Cooperative Effects in the Esterolytic Action of Synthetic Macromolecules Containing Imidazole and Hydroxyl Functions[1]

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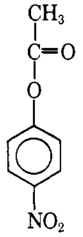
Abstract: The imidazole group of histidine and the hydroxyl group of serine are the purported participants in the active site of α-chymotrypsin. Copolymers of 4(5)-vinylimidazole with vinyl alcohol and with *p*-vinylphenol were prepared in order to investigate whether interactions between two different functional groups within a vinyl polymer chain would occur in esterolytic catalyses. The substrates investigated were neutral and negatively and positively charged phenyl esters. Rates of solvolyses were determined in either 28.5% ethanol–water or in 80% methanol–water. The copolymer of 4(5)-vinylimidazole and *p*-vinylphenol was found a highly superior catalyst at high pH values toward each substrate investigated than any of its monomeric or polymeric analogs. These rate enhancements are not associated with the apparent dissociation constants of the imidazole or phenol groups, but can be attributed to bifunctional catalysis by imidazole and anionic phenol. One probable mechanism is the nucleophilic attack of imidazole on the substrate, followed by reaction of anionic phenol acting as a general base on the tetrahedral intermediate. Considerable rate enhancements were also observed for reactions catalyzed by copolymers of 4(5)-vinylimidazole and vinyl alcohol.

Bifunctional esterolytic catalyses displayed by homopolymers containing pendent imidazole or benzimidazole groups were shown to be more efficient than the esterolytic catalyses exhibited by their respective monomeric analogs, imidazole and benzimidazole.[3] A substantial decrease in the enthalpy of activation was associated with the esterolytic action of the homopolymers (relative to the esterolytic action of the monomeric analogs), with a smaller decrease in the entropy of activation. This combination, with its consequential higher catalytic efficiency, is a result of an over-all second-order but terfunctional interaction of the pendent catalytic groups and the substrate.[4] Multifunctional catalyses displayed by the functional groups comprising the active sites of enzymes resemble the polymers in this respect. Apparently the high local concentration of pendent, catalytically active imidazole or benzimidazole groups facilitates their ability to act as multifunctional catalysts.

The role of the imidazole function in α-chymotrypsin catalyses has been well investigated.[5] A cooperative interaction of imidazole and hydroxyl groups underlies most of the mechanisms proposed.[5,6] Although the major portion of chemical and physical evidence indicates the involvement of the serine hydroxyl, the low pK_a value inferred from the descending slope of the bell-shaped pH–rate profile of α-chymotrypsin with certain substrates promoted the suggestion of the involvement of the phenol group of tyrosine.[7a]

It was natural to extend our investigation of synthetic, polymeric enzyme models containing pendent imidazole groups to study whether the introduction of hydroxyl or phenol functions improves the catalytic action of the polymer. Furthermore, if such an enhancement exists, it would be of interest to determine if such an effect is a result of bifunctional catalysis involving imidazole and hydroxyl or imidazole and phenol functions. Copolymers of 4(5)-vinylimidazole with vinyl alcohol[8] and with *p*-vinylphenol were prepared and their catalytic effects toward a neutral substrate, *p*-nitrophenyl acetate (PNPA, I), a positively charged substrate, 3-acetoxy-N-trimethylanilinium iodide (ANTI, II), and two negatively charged substrates, 4-acetoxy-3-nitrobenzoic acid (NABA, III) and sodium 4-acetoxy-3-nitrobenzenesulfonate (NABS, IV), were studied. In addition, the above solvolytic reactions were compared to those of their respective monomeric and polymeric analogs, *i.e.*, imidazole, poly-4(5)-vinylimidazole, phenol, poly-*p*-vinylphenol, and a copolymer of 4(5)-vinylimidazole and *p*-methoxystyrene.[9]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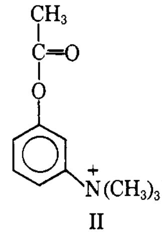
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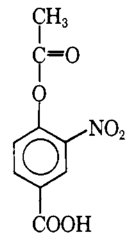
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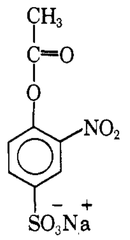
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[1] This paper comprises a portion of the dissertation submitted by J. C. Salamone in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Polytechnic Institute of Brooklyn.

[2] (a) Address correspondence to The University of Michigan, Ann Arbor, Michigan. (b) Deceased July 1967. The surviving authors and friends wish to dedicate this paper in memory of Dr. Samuel Yaroslavsky, dear friend and scientist.

[3] C. G. Overberger, T. St. Pierre, N. Vorchheimer, and S. Yaroslavsky, *J. Am. Chem. Soc.*, **85**, 3513 (1963); C. G. Overberger, T. St. Pierre, N. Vorchheimer, J. Lee, and S. Yaroslavsky, *ibid.*, **87**, 296 (1965).

[4] C. G. Overberger, T. St. Pierre, N. Vorchheimer, C. Yaroslavsky, J. Lee, J. C. Salamone, R. Sitaramaiah, and S. Yaroslavsky, *Trans. N. Y. Acad. Sci.*, **27**, 790 (1965); C. G. Overberger, T. St. Pierre, C. Yaroslavsky, and S. Yaroslavsky, *J. Am. Chem. Soc.*, **88**, 3270 (1966).

