Acknowledgment. We are grateful to Dr. Hoying Hung of the Analytical Services Laboratory of the Chemistry Department for assistance in obtaining GC-mass spectra. This investigation was supported in part by a grant from the National Science Foundation.

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$$f_{CH_3C^{18}O_2H} = (1 - f_{H_2{}^{18}O})[\alpha/(1 + \alpha)] + f_{H_2{}^{18}O}$$

where $f_{H_2{}^{18}O}$ is equal to the fraction of $H_2{}^{18}O$ in the sample. For our present purposes, eq. 9 suffices.
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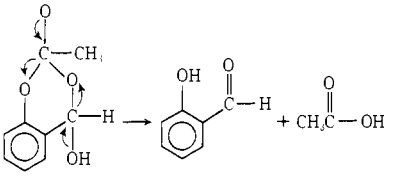

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Catalysis of the Hydrolysis of a Nitrophenyl Ester by *o*-Hydroxybenzaldehyde in the Presence of a Poly(ethylenimine) Derivative

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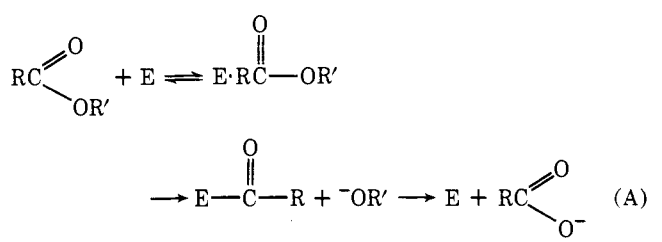
Abstract: *o*-Hydroxybenzaldehyde, a bifunctional reagent, has been shown to be effective in catalyzing the hydrolysis of nitrophenyl esters. The phenoxide ion acts as the primary nucleophile, attacking the ester and forming an acylated intermediate; the proximal hydrated formyl group acts as the secondary nucleophile to deacylate the acylated intermediate. For the reaction in the presence of a binding polymer, lauryl quaternized poly(ethylenimine), a kinetic equation has been derived under the conditions of concentration of polymer > *o*-hydroxybenzaldehyde > 3-nitro-4-acetoxybenzoic acid. The second-order rate constant calculated using this equation is 160 times greater than the value observed in the absence of polymer. This acceleration reflects the binding of the anionic reactants to the positively charged polymer and also a decrease in the pK_a of the phenolic group (by 0.9 units) of *o*-hydroxybenzaldehyde. That true catalysis occurs was demonstrated in the regeneration of 96% of the original *o*-hydroxybenzaldehyde in an experiment in which a 2.5-fold excess of ester was hydrolyzed through the acylsalicylaldehyde pathway.

There have been numerous attempts to reproduce enzyme-like catalytic behavior with synthetic polymers.[1-4] Most of the investigators in this area have tried to reproduce the esterolytic properties of the serine proteases, since this class of enzymes has been well characterized.[5]

It is generally agreed that, in the enzymatic hydrolysis of labile ester substrates, such as *p*-nitrophenyl acetate, the first step is the formation of a noncovalent complex between the enzyme and ester. This is followed by the formation of an acyl enzyme intermediate, which is subsequently hydrolyzed to regenerate the original enzyme (reaction A).



(A)

Since the first step in the overall process is the binding of the ester substrate to the macromolecule, the polymer selected for

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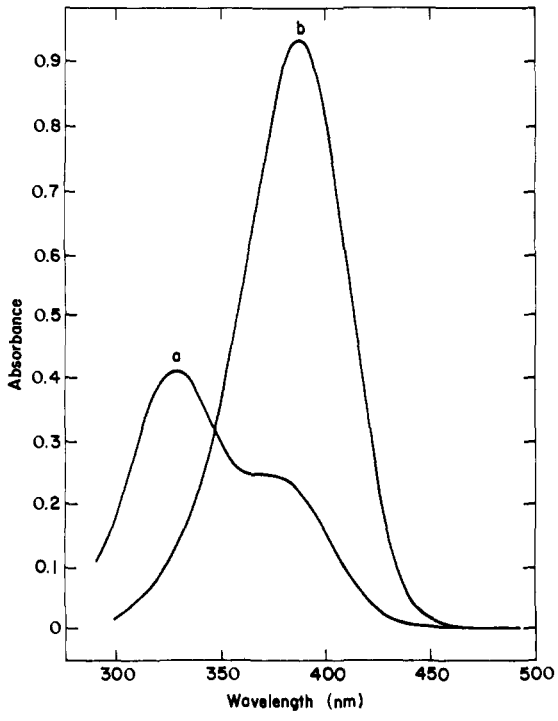


