheme in eq 2 is

$$k_0 = k_2 C_0/(K_M + S_0) \qquad (6)$$

where k_0 is the initial rate constant, V_0/S_0. Again if the n

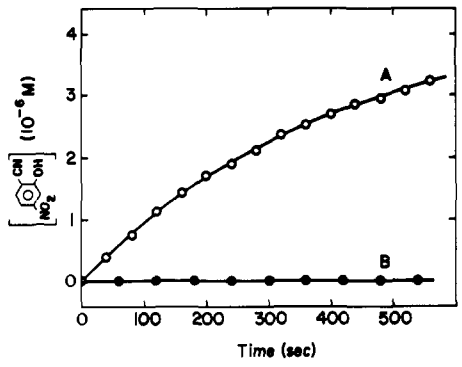


Figure 1. Rate of release of 5-nitro-2-cyanophenolate during decarboxylation of 6-nitrobenzisoxazole-3-carboxylate at 25 °C, pH 7.4, μ = 0.05. Initial concentration of substrate, $S_0 = 4.18 \times 10^{-6}$ M. A. In the presence of quaternized polyethylenimine $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_5)_{1.75m}$, $P_0 = 7.47 \times 10^{-6}$ M. Molecular weight of modified polymer is 240 000 with chloride ions. B. Spontaneous decarboxylation in the absence of polymer.

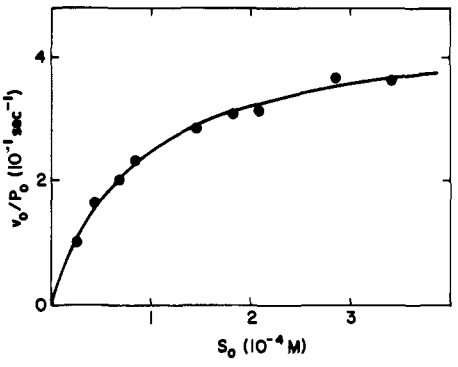


Figure 3. Variation of initial rate constant V_0/P_0 for decarboxylation of 6-nitrobenzisoxazole-3-carboxylate as a function of substrate concentration under conditions of excess substrate. Initial velocities were corrected for the spontaneous hydrolysis of substrate in the absence of polymer. Polymer is $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_5)_{1.75m}$. The line was drawn according to eq 6 with $nk_2 = 0.458$ s^{-1} and $K_M = 8.59 \times 10^{-5}$ M.

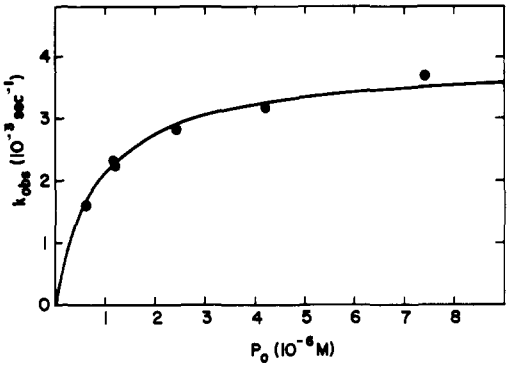


Figure 2. Variation of pseudo-first-order rate constant for decarboxylation of 6-nitrobenzisoxazole-3-carboxylate as a function of catalyst concentration under conditions of excess catalyst. Polymer is quaternized polyethylenimine $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_5)_{1.75m}$. The line was drawn according to eq 4 with $k_2 = 3.92 \times 10^{-3}$ s^{-1} and $K_M/n = 8.47 \times 10^{-7}$ M.

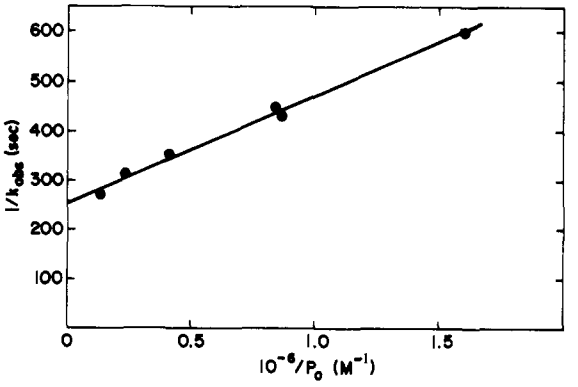


Figure 4. Linear transform, following eq 5, of data illustrated in Figure 2.