[5] For a general review, see M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).

[6] M. L. Bender and F. J. Kézdy, *J. Am. Chem. Soc.*, **86**, 3704 (1964).

[7] (a) M. L. Bender, G. E. Clement, F. J. Kézdy, and B. Zerner, *ibid.*, **85**, 358 (1963); (b) A. Himoe and G. P. Hess, *Biochem. Biophys. Res. Commun.*, **23**, 234 (1966); M. Bender, M. J. Gibian, and D. J. Whelan, *Proc. Natl. Acad. Sci. U. S.*, **56**, 833 (1966).

[8] C. G. Overberger and N. Vorchheimer, *J. Am. Chem. Soc.*, **85**, 951 (1963).

[9] This copolymer, suggested by Professor H. Morawetz, appears to be an appropriate polymeric analog to the copolymer of 4(5)-vinylimidazole and *p*-vinylphenol.

Experimental Section

4(5)-Vinylimidazole was prepared by the procedure of Overberger and Vorchheimer, as also was poly-4(5)-vinylimidazole.[8]

***p*-Vinylphenol.** Upon heating 7.0 g (0.043 mole) of *p*-hydroxycinnamic acid in a sublimator at 220° (0.01 mm), gas was liberated and 5.3 g of white solid condensed on the cold finger. The solid was washed with benzene and the mixture filtered to remove the unreacted *p*-hydroxycinnamic acid. After lyophilizing the benzene solution, 2.0 g of *p*-vinylphenol was obtained in 58% yield based on recovered starting material. *p*-Vinylphenol was purified by sublimation at room temperature (0.01 mm), mp 72–73° (lit.[10] 72–73°).

Poly-*p*-vinylphenol was prepared by allowing *p*-vinylphenol to polymerize on standing at room temperature in the solid state.[10] The polymer was dissolved in methyl ethyl ketone and twice precipitated into benzene. The infrared spectrum (potassium bromide pellet) was similar to that reported by Sovish.[10] A sample of reprecipitated polymer was shown to have a molecular weight of 4840 ($\overline{DP}$ *ca.* 40).

Anal. Calcd for C_8H_8O: C, 80.0; H, 6.7; Found: C, 77.9; H, 6.7.

Copolymerization of 4(5)-Vinylimidazole and *p*-Vinylphenol. A solution of 0.97 g (0.007 mole) of *p*-vinylphenol, 0.25 g (0.0027 mole) of 4(5)-vinylimidazole, and 0.011 g of azobisisobutyronitrile in 10 ml of benzene in a polymerization tube was cooled to −78°, flushed with nitrogen, degassed, and sealed under reduced pressure. The tube was heated for 24 hr in refluxing hexane, during which time polymer had precipitated. After filtration and washing with benzene, the off-white polymer was dissolved in methanol and twice precipitated into ethyl acetate. Infrared spectrum (potassium bromide pellet) revealed the presence of imidazole and phenol groups.

Anal. Found: C, 75.2; H, 6.9; N, 8.5 (0.30 mole of imidazole and 0.59 mole of phenol per 100 g of polymer).

Copolymerization of 4(5)-Vinylimidazole and *p*-Methoxystyrene. In a polymerization tube was placed a solution of 1.00 g of *p*-methoxystyrene (0.0075 mole), 0.25 g (0.0027 mole) of 4(5)-vinylimidazole, and 0.018 g of azobisisobutyronitrile in 25 ml of benzene. After cooling and degassing, the tube was sealed *in vacuo* and heated in a refluxing methanol bath for 24 hr. The polymer was filtered and washed with benzene giving 0.37 g (30% conversion) of white powder. The polymer was purified by twice precipitating from methanol into ethyl acetate.

Anal. Found: C, 67.3; H, 7.3; N, 17.6 (0.63 mole of imidazole and 0.30 mole of *p*-methoxystyrene per 100 g of polymer).

Copolymerization of 4(5)-Vinylimidazole and Vinyl Acetate. a. A solution of 4.00 g (0.047 mole) of freshly distilled vinyl acetate, 0.52 g (0.0055 mole) of 4(5)-vinylimidazole, and 0.008 g of azobisisobutyronitrile in 14 ml of benzene was placed in a polymerization tube, cooled to −70°, flushed with nitrogen, and sealed under reduced pressure. The tube was heated in a refluxing hexane bath for 30 hr. The precipitated polymer was filtered and washed with 25 ml of benzene, yielding 0.62 g (13.7% conversion) of an off-white solid. The infrared spectrum (potassium bromide pellet) revealed the presence of imidazole and acetate groups.

Anal. Found: C, 60.1; H, 7.0; N, 23.2 (0.83 mole of imidazole and 0.26 mole of acetate per 100 g of polymer).

b. In the bulk, 1.1 g (0.012 mole) of 4(5)-vinylimidazole and 20.0 g (0.24 mole) of freshly distilled vinyl acetate were copolymerized according to the procedure of Overberger and Vorchheimer.[8]

Anal. Found: C, 59.5; H, 5.7; N, 18.7 (0.66 mole of imidazole and 0.42 mole of acetate per 100 g of polymer).