Figure 1. (a) Absorption spectrum of 1.4×10^{-4} M *o*-hydroxybenzaldehyde in 0.1 *M* Bis-Tris, pH 7.8. (b) Absorption spectrum of 1.4×10^{-4} M *o*-hydroxybenzaldehyde in the presence of 9.0×10^{-2} residue-molar lauryl quarternized poly(ethylenimine) in 0.1 M Bis-Tris, pH 7.8.

a synthetic analogue of the enzyme should display a strong affinity for the substrate molecules. Poly(ethylenimine), a water-soluble highly branched polymer, has previously been demonstrated to have a strong affinity for small molecules,[6] an affinity much higher than that observed for other synthetic polymers. Therefore, poly(ethylenimine) is an attractive macromolecular matrix for the introduction of nucleophilic catalysts capable of cleaving esters.

A potential bifunctional catalyst for ester hydrolysis is *o*-hydroxybenzaldehyde (salicylaldehyde). As has been described in the preceding paper, a well-approximated hydrated aldehyde is a very efficient intramolecular catalyst for the hydrolysis of an ester.[7] Therefore, the juxtaposition of an aldehyde with a functional group (e.g., a phenolic hydroxide) which can engage in an intermolecular reaction with an ester substrate, may provide a bifunctional molecule that will catalyze the hydrolysis of labile ester substrates. To test this hypothesis, the esterolytic properties of *o*-hydroxybenzaldehyde (salicylaldehyde) have been examined. The effect of a strong binding derivative of poly(ethylenimine) on the overall catalytic efficiency of salicylaldehyde has also been studied.

Experimental Section

o-Hydroxybenzaldehyde, benzaldehyde, and *p*-nitrophenyl acetate were purchased from Aldrich Chemical Co. Poly(ethylenimine), PEI 600, with an average molecular weight of 60 000 was obtained from Dow Chemical Co. as a 33% aqueous solution. *o*-Acetoxybenzaldehyde was synthesized by the procedure of Neuberger[8] and purified by vacuum fractional distillation: mp 36.5–37.3 °C (lit.[8] mp 37 °C). 3-Nitro-4-acetoxybenzoic acid was prepared by the method of Overberger et al.:[9] mp 152–153.8 °C (lit.[9] mp 152 °C).

Lauryl quaternized poly(ethylenimine) was synthesized by a procedure reported previously.[10,11] Reduction of this modified poly(ethylenimine) with $NaBH_4$ was carried out to eliminate an unidentified oxidant present on the polymer. For this purpose 1.01 g of modified polymer was dissolved in 75 mL of double-distilled water, 8 mg of $NaBH_4$ was added, and the reaction mixture was stirred at 25 °C for 2 h. An additional 8 mg of $NaBH_4$ was then added and the reaction mixture stirred for another 2 h. The mixture was then ultrafiltered first against 10 L of 0.02 M NaCl and then against 10 L of double-distilled water, in an Amicon ultrafiltration vessel with a XM50 (76 mm) membrane. The sample was then lyophilized. Proton magnetic resonance measurements of the modified polymer led to the stoichiometric composition $[(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}]Cl$, where $m = 1400$.

Reaction rates were determined from the rate of formation of phenolic products, measured spectrophotometrically in thermostated cell compartments, maintained at 25 °C, of a Cary 14 spectrophotometer. The appearance of *p*-nitrophenol and of 3-nitro-4-hydroxybenzoic acid, respectively, was followed at 450 nm. The reactions were initiated by the addition of 10 μL of a stock solution of the ester in dioxane to 3 mL of aqueous 0.1 M Bis-Tris·HCl buffer containing the appropriate reagents. In the hydrolysis of *p*-nitrophenyl acetate by *o*-hydroxybenzaldehyde, the reaction mixture also contained 1 M KCl. The appearance of *o*-hydroxybenzaldehyde, in the hydrolysis of *o*-acetoxybenzaldehyde, was followed at 320 nm. This reaction was initiated by the addition of 10 μL of a stock solution of the ester in acetonitrile to 3 mL of aqueous 0.05 M veronal buffer at pH 7.8.