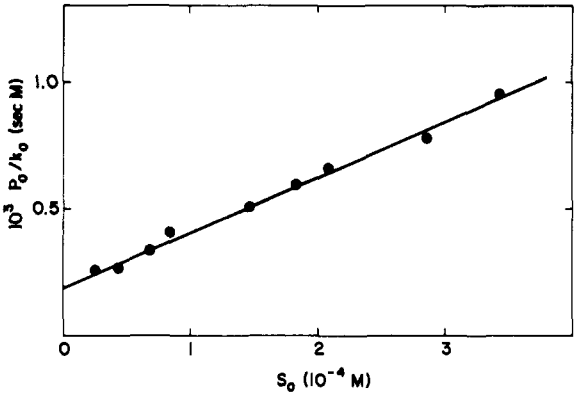


Figure 5. Linear transform, following eq 8, of data illustrated in Figure 3.

catalytic sites on a polymer molecule behave independently, then

$$k_0 = nk_2P_0/(K_M + S_0) \quad (7)$$

and

$$P_0/k_0 = K_M/nk_2 + (1/nk_2)S_0 \quad (8)$$

Fitting experimental data at $P_0 \gg S_0$ to eq 5, one can evaluate k_2 and K_M/n. A corresponding treatment of observations for $S_0 \gg P_0$ by means of eq 8 gives nk_2 and K_M. Combination of the information provides an explicit value for n, as well as of k_2 and K_M.

Figure 1 illustrates a typical decarboxylation experiment with nitrobenzisoxazolecarboxylate under conditions of $C_0 \gg S_0$ (curve A). With low concentrations of substrate, spontaneous decomposition (curve B) cannot be detected. An accumulation of experiments such as those in Figure 1, for a series of polymer concentrations, provides the data for Figure 2. This illustrates the saturation behavior required by the scheme of eq 2. Correpsondingly, a series of experiments with $S_0 \gg C_0$ is summarized in Figure 3. Again we see that a plateau is reached with increasing substrate concentration. Since S and C appear symmetrically in the rate steps of eq 2, each must exhibit saturation behavior, and each does in this decarboxylation reaction.

Since saturation was observed at relatively low polymer concentration, changes in ionic strength caused by the addition of polymer are negligible. For example, in Figure 2, saturation is evident at about 2.5×10^{-6} M polymer, corresponding to 3.5×10^{-3} M total cationic residues on the polymer, in a solution of 0.05 ionic strength.

Figure 4 illustrates the fit of the data for $C_0 \gg S_0$ to the linear eq 5. The parameters of the line shown are $k_2 = 3.92 \times 10^{-3}$ s^{-1} and $K_M/n = 0.847 \times 10^{-6}$ M. Correspondingly, the data for $S_0 \gg C_0$ have been fitted to eq 8 as is illustrated in Figure 5. From these results the following parameters have been obtained: $nk_2 = 0.458$ s^{-1}; $K_M = 0.859 \times 10^{-4}$ M. Table I summarizes the kinetic parameters derived from the decarboxylation rates of nitrobenzisoxazolecarboxylate catalyzed by each of four different laurylpolyethylenimines.

Table I. Kinetic Parameters for Catalyzed Decarboxylation of Nitrobenzisoxazolecarboxylate by Modified Polyethylenimines[a]

Polymer derivative	nk_2/K_M, 10^3 s^{-1} M^{-1}	k_2, 10^{-3} s^{-1}	nk_2, 10^{-2} s^{-1}	K_M, 10^{-5} M	K_M/n, 10^{-7} M	n[b]	Av no.[b] of ethylenimine units per catalytic site	Av no.[b] of lauryl residues per catalytic site
A. $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}$								
$C_0 \gg S_0$	1.44	1.30			9.0	56, 72	25, 19	6.2, 4.9
$C_0 \ll S_0$	1.12		7.3	6.5				
B. $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_5)_{1.75m}$								
$C_0 \gg S_0$	4.63	3.92			8.5	117, 107	12, 13	3.0, 3.2
$C_0 \ll S_0$	5.33		45.8	8.6				
C. $(C_2H_4N)_m(C_{12}H_{25})_{0.1m}(CH_3)_{1.9m}$								
$C_0 \gg S_0$	0.11	0.27			25			
D. $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}$								
$C_0 \gg S_0$	0.50	0.65			13	86, 78	16, 18	4.1, 4.5
$C_0 \ll S_0$	0.55		5.6	10.2				
E. None		0.003[c]						

[a] Reactions followed at 25 °C, pH 7.4, ionic strength 0.05. [b] The first entry has been calculated from nk_2 and k_2, the second from K_M/n and K_M. [c] Spontaneous first-order rate constant in absence of polymer; the value listed is from ref 1 and our less precise measurements gave similar results.