Hydrolyses of Copolymers of 4(5)-Vinylimidazole and Vinyl Acetate. a. A solution of 0.30 g of the 1:0.31 copolymer of 4(5)-vinylimidazole and vinyl acetate (prepared by method a above) in 100 ml of water was heated for 20 hr at reflux. The solution was cooled, filtered, and lyophilized, giving 0.23 g of an off-white solid. The infrared spectrum (potassium bromide pellet) indicated that no acetyl functions were present.

Anal. Found: C, 54.3; H, 6.7; N, 21.7 (0.78 mole of imidazole/100 g of polymer).

b. An opaque solution of 0.31 g of the 1:0.65 copolymer of 4(5)-vinylimidazole and vinyl acetate (prepared by method b above) in 100 ml of water was refluxed for 2 weeks. The clear solution obtained was cooled, filtered, and lyophilized, yielding 0.25 g of an off-white polymer. The infrared spectrum (potassium bromide pellet) indicated the disappearance of acetyl groups.

Anal. Found: C, 57.7; H, 6.6; N, 19.4 (0.69 mole of imidazole/100 g of polymer).

***p*-Nitrophenyl acetate, 4-acetoxy-3-nitrobenzoic acid, and sodium 4-acetoxy-3-nitrobenzenesulfonate** were prepared as previously reported.[3,11]

3-Acetoxy-N-trimethylanilinium Iodide. To 1.0 g (0.0056 mole) of 3-acetoxy-N-dimethylaniline (prepared by treating 3-hydroxy-N-dimethylaniline in dilute alkali with acetic anhydride) in 15 ml of dry benzene was added 3.0 g (0.021 mole) of methyl iodide. After heating overnight at 70°, 3-acetoxy-N-trimethylanilinium iodide was obtained in high yield. This compound was twice recrystallized from nitromethane, mp 209–210°.

Anal. Calcd for $C_{11}H_{16}NO_2I$: C, 40.83; H, 5.00; N, 4.36; I, 39.51. Found: C, 40.94; H, 4.88; N, 4.43; I, 39.70.

Spectrophotometric Titrations. Measurements of the state of ionization of phenol and the phenol groups in poly-*p*-vinylphenol and in the 1:1.95 imidazole–phenol copolymer were performed in 80% methanol–water solutions at concentrations of approximately 10^{-4} M in phenol. Both the imidazole–phenol copolymer and poly-*p*-vinylphenol were insoluble in systems containing lower percentages of alcohol. The ionic strength was adjusted to 0.02 by hydrochloric acid for measurements at low and intermediate pH. The optical density was recorded at 286 mμ.[12] The method of calculation of the fraction of phenolate ions was as previously described.[3,12]

The plots of pH *vs.* α_{PhO^-} (the fraction of anionic phenol functions) for phenol and the phenol groups in poly-*p*-vinylphenol and in the 1:1.95 imidazole–phenol copolymer appear in Figure 1. The pK_a of phenol in 80% methanol–water as determined from Figure 1 is 10.9 at the point where $\alpha_{\text{PhO}^-} = 0.5$. Similarly, the apparent pK_a's of the phenol groups at half-neutralization in poly-*p*-vinylphenol and in the imidazole–phenol copolymer are determined to be 11.9 and 11.3, respectively.

Kinetic Measurements. In the case of the 1:1.95 copolymer of 4(5)-vinylimidazole and *p*-vinylphenol, 2.6×10^{-4} M (in moles of imidazole) catalyst solutions were prepared in 80% methanol–water. At pH 7 and above, solutions were buffered with 0.02 M tris(hydroxymethyl)aminomethane (Tris) and hydrochloric acid. At pH's 5.1 and 6.1, solutions were buffered with 0.02 M sodium acetate and acetic acid. At pH 3.3 hydrochloric acid was employed. In each instance the ionic strength was adjusted to 0.02 with potassium chloride. For comparison, 2.7×10^{-4} M solutions of poly-4(5)-vinylimidazole and 2.5×10^{-4} M solutions of imidazole and of the 1:0.48 copolymer of 4(5)-vinylimidazole and *p*-methoxystyrene were prepared under the same conditions. Similarly, 3.3 and 2.7×10^{-4} M (in moles of phenol) of phenol and poly-*p*-vinylphenol were respectively prepared.

For the 1:0.31 and the 1:0.65 copolymers of 4(5)-vinylimidazole and vinyl alcohol, 2.6 and 2.5×10^{-4} M catalyst solutions (in moles of imidazole) were respectively prepared in 28.5% ethanol–water with sufficient potassium chloride added to adjust the ionic strength to 0.02. Between pH values 7 and 9, solutions were buffered with 0.02 M Tris and hydrochloric acid; pH values less than 7 were buffered with 0.02 M sodium acetate–acetic acid. At pH 10, solutions were buffered with 0.02 M triethylamine and hydrochloric acid. For comparison, 2.5×10^{-4} M solutions of imidazole and of poly-4(5)-vinylimidazole were prepared under the same conditions.

