Catalysis of Decarboxylation of Oxalacetic Acid by Modified Poly(ethylenimines)

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Abstract: A modified poly(ethylenimine) has been prepared containing free primary amines on 10% of its residues, apolar lauryl groups on 20%, and sufficient methyl groups to quaternize the nitrogens except for some of the primary amines. This polymer catalyzes markedly the rate of decarboxylation of oxalacetate. The rates of decarboxylation fit equations for saturation kinetics similar to those for enzymes. Preequilibrium binding constants are in the range of 10^3 M^{-1}, and the catalytic constant, k_2, is 2.1 min^{-1} at the pH of maximum activity. Compared with a small-molecule amine, the second-order rate constant with the polymer is about 10^5 times greater. The turnover number at a polymer catalytic site corresponds to a rate 10^2–10^3 times faster than the spontaneous rate in water. Spectroscopic observations (ultraviolet, infrared, proton magnetic resonance) and chemical studies are consistent with a mechanistic pathway involving Schiff base formation.

Amine catalysis of decarboxylation of β-keto acids has been carefully studied for a long time.[1-7] With oxalacetate as reactant, investigators are in agreement that primary amines are the most effective in such catalysis, secondary amines are much less so, and tertiary amines are ineffective.[4,6] Mechanistic evidence[1,3,5,7,8] points to the formation of a Schiff base intermediate in the decarboxylation pathway, although in aqueous solution the hydrated derivative, a carbinolamine, may be the dominant structure. In any event, it seems clear that the keto carbonyl carbon is subject to nucleophilic attack by the amine catalyst and that the intermediate formed subsequently loses its carboxyl group, releases CO_2, and leaves pyruvic acid. The overall reaction, for oxalacetate as monoanion, is

$$HO_2CC(=O)CH_2CO_2^- + RNH_2 + H^+ \longrightarrow HO_2CC(=O)CH_3 + CO_2 + RNH_2 \quad (1)$$

We have previously described high-molecular-weight poly(ethylenimines) containing intrinsic or added nucleophilic groups. These polymers markedly enhance rates of reactions sensitive to nucleophiles, particularly hydrolytic reactions.[9-12] Others have demonstrated polyamines to be effective catalysts in deuterium exchange reactions involving Schiff bases.[13,14] We have now observed that very substantial catalytic effects are also produced in the decarboxylation of oxalacetate by a suitably modified poly(ethylenimine) containing primary amines. Furthermore, strong evidence has been obtained that the reaction proceeds through a Schiff base intermediate, and that during the reaction there is no accumulation of a long-lived species formed prior to the product, pyruvate. Thus the versatility of the poly(ethylenimines) as macromolecular catalysts has been significantly expanded.

Experimental Section

Oxalacetic acid was purchased from Sigma Chemical Co. and recrystallized from acetone/benzene (1:10). Oxalacetate-4-ethyl ester was purchased from ICN and recrystallized from ether/benzene (1:10). Lactate dehydrogenase and NADH were obtained from Sigma Chemical Co. and were used as received.

Quaternized laurylated poly(ethylenimines) were synthesized by procedures reported recently.[12,15] The fully quaternized polymer, PEIQ, had a composition corresponding to $[(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}]Cl$, where m is about 1400. The partially quaternized polymer, in which 10% of the residues are retained as primary amines, PEIQ-NH_2, had the composition $[(C_2H_4N)_{0.90m}(C_2H_4NH_2)_{0.10m}(C_{12}H_{25})_{0.20m}(CH_3)_m]Cl$.

The rates of decarboxylation were followed spectrophotometrically with a Cary 14 spectrophotometer. The sample compartments were thermostated at 25 °C by a circulating water bath. All kinetic data were obtained in fully aqueous solutions. Acetate, cacodylate, or [bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane] (Bis-Tris) buffers were used to maintain the desired pH. All reactions were carried out at approximately 0.05 μ ionic strength. An aliquot from a freshly prepared stock solution of oxalacetate was added to the buffered polymer solution to initiate the decarboxylation reaction. The rate of spontaneous decarboxylation was measured in the presence of equal residue molar concentrations of fully quaternized poly(ethylenimine). This rate never exceeded 5% of the catalyzed rate, and in many cases it was negligible.

Proton magnetic resonance measurements were made with a Hitachi Perkin-Elmer R-20B spectrometer operating at 60 MHz and a probe temperature maintained at 35 °C. Infrared absorptions were obtained between 1500 and 1900 cm^{-1} in D_2O solutions in a Beckman IR-12 spectrophotometer. Disposable AgCl cells with 0.05 mm path length were used for infrared spectra.