Rate constants for the hydrolysis of *o*-acetoxybenzaldehyde were calculated from first-order plots. For the hydrolysis of *p*-nitrophenyl acetate and of 3-nitro-4-acetoxybenzoic acid, concentrations were adjusted to permit observation of initial rates for kinetic runs in the absence of polymer, or pseudo-first-order rates in its presence. For initial-rate experiments, <5% reaction, a plot of absorbance, A, vs. time, t, gives a straight line from which initial rates can be calculated. The slope of this line is related to k_{obsd} by the equation, $k_{obsd} = [(A_2 - A_1)/(t_2 - t_1)][1/(A_\infty - A_0)]$. Pseudo-first-order rate constants were obtained from the slope of plots of 2.303 log $(A_\infty - A_t)$ against time, which were linear for at least three half-lives.

The pK_a values of *o*-hydroxybenzaldehyde in the presence of lauryl quaternized poly(ethylenimine) in H_2O and in D_2O without polymer were determined by the method of Albert and Serjeant.[12]

Results

Establishment of Phenoxide Ion as Primary Nucleophile. The rate of the reaction of *p*-nitrophenyl acetate with *o*-hydroxybenzaldehyde was determined over a pH range of 6.5–8.0. The second-order rate constant for this reaction, calculated on the basis of the phenoxide ion concentration (pK_a of salicylaldehyde is 8.37),[12] is 1.84 min^{-1} M^{-1}. This value lies on the line of a Brønsted plot previously obtained[13] in the reaction of *p*-nitrophenyl acetate with a series of compounds in which the nucleophilic species is an alkoxide ion. Furthermore in D_2O the value of this rate constant was found to be 2.15 min^{-1} M^{-1} (pK_a of salicylaldehyde in D_2O is 8.9). Thus $k_{H_2O}/k_{D_2O} = 0.9$. Clearly, the phenoxide ion of *o*-hydroxybenzaldehyde is acting as a nucleophile in this reaction. Further corroboration of this conclusion was obtained from the observation that benzaldehyde, lacking the phenoxide moiety, does not increase the hydrolysis of *p*-nitrophenyl acetate over background values.

Interaction of *o*-Hydroxybenzaldehyde with Lauryl Quaternized Poly(ethylenimine). The dissociation constant for the binary complex between *o*-hydroxybenzaldehyde and lauryl quaternized poly(ethylenimine) can be determined from the spectroscopic shift associated with the binding of the small molecule to the polymer (Figure 1). The equilibrium constant, K_D', for the binding of salicylaldehyde, N, in a domain, D, of the polymer is

$$K_D' = (D)(N)/(DN) \quad (1)$$

Under the condition of $(D)_0 > (N)_0$ and with the assumption that there are ν' monomer residues per binding domain, eq 1 can be rewritten as

$$\nu' K_D' = (P)_0(N)/(DN) \quad (2)$$

where $(P)_0$ is the initial polymer concentration in residue-molar units and $(P)_0/\nu' = (D)_0$. Analysis of the spectroscopic data[14] yields a value of 1.11×10^{-2} M for $\nu' K_D'$.

The pK_a of *o*-hydroxybenzaldehyde in the presence of the

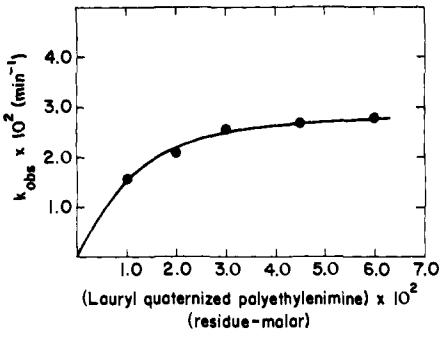


Figure 2. Variation of pseudo-first-order rate constant for the hydrolysis of 3-nitro-4-acetoxybenzoic acid as a function of polymer concentration. (3-nitro-4-acetoxybenzoic acid) = 1.5×10^{-5} M.