Kinetic measurements and the methods of calculation of the first-order observed rate constants ($k_{\text{obsd}} = k_{\text{measd}} - k_{\text{blank}}$) were performed as previously described.[3] The temperature employed was 26°. All values of the first-order observed rate constants are reported for catalyst concentrations of 2.5×10^{-4} M.

Results and Discussion

Synthesis. The previously reported procedures for the preparation of *p*-vinylphenol were usually laborious, and resulted in poor yields.[10] It was found, however, that the preparation could be simplified. Pyrolysis of *p*-hydroxycinnamic acid in a sublimator at 220° (0.01 mm) resulted in a 58% yield of *p*-vinylphenol

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(10) R. C. Sovish, *J. Org. Chem.*, **24**, 1345 (1959), and references cited therein.

(11) C. G. Overberger, T. St. Pierre, and S. Yaroslavsky, *J. Am. Chem. Soc.*, **87**, 4310 (1965).

(12) W. Stenstrom and N. Goldsmith, *J. Phys. Chem.*, 30, 1683 (1926).

(based on recovered starting material). The monomer underwent free-radical copolymerization with 4(5)-vinylimidazole in solution. It also polymerized in the solid state on standing at room temperature to give a polymer of $\overline{DP}$ *ca.* 40.

Copolymerization of 4(5)-vinylimidazole with vinyl acetate afforded copolymers of different compositions. It was found that the acetyl groups in these copolymers hydrolyzed conveniently in boiling water, the copolymer with the higher imidazole content at a faster rate.[13] The ease of hydrolysis of the acetyl groups is probably related to a neighboring group effect of the imidazole functions.

Kinetics. The reactions of imidazole-containing polymers with substrates have been shown to be first order in catalyst and first order in substrate (for the concentration ranges investigated). Similar results were obtained for the 1:1.95 copolymer of 4(5)-vinylimidazole and *p*-vinylphenol. The first-order observed rate constants for the solvolyses of PNPA catalyzed by imidazole, by poly-4(5)-vinylimidazole, and by the 1:1.95 copolymer of 4(5)-vinylimidazole and *p*-vinylphenol in 80% methanol–water are reported in Table I. It is apparent that the imidazole–phenol copolymer greatly enhances the solvolytic rate of PNPA as the pH of the solution is increased. This strong enhancement was not observed with either poly-4(5)-vinylimidazole or imidazole, both of which had nearly identical reactivities in the pH region studied. It was also observed that neither phenol, nor poly-*p*-vinylphenol, nor the 1:0.48 copolymer of 4(5)-vinylimidazole and *p*-methoxystyrene affected the solvolytic rate of PNPA to a detectable extent.

Table I. First-Order Observed Rate Constants for 1:1.95 Imidazole–Phenol Copolymer, Poly-4(5)-vinylimidazole, and Imidazole Catalyzed Solvolyses of PNPA[a]

	$k_{obsd} \times 10^4$, min^{-1}		
pH	1:1.95 copolymer of 4(5)-vinylimidazole and *p*-vinylphenol	Poly-4(5)-vinylimidazole	Imidazole
7.4	3.0	2.1	2.6
8.2	5.1	3.0	2.4
9.1	28.6	3.2	2.7

[a] In 80% methanol–water at an ionic strength of 0.02.

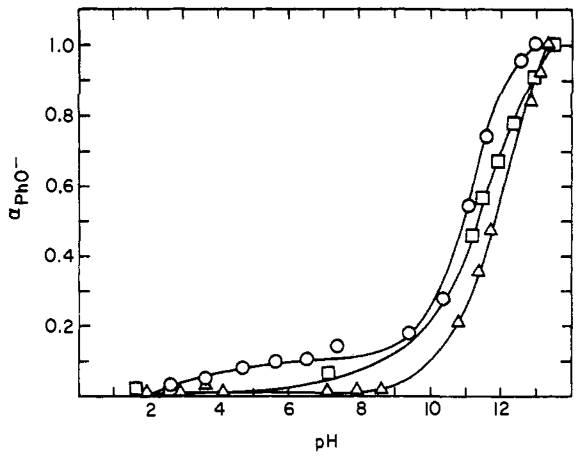

Figure 1. Spectrophotometric titrations. Plots of α_{PhO^-} *vs.* pH for phenol (○) and the phenol groups in poly-*p*-vinylphenol (△) and in the 1:1.95 copolymer of 4(5)-vinylimidazole and *p*-vinylphenol (□) in 80% methanol–water, μ = 0.02.