Results

Rates of Decarboxylation from Ultraviolet Absorbance. The ultraviolet absorption spectrum of aqueous solutions of oxalacetate has been described by Gelles and Hay.[16] Although the ketonic form absorbs weakly, the enolic tautomer exhibits a substantial maximum at 260 nm. This absorption has been used as a convenient probe in the decarboxylation reaction of oxalacetate.[17] We have examined the spectrum of oxalacetate in the presence of quaternized modifications of poly(ethylenimine), PEIQ and PEIQ-NH_2. Although these polymers do not shift the 260-nm peak, there is a rapid drop in absorption intensity with time in the presence of the (primary amine)-containing polymer (Figure 1), and no new band is observed between 220 and 400 nm. Clearly the oxalacetate is decaying.

A typical kinetic run is illustrated in Figure 2. Two processes are evident in the recording of absorbance (at 260 nm) with time. The first, manifested by an initial increase in absorbance, probably reflects the binding of the substrate to the polymer matrices. Such binding could be reasonably expected to shift the oxalacetate tautomeric equilibrium toward the enol form since the polymer is highly cationic and would bind the anionic form of the substrate preferentially.

It is evident from Figure 2 that the tautomeric shift upon binding to the polymer is much faster than the subsequent decarboxylation. Since the time scales for these successive processes are sufficiently different, the second stage can be analyzed independently of the first. The decreasing absorbance in the second stage follows very well a first-order rate law.

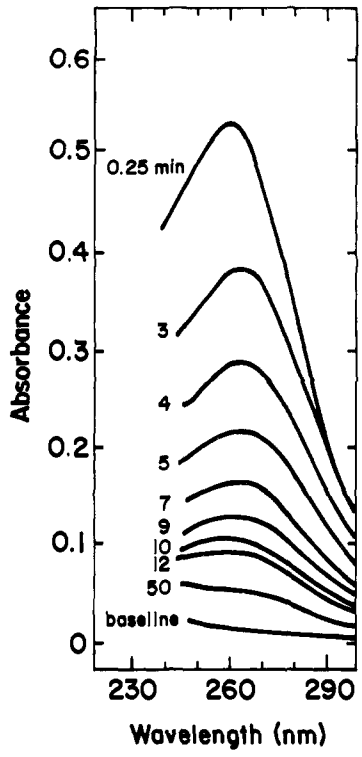


Figure 1. Ultraviolet spectrum of oxalacetate during decarboxylation by 6×10^{-2} residue molar PEIQ-NH_2. Initial concentration of oxalacetate 5×10^{-4} M.

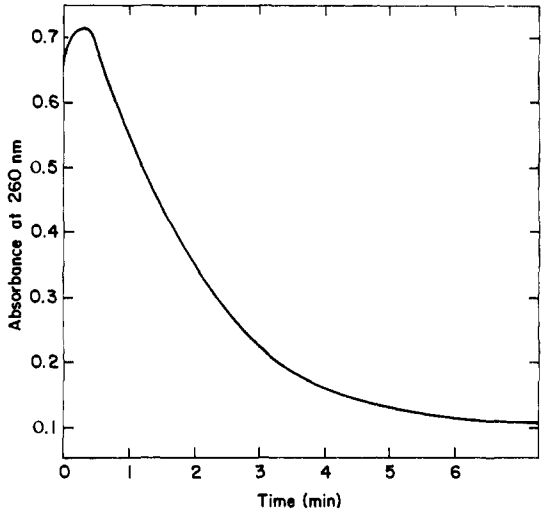


Figure 2. Rate of disappearance of oxalacetate during decarboxylation by PEIQ-NH_2 at 25 °C, pH 7.0, μ = 0.05. Initial concentration of oxalacetate was 5.0×10^{-4} M.

Thus, the decarboxylation of oxalacetate in the presence of the PEIQ-NH_2 catalyst acts as a first-order process.