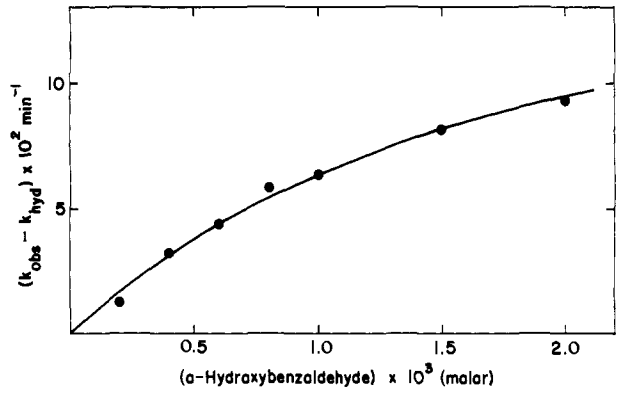


Figure 3. Variation of pseudo-first-order rate constant for reaction of 3-nitro-4-acetoxybenzoic acid with *o*-hydroxybenzaldehyde in presence of lauryl quarternized poly(ethylenimine). (3-nitro-4-acetoxybenzoic acid) = 1.5×10^{-5} M; (polymer) = 2.5×10^{-2} residue-molar; pH is 7.8. The curve was drawn according to eq 7.

polymer can also be determined from the spectroscopic shifts at a series of pH values. Following the method of Albert and Serjeant,[12] and using solutions of 1.4×10^{-4} M salicylaldehyde in the presence of 2.5×10^{-2} residue-molar polymer, we found a pK_a of 7.42.

Hydrolysis of 3-Nitro-4-acetoxybenzoic Acid in the Presence of Lauryl Quarternized Poly(ethylenimine). In the absence of polymer, the first-order rate constant for the hydrolysis of 3-nitro-4-acetoxybenzoic, at pH 7.8, is 4.6×10^{-3} min^{-1}. The rate of cleavage is increased in the presence of excess polymer.

The hydrolysis of the ester substrate in the presence of polymer can be formulated as

$$D + S \overset{K_D''}{\rightleftharpoons} DS \xrightarrow{k_1} D + X_1 + X_2 \qquad (3)$$

where D represents a binding domain on the polymer, S the 3-nitro-4-acetoxybenzoic acid, X_1 the 3-nitro-4-hydroxybenzoic acid, and X_2 the acetate. When polymer is in excess and if the pre-equilibrium (eq 3) is rapid, the following equation can be derived[11]

$$k_{obsd} = \frac{k_1(P)_0/\nu''}{K_D'' + (P)_0/\nu''} \qquad (4)$$

where ν'' is the number of monomer residues per binding domain (i.e., $(D)_0 = (P)_0/\nu''$) and k_{obsd} is the observed first-order rate constant.

The experimental rate constants for the hydrolysis of 3-nitro-4-acetoxybenzoic acid in the presence of polymer are shown in Figure 2. Analysis of the kinetic results on the basis of a linear transform of eq 4 yields a k_1 of 3.28×10^{-2} min^{-1} and $\nu''K_D''$ of 1.10×10^{-2} M.

Reaction of *o*-Hydroxybenzaldehyde with 3-Nitro-4-acetoxybenzoic Acid in Presence and Absence of Lauryl Quarternized Poly(ethylenimine). In the absence of polymer, the second-order rate constant for the reaction of 3-nitro-4-acetoxybenzoic acid with *o*-hydroxybenzaldehyde, at pH 7.8, is 0.60 min^{-1} M^{-1}.

In the presence of polymer, the various steps in this reaction may be visualized as eq 5 where D is a binding domain on the

$$\begin{array}{l} D + N \overset{K_D'}{\rightleftharpoons} DN \overset{S}{\rightleftharpoons} \\ \qquad\qquad \overset{K_D''}{\rightleftharpoons} DNS \xrightarrow{k_2} DN' + X_1 \overset{K_D'''}{\rightleftharpoons} D + N' \\ D + S \overset{K_D''}{\rightleftharpoons} DS \overset{K_D'}{\rightleftharpoons} \\ \qquad\qquad \overset{N}{\rightleftharpoons} \\ \qquad\qquad \xrightarrow{k_3} D + N + X_2 \end{array} \qquad (5)$$

polymer, S is 3-nitro-4-acetoxybenzoic acid, N is *o*-hydroxybenzaldehyde, DN′ is a binary complex between polymer and *o*-acetoxybenzaldehyde, X_1 is 3-nitro-4-hydroxybenzoic acid, N′ is *o*-acetoxybenzaldehyde, and X_2 is acetate.