The second-order catalytic rate constant for the reaction of imidazole with PNPA in 80% methanol–water at pH 9.1 can be calculated as 1.1 l./mole min. The same constant in 28.5% ethanol–water at pH 9.0 is 17 l./mole min.[3] Bruice and Lapinsky determined the catalytic rate constant for the reaction of phenolate ions with PNPA in 28.5% ethanol–water to be 19 l./mole min.[14] According to the ultraviolet titrations performed, the fractions of anionic phenol in 80% methanol–water at pH 9 are *ca.* 0.10, 0.14, and 0.04 for the imidazole–phenol copolymer, phenol, and poly-*p*-vinylphenol, respectively. Assuming a similar rate depression to that of imidazole in 80% methanol–water, at the practical concentrations used, no significant solvolytic rates due to the reaction of phenolate ions should be detected in the pH region studied. Indeed, no appreciable solvolytic reactions were observed with either phenol (pK_a = 10.9) or poly-*p*-vinylphenol (pK_a = 11.9) with PNPA up to pH 9.1. The high solvolytic rate observed for the imidazole–phenol copolymer (pK_a's of phenol and imidazole[15] groups of 11.3 and 5.6, respectively) cannot be attributed to an additive contribution of the phenolate ions to the imidazole-catalyzed reaction.[16] Furthermore, since there was no significant rate of solvolysis of PNPA by the 1:0.48 copolymer of 4(5)-vinylimidazole and *p*-methoxystyrene (where there are no phenoxide groups present), the pH–rate profiles (for data in Table I) indicate that a contribution of bifunctional imidazole–phenolate catalysis exists. The catalytic rate constant for the reaction of the copolymer with PNPA can then be described as a composite of the following terms[3,11]

$$k_{cat} = k_1\alpha_{IM} + k_2\alpha_{IM}^2 + k_3\alpha_{IM}\alpha_{IM^-} + k_4\alpha_{IM}\alpha_{PhO^-} + k_5\alpha_{PhO^-}$$

where α_{IM} is the fraction of neutral imidazole, α_{IM^-} that of anionic imidazole, and α_{PhO^-} that of anionic phenol. The over-all contribution of the first three terms can be estimated from the observed rate constants of imidazole and poly-4(5)-vinylimidazole (Table I), while $k_5\alpha_{PhO^-}$ must be negligible in the pH region studied. Therefore, k_4 can be estimated as *ca.* 100 l./mole min. Three mechanisms may describe the bifunctional action of the phenolate ions and the imidazole groups.

These conclusions were further substantiated by the action of the imidazole–phenol copolymer on the negatively charged substrates. The first-order observed rate constants for the solvolyses of NABA and NABS,

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[13] N. Vorchheimer, Ph.D. Thesis, Polytechnic Institute of Brooklyn, 1962.

[14] T. C. Bruice and R. Lapinsky, *J. Am. Chem. Soc.*, 80, 2265 (1958).

[15] Determined potentiometrically in 80% methanol–water according to the procedure in ref 3.

[16] Under similar experimental conditions a copolymer of *p*-vinylphenol and 1-vinyl-3-methylimidazolium iodide, with an apparent pK_a of the phenol groups of 10.2, was observed to have no measurable catalytic effect on PNPA or ANTI.[17]

[17] H. Morawetz, C. G. Overberger, J. C. Salamone, and S. Yaroslavsky, *J. Am. Chem. Soc.*, in press.

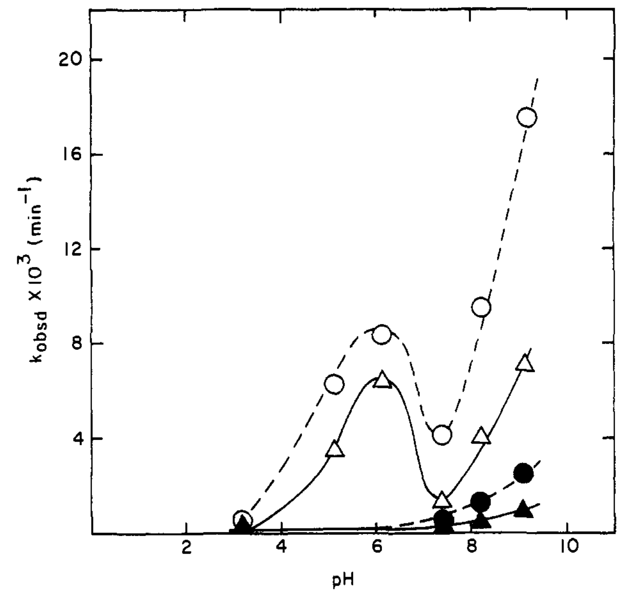
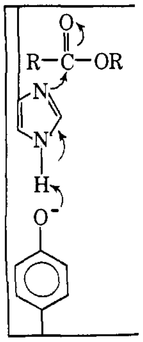


Figure 2. pH–rate profiles for the solvolyses of NABA and NABS catalyzed by 1:1.95 copolymer of 4(5)-vinylimidazole and *p*-vinylphenol (△, ○) and imidazole (▲, ●), respectively, in 80% methanol–water, μ = 0.02.

each catalyzed by the imidazole–phenol copolymer, by poly-4(5)-vinylimidazole, and by imidazole, are reported in Tables II and III, respectively. The data for the imidazole–phenol copolymer and imidazole catalyzed reactions are represented in Figure 2. Although neither phenol nor poly-*p*-vinylphenol affected the solvolytic rates of NABA and NABS at pH 9.1, the first-order observed rate constants for the 1:0.48 copolymer of 4(5)-vinylimidazole and *p*-methoxystyrene catalyzed solvolyses of NABA and NABS at pH 9.2 are 1.1 and 2.9 × 10^{-3} min^{-1}, respectively.