Probe of Tautomeric Shift Using Infrared Spectra. We have tested the interpretation that the polymer shifts the tautomeric equilibrium by examining the infrared spectrum, between 1500 and 1900 cm^{-1}, of oxalacetate-4-ethyl ester (Figure 3). The monoethyl ester shows two vibrational bands, at 1610 and 1720 cm^{-1}, in the presence or absence of fully quaternized PEIQ. The lower energy band can readily be assigned to the carbonyl stretching mode of the anionic carboxylate group. The higher energy band is probably a combination of the ester carbonyl stretching frequency and the keto carbonyl vibration. A significant change in the observed intensity ratios for these bands is observed when the PEIQ is added to the solution (Figure 3). Such behavior is consistent with a shift in the tautomeric

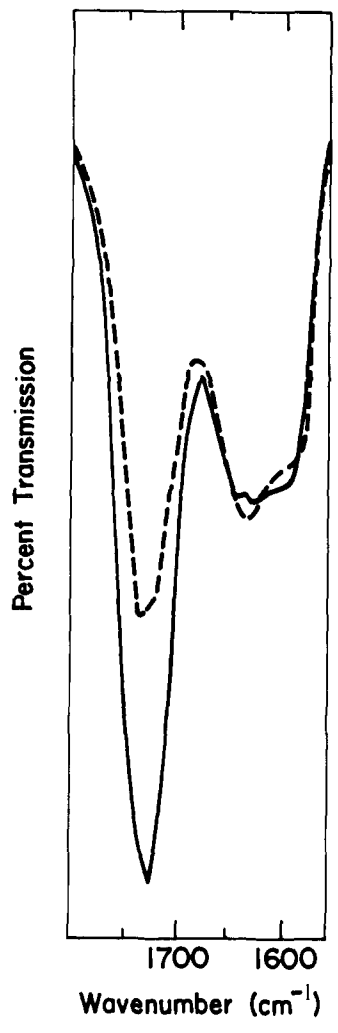


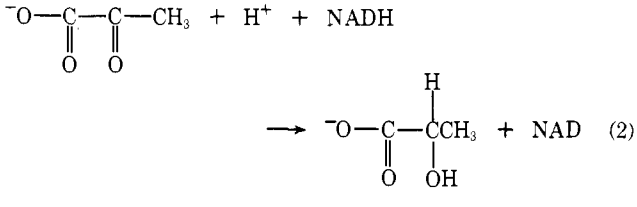
Figure 3. Infrared spectrum in D_2O of oxalacetate-4-ethyl ester with (- - -) and without (——) PEIQ.

equilibrium toward the enol isomer, for this would result in a decreased contribution to absorption at 1720 cm^{-1} due to the decrease in concentration of the keto group.

Analysis of Decarboxylation Product by Thin-Layer Chromatography. It seemed prudent to verify that the product of the decarboxylation in the presence of polymer is indeed pyruvic acid (eq 1). This has been achieved by thin-layer chromatography and by enzymatic assay.

Table I compares the migration of the product of the polymer-catalyzed decarboxylation, in an unbuffered solution (pH 6-7), with that of a prepared mixture of pyruvic acid and PEIQ-NH_2. Comparable values for reaction product and control pyruvic acid in all four of these thin-layer chromatographic systems provides strong evidence that the product is actually pyruvic acid. In addition, it was observed that the spot corresponding to the decarboxylation product was sensitive to 2,4-dinitrophenylhydrazine and gave a yellow derivative just as did the pyruvate standard.

Enzymic Assays. The polymer-catalyzed reaction was also assayed for pyruvate by enzymatic means using lactate dehydrogenase in the presence of reduced nicotinamide adenine dinucleotide (NADH).[18,19] The overall reaction can be fol-



(2)

Table I. Comparison of Migration of Decarboxylation Product and of Reactant

Support	Eluent	R_f Product	R_f Pyruvic acid and PEIQ-NH_2	R_f Pyruvic acid and PEIQ	R_f Oxalacetic acid and PEIQ
Silica gel	C_2H_5OH/NH_4OH (4:1)	0.73	0.73	0.70	0.59
Silica gel	Pyridine/heptane (1:2)	~0.02	~0.02	~0.00	~0.00
Cellulose	C_2H_5OH/NH_4OH (4:1)	0.60	0.60	0.56	0.19
Cellulose	2-Propanol/5 M formic acid (2:3)	0.80	0.80	0.82	0.69

Table II. Kinetic Parameters for Catalyzed Decarboxylation of Oxalacetate by Modified Poly(ethylenimine)

