Deacylation Behavior of *N*-Acylpoly[4(5)-vinylimidazole] and Its Consequences

C. G. Overberger* and R. C. Glowaky

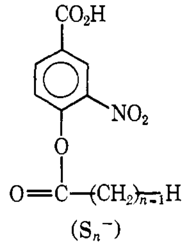
Contribution from the Department of Chemistry
and the Macromolecular Research Center, The University of Michigan,
Ann Arbor, Michigan 48104. *Received March 15, 1973*

Abstract: In certain cases (with the polymeric catalyst), the hydrolysis of 3-nitro-4-acyloxybenzoic acid substrates (S_n^-) catalyzed by poly[4(5)-vinylimidazole] (PVIm) and imidazole (Im) displayed a deviation (accelerative) from the expected pseudo-first-order kinetic behavior. This deviation was attributed to an appreciable buildup of a partially acylated PVIm intermediate which is relatively long lived and is more hydrophobic than the original catalyst. The rates of acylimidazole formation and dissipation were determined for various systems. In all cases where accelerative kinetic behavior was observed, a knowledge of the relative rates of acylation and deacylation allow one to predict a buildup of acylated catalyst. The presence of this acylated-catalyst intermediate and the fact that it is more hydrophobic than the original catalyst were demonstrated by double addition (of substrate) experiments and by destruction of the intermediate by the addition of hydroxylamine to the reaction solution.

The dramatic rate enhancing effect of apolar forces on the esterolytic action of polymeric imidazole[1-3] and other synthetic macromolecular catalysts[4,5] is now well documented. In the accompanying paper[6] we reported large rate enhancements for the poly[4(5)-vinylimidazole] (PVIm) catalyzed hydrolyses of long-chain esters, relative to monomeric imidazole (Im). During the course of this study, an autocatalytic or accelerative phenomena was observed. In this paper we report evidence detailing the involvement of the acylated PVIm species in the accelerative hydrolysis. This unusual phenomena is in principle reminiscent of the allosteric effect[7] observed in certain enzyme systems. The deacylation behavior of the *N*-acylpoly[4(5)-vinylimidazole] permits this insight into the total reaction scheme for polymeric imidazole catalysis.

Results and Discussion

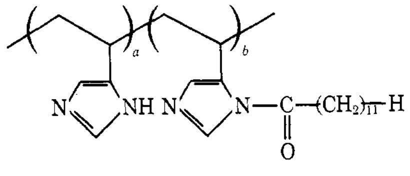
Accelerative Kinetic Behavior. In the course of the study of the poly[4(5)-vinylimidazole] (PVIm) catalyzed hydrolysis of S_n^-, an unusual kinetic behavior was sometimes observed. The hydrolysis conditions used in this study were generally pseudo first order, *i.e.*, the catalyst was in excess (~tenfold) of substrate. Under these conditions, treatment of the raw data from a kinetic run as a plot of ln ($OD_\infty - OD_t$) *vs.* time should define a straight line. The slope of this line will then be $-k_{obsd}$, the pseudo-first-order rate constant (after subtracting the blank rate). This was indeed the case for *all* the Im catalyzed hydrolyses of all the substrates investigated and also for the PVIm catalyzed hydrolysis of S_2^-. However, an anomalous kinetic behavior was frequently observed for the PVIm catalyzed hydrolyses of the long-chain substrates as mentioned in the previous paper.[6] The pseudo-first-order rate plots deviated in such a way that the rate constant (as determined by the slope of the tangent at any point along the curve) increased throughout the course of the hydrolysis. This indicated that some form of autocatalysis was occurring during the reaction. A typical example of this *accelerative kinetic behavior* is described in Figure 1. The final rate constant (~90% reaction, k_{obsd} = 0.126 min^{-1}) was about five times faster than the initial rate constant (<10% reaction, k_{obsd} = 0.022 min^{-1}).



(S_n^-)

n = 2, 7, 12, and 18

An accelerative behavior was also recognized by Overberger and Morimoto[1] under reaction conditions similar to those in Figure 1. They speculated that this behavior, which was not fully characterized, was due to the formation of a partially dodecanoylated PVIm intermediate (PVIm-Ac_{12}) during the hydrolysis.