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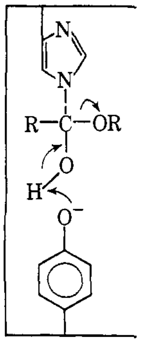

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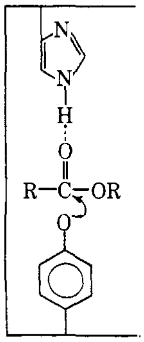

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Table II. First-Order Observed Rate Constants for 1:1.95 Imidazole–Phenol Copolymer, Poly-4(5)-vinylimidazole, and Imidazole Catalyzed Solvolyses of NABA[a]

	$k_{obsd} \times 10^3$, min^{-1}		
pH	1:1.95 copolymer of 4(5)-vinyl imidazole and *p*-vinylphenol	Poly-4(5)-vinyl-imidazole	Imidazole
3.3	0.2	0.1	0.1
5.1	3.5	5.2	...
6.1	6.4	6.9	...
7.4	1.3	3.6	0.3
8.2	4.0	1.5	0.5
9.1	7.1	1.0	1.0

[a] In 80% methanol–water at an ionic strength of 0.02.

Table III. First-Order Observed Rate Constants for 1:1.95 Imidazole–Phenol Copolymer, Poly-4(5)-vinylimidazole, and Imidazole Catalyzed Solvolyses of NABS[a]

	$k_{obsd} \times 10^3$, min^{-1}		
pH	1:1.95 copolymer of 4(5)-vinyl-imidazole and *p*-vinylphenol	Poly-4(5)-vinyl-imidazole	Imidazole
3.3	0.4	0.7	0.0
5.1	6.2	8.9	...
6.1	8.3	10.3	...
7.4	4.1	6.9	0.5
8.2	9.5	3.8	1.2
9.1	17.5	1.3	2.5

[a] In 80% methanol–water at an ionic strength of 0.02.

The shapes of the pH–rate profiles for the imidazole–phenol copolymer catalyzed reactions are similar to that obtained for the reaction of NABS with poly-5(6)-vinylbenzimidazole.[11] The bell-shaped portions of the curves in Figure 2 must represent the solvolytic rates as a result of the electrostatic attraction of the negatively charged substrates to the partly protonated imidazole functions on the polymer chain.[11,18] At high pH values poly-5(6)-vinylbenzimidazole was found highly reactive toward negatively charged NABS, while no reaction ensued with monomeric benzimidazole anions (presumably due to the electrostatic repulsion).[11] This difference was rationalized by the existence of terfunctional interactions among substrate and the pendent benzimidazole groups on the polymer chain. Similarly, no solvolytic reactions were observed at high pH between the anions of phenol or poly-*p*-vinylphenol and the negatively charged substrates. The fact that the imidazole–phenol copolymer revealed much higher solvolytic rates than either imidazole, poly-4(5)-vinylimidazole, or the copolymer of 4(5)-vinylimidazole and *p*-methoxystyrene at high pH values with the negatively charged esters can again be correlated to bifunctional catalysis by imidazole and phenolate ions. For the reaction of the imidazole–phenol copolymer with NABA and NABS, k_4 can be estimated as *ca.* 250 and 700 l./mole min, respectively.

Employing the positively charged substrate ANTI, it was noted that neither phenol, poly-*p*-vinylphenol, poly-4(5)-vinylimidazole, nor the 1:0.48 copolymer of 4(5)-vinylimidazole and *p*-methoxystyrene had any effect on the solvolytic rate in the pH region investigated. However, with this substrate the imidazole–phenol copolymer at pH 9.1 revealed a rate enhancement of *ca.* 63 times that of imidazole (Table IV). This rate enhancement must be partly due to the electrostatic attraction of ANTI to the anionic sites in the copolymer, thereby facilitating a solvolytic attack from either imidazole or phenolate ions, or from their combined action on the substrate. From the results of the reaction of ANTI with a copolymer of 4(5)-vinylimidazole and acrylic acid,[19] it can be concluded that at a

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(18) R. L. Letsinger and T. J. Savereide, *J. Am. Chem. Soc.*, **84**, 114, 3122 (1962); R. L. Letsinger and I. Klaus, *ibid.*, **86**, 3884 (1964).

(19) C. G. Overberger, R. Sitaramaiah, T. St. Pierre, and S. Yaroslavsky, *ibid.*, **87**, 3270 (1965).

Table IV. First-Order Observed Rate Constants for 1:1.95 Imidazole–Phenol Copolymer and Imidazole Catalyzed Solvolyses of ANTI[a]

	$k_{obsd} \times 10^3$, min^{-1}	
pH	1:1.95 copolymer of 4(5)-vinylimidazole and *p*-vinylphenol	Imidazole
7.4	0.1	0.1
8.2	2.1	0.2
9.1	37.7	0.6

[a] In 80% methanol–water at an ionic strength of 0.02.

concentration of phenolate ions of 10% (at pH 9.0) the first possibility would not appear in a high rate of reaction. The results obtained with poly-*p*-vinylphenol also rule out the second possibility. Bifunctional catalysis involving imidazole and phenolate ions with a substrate attracted to the copolymer chain by electrostatic forces, or a terfunctional reaction in which the frequency factor is enhanced due to the opposite charges of the reactants,[20] must account for the high rate.