Catalyst	k_2, min^{-1}	K_M, M	n	k_2/K_M, M^{-1} min^{-1}	pH
PEIQ-NH_2; $[(C_2H_4N)_{0.90m}(C_2H_4NH_2)_{0.10m}(C_{12}H_{25})_{0.20m}(CH_3)_m]Cl$	2.1	3.5×10^{-4}	90	6.0×10^3	4.5
	0.8	2.3×10^{-3}	150	3.4×10^2	7.0
$C_2H_5NH_2$[a]				4.5×10^{-2}	4.5
				4.8×10^{-2}	7.0
PEIQ:[b] $[(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}]Cl$	3.6×10^{-2}				5.0
	7.9×10^{-3}				7.0
None[c]	4.3×10^{-3}				4.5
	1.0×10^{-3}				7.0

[a] The second-order rate constants were calculated from the data of Pedersen.[6] [b] Spontaneous decarboxylation in the presence of 6.7×10^{-2} residue molar polymer. [c] Spontaneous decarboxylation reported in ref 24.

lowed spectrophotometrically by the decreasing absorbance at 340 nm, the absorption peak for NADH. The presence of pyruvate was clearly established.

Furthermore, the enzymatic assay was used to examine the kinetics of decarboxylation. When the release of pyruvate (eq 1) was followed by the decrease in absorption by NADH at 340 nm, due to the reaction of eq 2, a pseudo-first-order process was observed. The rate constant calculated, 0.36 min^{-1}, was comparable with that observed for decarboxylation by means of ultraviolet absorption at 260 nm, 0.33 min^{-1}.

Catalytic Turnover by Polymer. We have demonstrated that turnover does take place in our catalytic polymer system. A 60-fold excess of substrate over the polymer primary amine concentration was completely decarboxylated. Furthermore, the decrease in oxalacetate absorbance at 260 nm with time was first order for at least 3 half-lives. This indicates not only that the catalyst is regenerated, but also that this release of catalyst is relatively rapid. Evidently there is no long-lived intermediate formed by the polymer amine catalyst.

Additional demonstration of turnover was provided by repeated injections of the substrate into the buffered amine-containing polymer solution. When equimolar amounts of primary amine and substrate were mixed, a pseudo-first-order kinetic decay was observed. At the completion of this reaction, another equimolar amount of substrate was added, and the absorption decay was again first order. The rate constants associated with these reactions were nearly identical in both cases (0.279 and 0.277 min^{-1}, respectively, under the conditions used). Further injections gave similar results.

Kinetic Analysis of Decarboxylation Pathway. Since the polymer acts catalytically, the reaction may be described schematically by the equation

$$C + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CS \xrightarrow{k_2} C + P \qquad (3)$$

where C represents a catalytic site on the polymer and S is the oxalacetate substrate. The first step in eq 3 represents the binding of substrate by polymer, the second the catalytic decarboxylation. A detailed kinetic analysis, based on eq 3 and appropriate steady-state approximations for poly(ethylenimine) catalysis, has been described earlier.[15] When the initial concentration of nucleophile (C_0) is much greater than that of the substrate (S_0), then

$$k_{obsd} = \frac{nk_2P_0}{K_M + nP_0} \qquad (4)$$

where k_{obsd} is the observed first-order rate constant for decarboxylation, P_0 is the molar concentration of polymer, containing n catalytic sites per macromolecule, and $K_M = (k_{-1} + k_2)/k_1$.

If the substrate S_0 is in much larger concentration than C_0, then

$$k_0 = \frac{nk_2P_0}{K_M + S_0} \qquad (5)$$

where k_0 is the initial rate constant, V_0/S_0, i.e., initial velocity, V_0, divided by initial concentration of substrate S_0. When the data were analyzed according to linear transforms of eq 4 and 5, good linear fits were obtained. Evaluation of the slopes and intercepts provides the parameters n, k_2, and K_M. These parameters for reactions at pH 4.5 and 7.0 are shown in Table II.

pH Dependence of Catalytic Constant. In this particular system, k_2 values were easily measured in solutions with large excesses (ca. 150:1) of catalytic sites over substrate concentrations. Under these conditions, the observed first-order rate constant was unaffected by increasing polymer concentration. The variation of k_2 over the pH range 3-7 is displayed in Figure 4. It is bell shaped and exhibits a maximum at pH 4.5. This bell-shaped pH-rate profile is similar to that of other model primary amines[6,7,20] as well as the enzyme acetoacetate decarboxylase.[21]

Schiff Base Formation. The similarity in pH-rate profile for the polymer and for simple primary amines or acetoacetate decarboxylase suggests that the mechanistic pathways of the decarboxylation reaction may also be alike. In the model amine