To follow the decarboxylation of cyanophenylacetic acid spectrophotometrically, one must measure absorbance changes near 225 nm. In this region the polymer scatters light strongly and interferes with absorbance measurements. Consequently it was not feasible to follow the kinetics of decarboxylation at high polymer concentrations, i.e., at $C_0 \gg S_0$. On the other hand, rate measurements could be made at $S_0 \gg C_0$. Under these conditions only nk_2 and K_M can be evaluated, however. The kinetic parameters for decarboxylation of cyanophenylacetic acid are listed in Table II.

Although it is not feasible to evaluate k_2 explicitly, it is possible to estimate a lower limit for this constant from measurement of rates for solutions with $C_0 \simeq S_0$. Since k_2 is the first-order rate constant for fully bound substrate in the polymer environment, the experimental initial rate, V_0/S_0, under other conditions where substrate is not fully bound must always be less than k_2. In experiments with S_0 varying from $(2.7\text{–}3.8) \times 10^{-4}$ M and P_0 in the range $(1.4\text{–}3.1) \times 10^{-6}$ M, the largest value of V_0/S_0 observed was 5.95×10^{-4} s^{-1}. We conclude, therefore, that $k_2 > 6 \times 10^{-4}$ s^{-1} (Table II). This is almost 600-fold greater than the pseudo-first-order constant for spontaneous decarboxylation in water.

Table II. Kinetic Parameters[a] for Decarboxylation of 2-Cyano-2-phenylacetic Acid Catalyzed by Laurylethylpolyethylenimine[b]

nk_2	0.183 s^{-1}
K_M	5.17×10^{-4} M
k_2 (estd)[c]	$> 6 \times 10^{-4}$ s^{-1}
k_{spont}[d]	1.1×10^{-6} s^{-1}

[a] 25 °C, pH 7.4, $\mu = 0.05$. [b] $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_5)_{1.75m}$. [c] $S_0 = 3.13 \times 10^{-4}$ M; $P_0 = 3.06 \times 10^{-6}$ M. [d] Spontaneous first-order rate constant in the absence of polymer.

Discussion

The kinetics of decarboxylation in the presence of polymer show concentration dependencies analogous to those found in the natural macromolecular catalysts—the enzymes. In such systems saturation of catalyst is reached at high concentrations of substrate. Such behavior is observed for the polyethylenimines, as is illustrated in Figure 3. Furthermore, since catalyst, C, and substrate, S, appear symmetrically in the kinetic reactants of eq 2, saturation behavior should also be observed at high concentrations of catalyst. Such indeed is seen for the polyethylenimines, as is illustrated in Figure 2. In enzyme model systems studied previously, such unequivocal saturation behavior was not observed. This was due largely to the low binding ability of the catalyst. For example, in micellar systems, simulation of plateau phenomena caused by salt effects at high micelle concentrations was not easily differentiated from true saturation effects. In other polymeric systems, the value of K_M was frequently so large compared to the solubility of the polymers or compared to practically attainable substrate concentrations that saturation phenomena could not be demonstrated. In cyclodextrin-catalyzed reactions,[12] saturation behavior was observed for the condition $C_0 \gg S_0$, that is, in the presence of large excess of catalyst. However, saturation behavior in the presence of excess substrate was not observed. Under conditions of $S_0 \gg C_0$, it is essential that $S_0 \geq K_M$ if saturation is to be attained; but under such conditions the rate of the catalyzed reaction was not sufficiently greater than the spontaneous rate because the concentration of catalyst, C_0, had to be kept low. In contrast, with our polymers with their strong binding affinity for substrates, and with a large difference between k_2 and k_{spont}, saturation phenomena were readily manifested at practically attainable concentrations of both polymer and substrate.

Comparing catalytic effects of different modified polyethylenimines on the decarboxylation of nitrobenzisoxazolecarboxylate, we can discern several interesting features. By comparison of the 25% laurylated polymer in the quaternized and nonquaternized forms, A and D, respectively, in Table I, we see that the former is about three times more effective as a catalyst than the latter. For the quaternized polymer, the first-order catalytic constant k_2 and the second-order rate constant nk_2/K_M are greater and the binding of substrate (measured by K_M^{-1}) is stronger. Stronger binding of substrate probably reflects the greater charge on the quaternized polymer at pH 7.4, since, in contrast to the nonquaternized polyethylenimine, it does not dissociate H^+ ions with increasing pH. However, stronger binding alone is not responsible for the increased catalytic effectiveness of the quaternized polymer, for k_2 in its environment is also twofold greater. Evidently the cationic environment it provides is more favorable for the anionic transition state[1] in the mechanistic pathway of the decarboxylation reaction.