The experiments were carried out under the condition $(D)_0 > (N)_0 > (S)_0$. If we assume rapid preequilibria and random binding of substrate and of nucleophile, the following equation can be derived[15] for the rate of appearance of the first product, X_1:

$$\frac{d(X_1)}{dt} = \left\{ k_{hyd(p)} + \frac{k_2}{\dfrac{[K_D'K_D''/(D)_0] + K_D' + K_D'' + (D)_0}{(N)_0} + 1} \right\} \times [(S)_0 - (X_1)] = k_{obsd}[(S)_0 - X_1)] \qquad (6)$$

Thus

$$k_{obsd} - k_{hyd(p)} = \frac{k_2}{[K_{app}/(N)_0] + 1} = \frac{k_2(N)_0}{K_{app} + (N)_0} \qquad (7)$$

where

$$K_{app} = [K_D'K_D''/(D)_0] + K_D' + K_D'' + (D)_0 \qquad (8)$$

From eq 7 it is apparent that a plot $k_{obsd} - k_{hyd(p)}$ vs. $(N)_0$, the total concentration of *o*-hydroxybenzaldehyde, should be hyperbolic. A plot of the experimental data, shown in Figure 3, is indeed hyperbolic. From a double-reciprocal plot, i.e., $1/(k_{obsd} - k_{hyd(p)})$ vs. $1/(N)_0$, which is linear, k_2 and K_{app} can be determined. The values of these parameters are listed in Table I.

From eq 7 and 8 it is apparent that as $(D)_0$ is increased progressively, $k_{obsd} - k_{hyd(p)}$ should initially increase and then gradually decrease. As is shown in Figure 4, such behavior is indeed followed by the experimental points. The solid curve in Figure 4 was calculated from eq 7 using the following constants. k_2 is 0.19 min^{-1} (Table I). The number of monomer residues per binding domain for molecules of size and charge similar to the reactants in this system has been shown previously[11] to be between 12 and 25. The best fit of the experimental data was obtained by assuming $\nu' = \nu'' = 24$. K_D' and K_D'' then are both equal to 4.6×10^{-4} M. ν, the number of monomer residues per reaction domain, was assumed to be equal to the sum of ν' and ν''. As can be seen from Figure 4, there is a good fit between the experimental data and the theoretical curve.

In separate experiments we found that the rate constant for the hydrolysis of *o*-acetoxybenzaldehyde in the presence of 2.5

Table I. Kinetic Parameters for Hydrolysis of 3-Nitro-4-acetoxybenzoic Acid

catalyst	kinetic parameter	values of parameter
$(C_2H_4N)_m$ $(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}$, pH 7.8	k_1	3.28×10^{-2} min^{-1}
	$\nu''K_D''$	1.10×10^{-2} M
$(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(CH_3)_{1.75m}$,[a] o-hydroxybenzaldehyde, pH 7.8	k_2	0.19 min^{-1}
	K_{app}	1.92×10^{-3} M
	k_2/K_{app}	98 min^{-1} M^{-1}
	$\nu'K_D'$	1.11×10^{-2} M
	k_3	0.48 min^{-1}
o-hydroxybenzaldehyde, pH 7.8	k	0.60 min^{-1} M^{-1}
H_2O, pH 7.8	k_{hyd}	4.6×10^{-3} min^{-1}
benzaldehyde, pH 7.8	k	no reaction

[a] 2.5×10^{-2} residue-molar polymer.