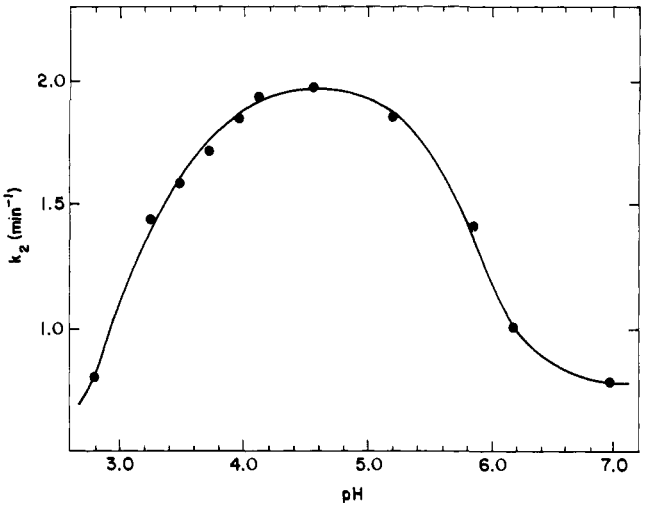
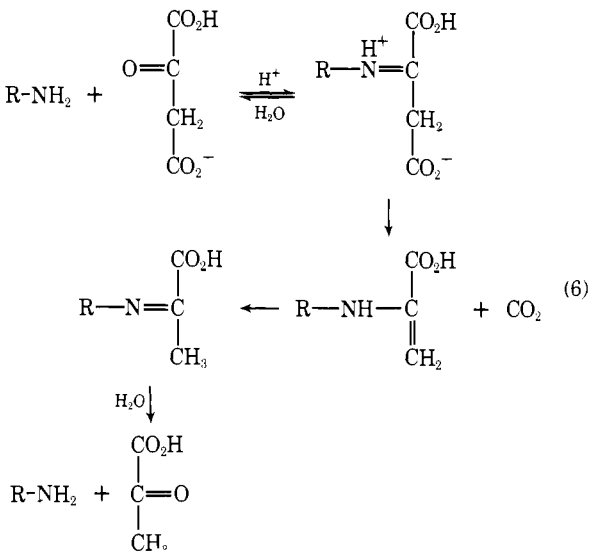


Figure 4. Rate constant–pH profile for PEIQ-NH_2-catalyzed decarboxylation of oxalacetate.

and enzyme systems there is good evidence for the pathway shown in the following equation:



(6)

It would be of interest to see if one could obtain any indication of Schiff base formation with the polymer. Since spectroscopic probes would be obscured with the actual substrate, oxalacetate, because of the progress of the decarboxylation reaction (eq 1), we have examined instead the spectra of oxalacetate-4-ethyl ester in solutions of the same modified poly(ethylenimine) PEIQ-NH_2. As Figure 5 illustrates, such solutions develop a new absorption band at 290 nm. Furthermore, this band is essentially abolished if $NaBH_4$ is added to the solution (Figure 6). As is well known, $NaBH_4$ reduces Schiff base linkages to amine groups.[22,23]

It has also been possible to confirm the presence of the reduction product of a Schiff base on the polymer by proton magnetic resonance. For this purpose we have used unmodified poly(ethylenimine) since it also catalyzes the decarboxylation of oxalacetate to its product, pyruvate. Unmodified poly(ethylenimine) (0.2 g) was mixed with oxalacetate-4-ethyl ester (0.2 g) in 100 ml of water. One-half of this solution was treated with $NaBH_4$ (0.5 g) over a 2-h period, with the pH being maintained near 7 by addition of HCl. The second-half was exposed to a similar environment but no $NaBH_4$ was added. After the 2-h period the solutions were adjusted to pH 3.5 with HCl, concentrated on a rotary evaporator, and applied to an A-25 DEAE Sephadex column. Fractionation was

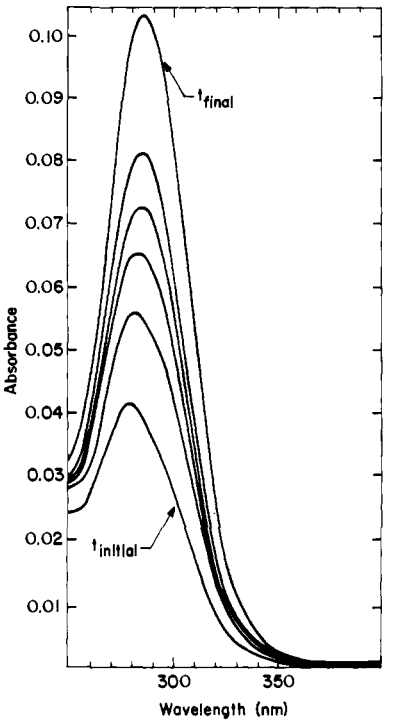


Figure 5. Ultraviolet spectrum during the reaction between 1.5×10^{-5} M oxalacetate-4-ethyl ester and 6×10^{-3} residue molar PEIQ-NH_2.