Table V. First-Order Observed Rate Constants for 1:0.31 and 1:0.65 Copolymers of 4(5)-Vinylimidazole and Vinyl Alcohol, Poly-4(5)-Vinylimidazole, and Imidazole Catalyzed Solvolyses of PNPA[a]

	$k_{obsd} \times 10^3$, min^{-1}			
pH	1:0.31 copolymer of 4(5)-vinylimidazole and vinyl alcohol	1:0.65 copolymer of 4(5)-vinylimidazole and vinyl alcohol	Poly-4(5)-vinylimidazole	Imidazole
6.0	1.5	1.3	1.2	0.8
7.1	4.1	3.3	2.8	2.9
8.0	6.4	4.6	4.6	...
9.1	9.4	6.6	5.6	4.2

[a] In 28.5% ethanol–water at an ionic strength of 0.02.

Considerable rate enhancements over imidazole and poly-4(5)-vinylimidazole in 28.5% ethanol–water were observed for the reaction of the two copolymers of 4(5)-vinylimidazole and vinyl alcohol with PNPA. The more obvious increase was with the copolymer of the higher imidazole content (Table V). In Figure 3 the first-order observed rate constants are plotted against pH. This plot is analogous to that previously reported for the imidazole and poly-4(5)-vinylimidazole catalyzed solvolyses of PNPA in that polymeric imidazole is a more efficient catalyst than imidazole at increased pH.[3]

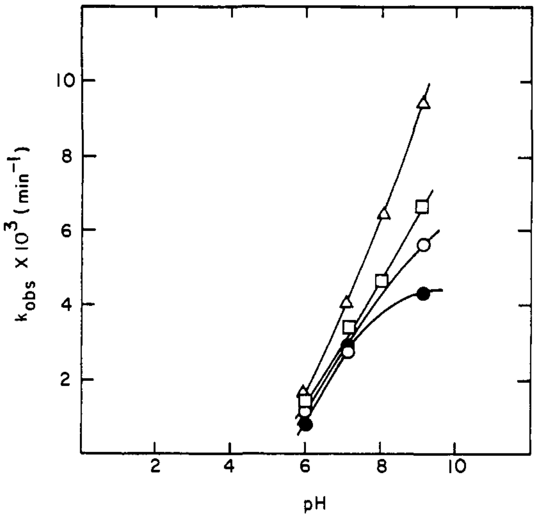

Figure 3. pH–rate profiles for the solvolyses of PNPA catalyzed by imidazole (●), poly-4(5)-vinylimidazole (○) and 1:0.31, (△) and 1:0.65 (□) copolymers of 4(5)-vinylimidazole and vinyl alcohol in 28.5% ethanol–water, μ = 0.02.

It is possible that these enhanced reactivities are due to a modification of the apparent dissociation constants of the hydroxyl functions in the copolymers. Bruice and co-workers have shown that the enhanced reactivity of N-acetylserinamide is a result of the lowered pK_a of the hydroxyl group.[21] In analogy, the apparent pK_a of the hydroxyl groups in a copolymer of vinyl alcohol and 1-vinyl-3-methylimidazolium iodide[17] could be expected to be affected to a larger extent than in the copolymers of 4(5)-vinylimidazole and vinyl alcohol. However, no significant enhanced reactivity of the quaternized imidazole–alcohol copolymer was observed with its reaction with PNPA under experimental conditions similar to those employed in this investigation. Therefore, in the copolymers of 4(5)-vinylimidazole and vinyl alcohol, the possibility of some bifunctional contributions involving imidazole or imidazole anions and the hydroxyl function cannot be overruled. Some sigmoid pH–rate profiles of α-chymotrypsin catalyzed reactions, if not describing the rate-determining deacylation step,[7b] can be interpreted as a result of the interactions of imidazole and later imidazole anions with the hydroxyl function.[22]

The imidazole–alcohol copolymers also exhibited enhanced activities in the catalyzed solvolyses of the negatively charged esters NABA (Table VI, Figure 4) and NABS (Table VII, Figure 5). At pH 6, the solvolytic rates of NABA and NABS catalyzed by the 1:0.31 copolymer of 4(5)-vinylimidazole and vinyl alcohol are nearly twice those of the poly-4(5)-vinyl-

Table VI. First-Order Observed Rate Constants for 1:0.31 and 1:0.65 Copolymers of 4(5)-Vinylimidazole and Vinyl Alcohol, Poly-4(5)-Vinylimidazole, and Imidazole Catalyzed Solvolyses of NABA[a]

	$k_{obsd} \times 10^2$, min^{-1}			
pH	1:0.31 copolymer of 4(5)-vinylimidazole and vinyl alcohol	1:0.65 copolymer of 4(5)-vinylimidazole and vinyl alcohol	Poly-4(5)-vinylimidazole	Imidazole
4.1	0.6	0.5	0.4	...
6.0	4.6	3.6	2.8	0.2
7.1	4.4	3.1	2.9	0.5
8.0	2.7	1.6	1.6	...
9.1	...	...	...	0.8
10.3	1.0	0.6	1.0	...