The ethyl-quaternized polymer is more effective as a catalyst than the comparable methyl-quaternized polymer (A and B, respectively, in Table I). This is due in large part to the threefold increase in k_2, which can be interpreted as a reflection of the contribution of the more apolar environment provided by ethyl as contrasted to methyl groups.

The very strong role played by the apolar environment is also evident in a comparison of 25 and 10% laurylated polyethylenimines (A and C, respectively, in Table I). The catalytic constant k_2, reflecting the effect of polymer environment on the transition state, drops by a factor of 5 in the less apolar environment.

All of the polymers show marked catalytic effects on the decarboxylation in the water solvent. In the polymer environment the intrinsic first-order rate constant, k_2, can be 10^3-fold greater than the pseudo-first-order rate constant in the aqueous solvent alone (B and E, respectively, in Table I). Furthermore, the polymer is an effective catalyst at concentrations of the order of 10^{-6} M, corresponding to catalytic site concentrations of about 10^{-4} M. This means that the decarboxylation rate can be enhanced more than 1000 times by addition of 5×10^{-6} M polymer to the aqueous solution. In contrast, for micellar systems, the maximum enhancement reported is at best 100-fold.[2]

Comparison of the catalytic effectiveness of the polyethylenimines on the two different substrates, nitrobenzisoxazolecarboxylate and cyanophenylacetic acid, is hampered by our inability to obtain data at high concentration of polymer, i.e., $C_0 \gg S_0$. Nevertheless, it is clear from the few experiments at $C_0 \sim S_0$ that k_2 for the cyanophenylacetic acid must be above 6×10^{-4} s^{-1}. The corresponding constant for nitrobenzisoxazolecarboxylate is 39.2×10^{-4} s^{-1}, at most, therefore, a factor of perhaps 6 greater. Since the spontaneous rate for cyanophenylacetate is some threefold slower than that for the benzisoxazole, we can see that the effect of the polymer environment on the stabilization of the transition state is approximately the same for each substrate. On the other hand, the binding affinity of the polymer for cyanophenylacetate, as measured by K_M^{-1}, is about tenfold weaker. This weaker affinity for the smaller substrate is not surprising in view of similar observations with other aromatic molecules and polyethylenimines.[4,16]

Thus it is evident that the modified polyethylenimines provide a matrix for achieving homogeneous catalysis of decarboxylation of anionic substrates in an aqueous environment. For nitrobenzisoxazolecarboxylate, as has been demonstrated by Kemp and Paul,[1] the transition state is a charge-delocalized anionic structure, stabilized in aprotic solvents by dispersion interactions. Clearly, the modified polyethylenimines also provide solvation features that stabilize the anionic transition structure in the state with particularly sensitive bonds.

The modified polyethylenimines described herein are only a few of many possibilities. The matrix of this polymer provides a framework for attachment of a variety of different types of apolar or polar (protic and aprotic) groups. Thus a wide range of local environments can be created on this macromolecular water-soluble catalyst. Large solvent effects have been observed in kinetic studies of many reactions involving anions.[1,17] Suitable derivatives of polyethylenimine should manifest interesting effects in many of these reactions.

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Substituent Effects on the Solution Conformation of Rifamycin S

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Abstract: The synthesis of several new rifamycin derivatives is presented. Correlations of their NMR spectra indicate that C-3 substitution effects the overall conformation of the ansa bridge. These conformational changes probably effect the enzyme inhibitory activity of these derivatives.

We have previously shown that the in vitro effectiveness of 3-substituted derivatives of rifamycin S as inhibitors of RNA synthesis by *Escherichia coli* DNA dependent RNA polymerase correlates with the electronegativity of the substituents, in particular with Hammett's σ_p.[1] These data, coupled with inhibition reversibility for the weaker inhibitors, were taken to implicate charge-transfer complexes (or some hydrophobic equivalent) in the inhibition interactions between the rifamycins and RNA polymerase. Since this conclusion is based on the assumption that the various rifamycin derivatives (Figure 1) possess similar conformations of the ansa bridge relative to the aromatic ring, we felt that this should be tested by carrying out a detailed high-field proton NMR examination of them. The results of this work are reported below, our main conclusion being that although the ansa bridge is relatively rigid, it appears to rock as a unit about pivot points at each end