achieved by elution with water. Polymer fractions after lyophilization were dissolved in D_2O, and the ^1H NMR spectrum was taken. The borohydride-treated polymer exhibited a strong triplet centered at 3.4 ppm upfield from the HOD resonance. This new feature would be expected from the terminal methyl protons of the oxalacetate ester attached to the polymer. Only a very weak triplet was found in the control sample not treated with borohydride. These observations are strong evidence for the formation of Schiff bases with the polymer primary amine groups and for their reduction by borohydride to secondary amines.

The infrared spectrum of this $NaBH_4$-reduced polymer in D_2O shows only a weak shoulder near 1720 cm^{-1}, the position of the carbonyl stretch, but retains a strong absorption band near 1610 cm^{-1}, the frequency for the ionized carboxylate group.

Oxalacetate-4-ethyl ester also acts as an inhibitor of the catalyzed decarboxylation of oxalacetate (Table III). This is an indication that both molecules occupy some common sites on the polymer.

Discussion

As is evident from Table II, the decarboxylation of oxalacetate is markedly faster in the presence of amine-containing modified poly(ethylenimine) (PEIQ-NH_2). The first-order rate constant, k_2, in the polymer environment is 10^2–10^3 times greater than that in aqueous solvent alone. Furthermore, at pH 4.5, the amine groups in the polymer matrix are 10^5 times more effective than those of the small molecule $C_2H_5NH_2$, as measured by the second-order rate constants. Alternatively, one can make the comparison by calculating from its second-order rate constant the concentration of $C_2H_5NH_2$ that would give a first-order constant equal to k_2 for the polymer; the small molecule amine would have to be 50 M in concentration.

The effectiveness of the polymer reflects several of its chemical and structural properties. Its cationic charge and apolar groups endow it with a very strong affinity for small molecules, particularly anions, as has been known for some time.[25] For oxalacetate specifically, binding by polymer is also

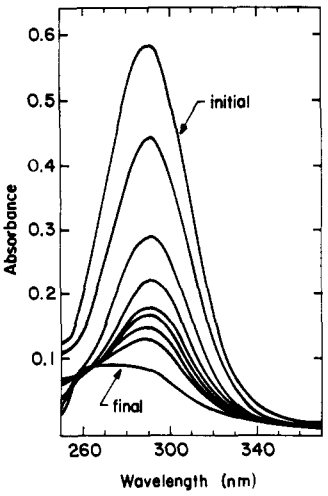


Figure 6. Changes in ultraviolet spectra upon addition of $NaBH_4$ to a mixture of oxalacetate-4-ethyl ester and PEIQ-NH_2.

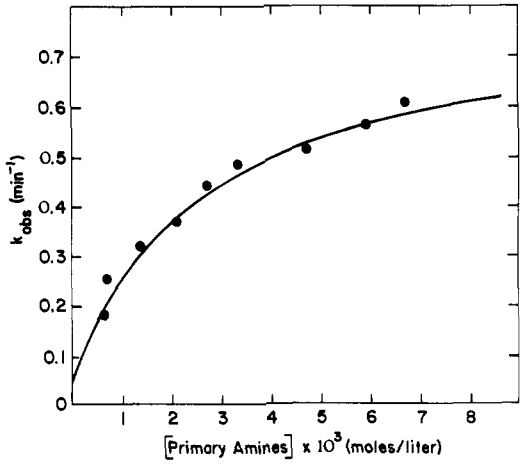


Figure 7. Variation of pseudo-first-order rate constant for decarboxylation of oxalacetate by PEIQ-NH_2 as a function of primary amine concentration, under conditions of excess primary amine; pH 7.0 and μ = 0.05.