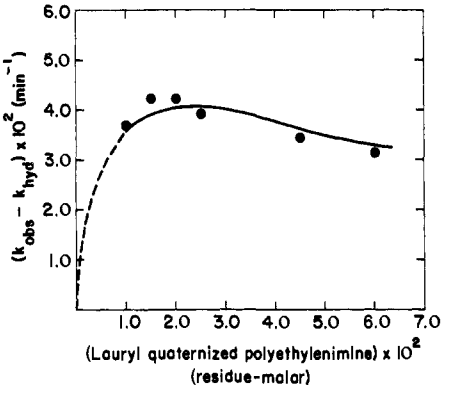


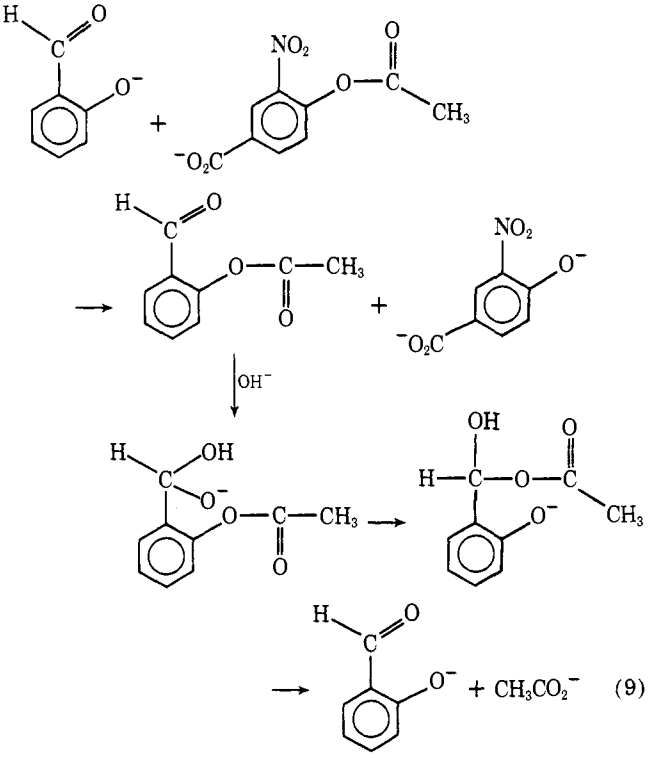
Figure 4. Variation of pseudo-first-order rate constant for hydrolysis of 3-nitro-4-acetoxybenzoic acid in presence of o-hydroxybenzaldehyde and polymer. (3-nitro-4-acetoxybenzoic acid) = 1.5×10^{-5} M; (o-hydroxybenzaldehyde) = 5.0×10^{-4} M.

$\times 10^{-2}$ residue-molar polymer, at pH 7.8, is 4.76×10^{-1} min^{-1}, whereas the value in the absence of the polymer is 4.52×10^{-1} min^{-1}. Clearly there is no significant acceleration of this hydrolysis by the polymer.

Demonstration of Turnover of o-Hydroxybenzaldehyde. The regeneration of o-hydroxybenzaldehyde after its initial reaction with 3-nitro-4-acetoxybenzoic acid to form an acylated intermediate was demonstrated under the experimental conditions, $(D)_0 > (S)_0 > (N)_0$. A fivefold excess of ester over o-hydroxybenzaldehyde was used. The pH was maintained between 7 and 8 by the addition of concentrated NaOH. After all of the ester had been hydrolyzed, as evidenced by the attainment of a constant pH, in less than an hour, Bis-Tris buffer reagent was added to give a final concentration of 0.1 M and the pH was adjusted to 7.8. A difference spectrum was taken, in which the reference cell contained modified polymer, 3-nitro-4-hydroxybenzoic acid, acetate, and Bis-Tris buffer at concentrations equal to those in the sample cell. The difference spectrum indicated that 96% of the o-hydroxybenzaldehyde initially added to the reaction mixture was still present after hydrolysis of the fivefold excess of the nitrophenyl ester. Under these conditions, 45% of the ester hydrolysis proceeded through the salicylaldehyde pathway.

Discussion

As the observations indicate, o-hydroxybenzaldehyde, a bifunctional reagent, is an efficient catalyst for the hydrolysis of labile ester substrates. The nucleophilic species in the intermolecular attack on the ester substrate is the phenoxide ion, as is evident from the lack of reactivity of benzaldehyde with nitrophenyl esters. The o-acetoxybenzaldehyde formed in the initial step is deacylated by a mechanism involving the hydrated formyl group.[7] Thus the overall pathway for the reaction of o-hydroxybenzaldehyde with 3-nitro-4-acetoxybenzoic acid may be formulated as reaction 9. o-Hydroxybenzaldehyde



(9)

displays all of the properties essential for an effective catalyst in the hydrolysis of labile ester substrates. (1) The phenoxide ion is nucleophilic enough to cleave the ester bond of the substrate. (2) The acyl intermediate, o-acetoxybenzaldehyde, that is formed in the initial step is more labile than an analogous nitrophenyl ester substrate. The first-order rate constant for the hydrolysis of o-acetoxybenzaldehyde is two orders of magnitude greater than the value for 3-nitro-4-acetoxybenzoic acid. (3) Only one of the functional groups can react with the ester substrate, and thus possible competition between the intermolecular attack on the ester substrate and the intramolecular deacylation of o-acetoxybenzaldehyde by the hydrated aldehyde is avoided.