[a] In 28.5% ethanol–water at an ionic strength of 0.02.

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(20) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1961, p 145.

(21) T. C. Bruice, T. H. Fife, J. J. Bruno, and N. E. Brandon, *Biochemistry*, 1, 7 (1962).

(22) See footnote 19 in T. C. Bruice and G. E. Schmir, *J. Am. Chem. Soc.*, 80, 148 (1958).

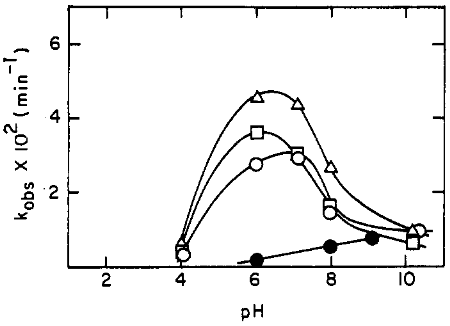

Figure 4. pH-rate profiles for the solvolyses of NABA catalyzed by imidazole (●), poly-4(5)-vinylimidazole (○), and 1:0.31 (△) and 1:0.65, (□) copolymers of 4(5)-vinylimidazole and vinyl alcohol in 28.5% ethanol-water, μ = 0.02.

Table VII. First-Order Observed Rate Constants for 1:0.31 and 1:0.65 Copolymers of 4(5)-Vinylimidazole and Vinyl Alcohol, Poly-4(5)-vinylimidazole, and Imidazole Catalyzed Solvolyses of NABS[a]

	$k_{obsd} \times 10^2$, min^{-1}			
pH	1:0.31 copolymer of 4(5)-vinylimidazole and vinyl alcohol	1:0.65 copolymer of 4(5)-vinylimidazole and vinyl alcohol	Poly-4(5)-vinylimidazole	Imidazole
4.1	1.2	1.3	0.9	0.0
6.0	10.1	8.2	6.1	0.3
7.1	9.4	7.4	6.2	1.2
8.0	5.7	3.4	3.4	1.3
9.1	...	...	...	1.6
10.3	3.3	2.2	2.7	...

[a] In 28.5% ethanol-water at an ionic strength of 0.02.

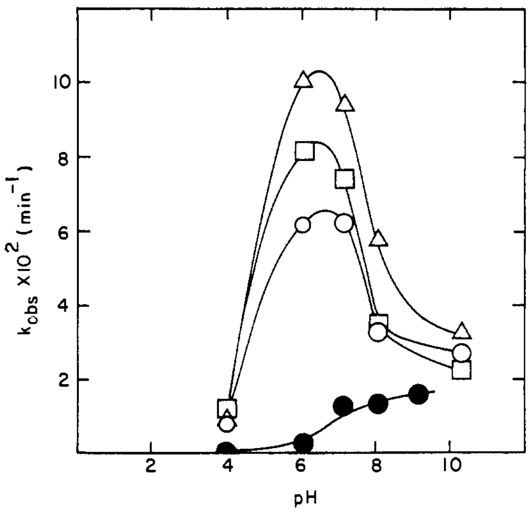

Figure 5. pH-rate profiles for the solvolyses of NABS catalyzed by imidazole (●), poly-4(5)-vinylimidazole (○), and 1:0.31 (△) and 1:0.65 (□) copolymers of 4(5)-vinylimidazole and vinyl alcohol in 28.5% ethanol-water, μ = 0.02.

imidazole catalyzed reactions. It is possible that the electrostatic attraction of the substrate to the protonated sites on the polymer chain will enhance the catalytic effect of the hydroxyl groups in a fashion similar to that of the imidazole groups. In several instances complexation has been demonstrated to increase the action of hydroxyl groups, *e.g.*, in cyclodextrins[23] and in cobalt-hydroxyl complexes.[24] Furthermore, the intramolecular assistance of hydroxyl groups or hydroxyl anions has been proven in several instances.[25] Since we have observed that a copolymer of 1-vinyl-3-methylimidazolium iodide and vinyl alcohol had no enhanced effect on the solvolytic rates of NABA and NABS,[17] it is possible that a bifunctional contribution of imidazole and hydroxyl functions also becomes apparent as a result of the electrostatic contribution of the substrate to the polymeric catalyst. An alternative possibility is that a proper spacing of the imidazole groups on the polymer chain (caused by a dilution with inert functional groups) could perhaps increase the over-all catalytic capabilities of the polymer. This possibility is now being investigated by diluting the imidazole functions with varying concentrations of inert groups.

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[23] F. Cramer and W. Kampe, *J. Am. Chem. Soc.*, **87**, 1115 (1965); H. Hennrich and F. Cramer, *ibid.*, **87**, 1121 (1965).

[24] J. P. Collman and D. A. Buckingham, *ibid.*, **85**, 3039 (1963).

[25] T. C. Bruice and F. Marquart, *ibid.*, **84**, 365 (1962); L. Zürn, *Ann.*, **613**, 56 (1960).