Table III. Inhibition of Decarboxylation of Oxalacetate by Poly(ethylenimine) in Presence of Oxalacetate-4-ethyl Ester

	Initial velocities[a] × 10^6, mol/(l. min)				
	Oxalacetate, M				
Oxalacetate-4-ethyl ester, M	5.0 × 10^{-4}	7.5 × 10^{-4}	1.0 × 10^{-3}	1.25 × 10^{-3}	1.5 × 10^{-3}
0	5.50	7.19	7.97	9.82	11.30
5.0 × 10^{-4}	2.59	3.37	3.75	4.59	5.03
1.0 × 10^{-3}	1.74	2.68	3.67	3.97	4.70

[a] Polymer was first incubated with inhibitor for 30 min at 25 °C in 0.05 M cacodylate buffer, pH 7.0. Initial velocities were measured upon addition of substrate to 2.5 × 10^{-4} residue molar PEIQ-NH_2.

directly evident in the ultraviolet and infrared spectroscopic changes accompanying the shift in tautomeric equilibrium in the environment of the macromolecule.

A further manifestation of an initial binding step is saturation kinetics. For example, in the presence of excess catalyst, eq 4 predicts a hyperbolic relation between k_{obsd} and concentration of nucleophilic sites C_0 (= nP_0). Such behavior is apparent in Figure 7.

The number of nucleophilic sites per macromolecule, n, can be established from kinetic measurements in the presence of excess substrate and complementary ones in the presence of excess polymer.[15] In the decarboxylation of oxalacetate, n is 90 at pH 4.5 and 150 at pH 7.0. The modified poly(ethylenimine) polymer used contains 140 primary amine groups per macromolecule. Thus 65–100% of these act as nucleophilic sites for the decarboxylation reaction.

The bell-shaped pH-rate profile (Figure 4) is very similar to that observed with small amines[6,7,20] and with the enzyme acetoacetate decarboxylase.[21] Thus a Schiff base mechanism (eq 6), as has been proposed for the small amines and the enzyme, also seems plausible for the catalytic decarboxylation by the polymer. Spectroscopic evidence, as well as rate inhibitions with oxalacetate ester, fit in with such an interpretation. The decrease in rate at pH values above the maximum may thus reflect the decreasing concentration of monoanionic oxalacetate, which is much more susceptible to nucleophilic attack than is the dianionic form.[6,7] Increased binding competition by the dianion may be manifested in the increased K_M, i.e., decreased affinity, for monoanion in the kinetic process (Table II).

The drop with lowered pH exhibited in left-hand limb of Figure 4 probably reflects the decreasing instantaneous concentration of uncharged -NH_2 state as the amine groups are increasingly protonated. The *average* pK_a of the primary amines on PEIQ-NH_2 is[12] near 8, substantially lower than that in small-molecule amines. Furthermore the titration curve is spread out over a wider pH range. Thus the maximum at pH 4.5 in the rate-pH profile for the polymer, lower than that of 7 for a sample amine computed from the data of Pedersen,[6] is consistent with the weaker basicity in the polymer environment. It is of interest to note that the pK_a of the amine group at the active site of acetoacetate decarboxylase,[26] approximately 6, is also much below that of an isolated lysine side chain (ca. 10.5).

In studies of the decarboxylation mechanism with aniline as nucleophile, Hay[7] found that a Schiff base adduct could be identified if oxalacetate-4-ethyl ester was the C=O donor. We have observed a similar correspondence with PEIQ-NH_2 as nucleophile. In both systems it seems likely that the slow step in the decarboxylation pathway (eq 6) is the Schiff base formation at the keto carbonyl carbon atom. We have found no spectroscopic evidence indicating an accumulation of any intermediate species in the decarboxylation reaction. Furthermore, the rates of disappearance of substrate and appearance of product are very similar. This suggests that the Schiff base adduct is decarboxylated and hydrolyzed in rapid steps in the reaction. We cannot exclude the possibility that the Schiff base equilibrium is established rapidly, followed by one or more slower steps. However, this would require that the equilibrium constant for Schiff base formation be dramatically different for oxalacetate and oxalacetate-4-ethyl ester under similar conditions. It is doubtful that this is so.

Thus it is clear that modified poly(ethylenimines) exhibit substantial advantages as catalysts, compared with small molecule nucleophiles, in a variety of chemical reactions.[9-12,15] Although these macromolecules, as so far modified, are weak in structural specificity toward substrates, they do have the potential of being effective catalysts in a broad spectrum of reactions with small molecules of widely differing structure and chemical nature.