In the presence of lauryl quaternized poly(ethylenimine), there is a substantial increase in the reactivity of o-hydroxybenzaldehyde toward 3-nitro-4-acetoxybenzoic acid. The second-order rate constant in the presence of the polymer, k_2/K_{app}, is 160 times greater than the corresponding second-order rate constant in the absence of the polymer. This rate enhancement can be ascribed to at least two factors. First, the positively charged matrix of the polymer lowers the pK_a of o-hydroxybenzaldehyde from 8.37 to 7.42. This shift of ~0.9 unit increases the concentration of the nucleophilic phenoxide

ion by about eightfold. Secondly, the cationic polymer binds both of the anionic reactants and thereby increases their local concentration and, consequently, the rate of reaction.

In the absence of polymer, the rate constant for the hydrolysis of *o*-acetoxybenzaldehyde, at pH 7.8, is 4.52×10^{-1} min^{-1}, and the first-order rate constants for the reaction of salicylaldehyde with 3-nitro-4-acetoxybenzoic acid, in the concentration range of $0.25-2.0 \times 10^{-3}$ M salicylaldehyde, vary from 0.16 to 1.21×10^{-3} min^{-1}. In the presence of polymer, the rate constant for the hydrolysis of *o*-acetoxybenzaldehyde is 4.76×10^{-1} min^{-1}, and the first-order rate constant for the reaction of salicylaldehyde with 3-nitro-4-acetoxybenzoic acid is 0.19 min^{-1}. Thus, in the presence or absence of polymer the rate-limiting step is the transfer of the acyl group from the nitrophenyl ester to *o*-hydroxybenzaldehyde. Although, the first step in this reaction is accelerated by the polymer, there is no significant acceleration of the second step, i.e., the hydrolysis of *o*-acetoxybenzaldehyde.

Since there is no significant increase in the rate of hydrolysis of *o*-acetoxybenzaldehyde in the presence of the polymer and, in this pH region, the rate is linear in (OH^-),[7] we presume that there is very little binding of this neutral small molecule to the macromolecule. Therefore, in the overall reaction scheme given in eq 5, the hydrolysis of *o*-acetoxybenzaldehyde is shown to occur free in solution.

An interesting feature of the catalyzed reaction in the presence of the polymer, illustrated in Figure 4, is the initial increase in the first-order rate constant, followed by the gradual decrease as the polymer concentration is increased. As the total number of binding domains is increased, the probability that the two reactants will be bound at separated sites also increases. Consequently, the amount of nonproductive binding increases at very high polymer concentration and the rate of reaction decreases.

Overall the results indicate that an efficient bifunctional reagent for the hydrolysis of labile ester substrates can be devised by having a formyl group proximal to a nucleophilic group that is capable of an intermolecular attack on the ester substrate. The catalytic efficiency of this system has been demonstrated to be enhanced by a binding polymer. Further studies are underway to devise other bifunctional reagents containing a carbonyl moiety proximal to a nucleophilic group possessing greater nucleophilicity than the phenoxide ion.

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Effect of Solvents of Decreasing Nucleophilicity (Sulfur Dioxide, Sulfuryl Chloride Fluoride, Sulfuryl Fluoride, and Methylene Fluoride) on the Complex Formation and Ionization of Alkyl Fluorides (Chlorides) with Antimony and Arsenic Pentafluoride[1]

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Abstract: C_1 to C_5 alkyl fluorides (chlorides) were complexed and/or ionized with antimony and arsenic pentafluoride in the following solvents of decreasing nucleophilicity: sulfur dioxide, sulfuryl chloride fluoride, sulfuryl fluoride, and methylene fluoride. ^1H and ^{13}C NMR spectroscopy were used to investigate the formation of the corresponding alkyl cations and/or complexes. Carbocation or complex formation is dependent on the stability of the species, the strength of Lewis acid, and the nucleophilicity of the solvent.

Introduction

Methods developed in our preceding work allowing the study of carbocations under stable ion conditions have helped to elucidate the structure of a large number of carbocationic reaction intermediates.[2] The use of higher valency Lewis acid fluorides such as antimony and arsenic pentafluoride and their derived conjugate protic superacids, together with the use of low nucleophilic solvents such as sulfur dioxide, sulfuryl chloride fluoride, and sulfuryl fluoride at low temperatures, has made possible the generation and study of a wide variety of stable carbocations. Despite the continuously increasing number of studies on carbocations, the role of the solvents in

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