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Nucleic Acid Related Compounds. 22. Transformation of Ribonucleoside 2′,3′-O-Ortho Esters into Halo, Deoxy, and Epoxy Sugar Nucleosides Using Acyl Halides. Mechanism and Structure of Products[1,2]

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Abstract: Treatment of 2′,3′-*O*-methoxyethylideneadenosine (**2**) with pivalic acid chloride in refluxing pyridine gave an unresolved mixture of 6-*N*-pivalamido-9-(3-chloro-3-deoxy-2-*O*-acetyl-5-*O*-pivalyl-β-D-xylofuranosyl)purine (**4a**) and its 2′-chloroarabino isomer (**3a**) as the major product. In addition, 6-*N*-pivalamido-9-(3-chloro-3-deoxy-2-*O*-[4,4-dimethyl-3-pivaloxypent-2-enoyl]-β-D-xylofuranosyl)purine (**4b**) and its 2′-chloroarabino isomer (**3b**) were produced by acylation reactions involving a 2′,3′-*O*-ketene acetal (**11**) which is in equilibrium with the initially formed 2′,3′-acetoxonium ion intermediate (**10**). The structure of the complex 4,4-dimethyl-3-pivaloxypent-2-enoate (DMPP) group was deduced by NMR and mass spectroscopy and verified by synthesis of ethyl DMPP (**9**) from ethyl orthoacetate and pivalyl chloride/sodium iodide. Treatment of **2** with pivalyl chloride and excess sodium iodide in refluxing pyridine gave the corresponding 3′-iodoxylo and 2′-iodoarabino DMPP-blocked nucleosides (**4c** and **3c**) in good combined yield accompanied by unsaturated products. The absence of the corresponding acetyl iodo derivatives was rationalized on the basis of greater acylating activity of pivalyl iodide (generated in situ) in the postulated mechanistic sequence involving ketene acetal intermediates. A pivalylketene acetal derivative (**14**) was isolated and found to be converted to **3c** and **4c** under the reaction conditions. Treatment of **3a**,**4a** with tri-*n*-butyltin hydride or of **3c** and **4c** under catalytic hydrogenolysis conditions gave 2′-deoxyadenosine (**7**) and 3′-deoxyadenosine (cordycepin) (**8**), respectively, after deblocking. The ribo epoxide, 9-(2,3-anhydro-β-D-ribofuranosyl)adenine (**6**), was formed upon treatment of **3a–c** and **4a–c** with methanolic sodium methoxide. This proved the 2′,3′-trans orientation of halo and acyloxy substituents and provides convenient access to the synthetically useful **6**. Spectroscopic identification of products, the acyloxonium ion mediated mechanism and comparison of the route with previously reported procedures are discussed.

Syntheses of purine nucleosides containing modified sugar moieties have usually employed coupling of a suitably blocked (and stereochemically selected) carbohydrate derivative with a derivatized base.[4] Intramolecular base participation via purine-X[8]-cyclonucleosides (X = nucleophilic hetero atom) has also been applied.[5] Preparation of an appropriately substituted sugar derivative followed by elaboration of the desired heterocyclic base has been used in recent approaches to C-nucleosides[6] as well as in convenient syntheses of β-D-arabinopyrimidine nucleosides and other "natural" N-nucleosides.[7] However, cyclonucleoside chemistry analogous to that in the purine-8 series[5] is precluded, for example, in the pyrazolopyrimidine antibiotic formycin[8] (whose structure contains a formal exchange of C-8 and N-9 relative to adenosine). Although coupling procedures have provided structure proofs of pyrrolopyrimidine antibiotics,[9] these total syntheses are somewhat lengthy and uninviting for parallel routes to various modified sugar structures, and most published studies have concentrated on base changes.[10] The situation is similar with respect to the ring-elaborated C-nucleosides. Anomeric stereochemistry is an additional problem involved with coupling procedures.

We were interested in developing generally applicable transformations of intact nucleosides into functionalized sugar products which were not dependent on any specific base structure or mode of attachment. Such an approach beginning with antibiotics obtained in quantity by fermentation has obvious advantages. The elegant pioneering work of Winstein and Meerwein on acyloxonium ion intermediacy and structure has been reviewed recently.[11] Examples of such intermediates involved in synthetic approaches[12] (and vide infra) or during neighboring group participation reactions[13] of carbohydrates and nucleosides have been reported. Preliminary accounts of our approach have been outlined.[14]

Adenosine (**1**) was conveniently converted into 2′,3′-*O*-methoxyethylideneadenosine (**2**) by modification of reported procedures.[15] Boron trifluoride etherate[12] or antimony pentachloride in the presence of added nucleophilic species invariably resulted in the formation of significant quantities of 2′(3′)-*O*-acetyladenosine after workup. Pyridine hydrohalides