PVIm-Ac_{12}, where a/b ~ 10/1

While pursuing this postulate, we found that under conditions which gave an accelerative pattern for the hydrolysis of S_{18}^- (30 vol % 1-propanol–water) both S_{18}^a and S_{18}^b also gave the accelerative kinetic pattern.

---

[1] C. G. Overberger and M. Morimoto, *J. Amer. Chem. Soc.*, **93**, 3222 (1971); C. G. Overberger, M. Morimoto, I. Cho, and J. C. Salamone, *ibid.*, **93**, 3228 (1971).

[2] C. G. Overberger, M. Morimoto, I. Cho, and J. C. Salamone, *Macromolecules*, **2**, 553 (1969).

[3] C. Aso, T. Kunitake, and F. Shimada, *J. Amer. Chem. Soc.*, **91**, 2716 (1969); T. Kunitake and S. Shinkai, *ibid.*, **93**, 4247, 4256 (1971).

[4] Y. E. Kirsh, V. A. Kabanov, and V. A. Kargin, *Dokl. Chem.*, 177, 976 (1967); S. K. Pluzhnov, Y. E. Kirsh, V. A. Kabanov, and V. A. Kargin, *ibid.*, **185**, 291 (1969).

[5] I. M. Klotz and V. H. Stryker, *J. Amer. Chem. Soc.*, **90**, 2717 (1968); I. M. Klotz and G. P. Royer, *ibid.*, **91**, 5885 (1969); I. M. Klotz, G. P. Royer, and A. R. Sloniewsky, *Biochemistry*, **8**, 4752 (1969); I. M. Klotz, G. P. Royer, and I. S. Scarpa, *Proc. Nat. Acad. Sci. U. S.*, **68**, 263 (1971).

[6] C. G. Overberger, R. C. Glowaky, and P. H. Vandewyer, *J. Amer. Chem. Soc.*, **95**, 6008 (1973).

[7] J. Monod, J. Wyman, and J.-P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965); D. Blangy, H. Buc, and J. Monod, *ibid.*, **31**, 13 (1968).

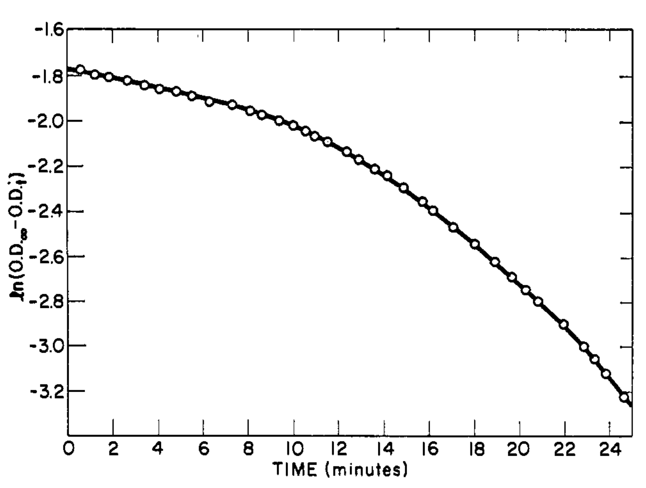
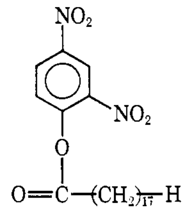
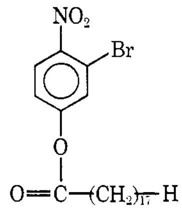


Figure 1. Accelerative pseudo-first-order rate plot: 40 vol % EtOH-H$_2$O, [PVIm] = 5.0×10^{-4} M, [S$_{12}^-$] = 5.0×10^{-5} M, μ = 0.02, [Tris] = 0.02 M, pH 8.0, 26°.

This result makes it doubtful that the negative charge on S_{18}^- is responsible for the acceleration; rather, it lends support to the involvement of the acylated polymeric intermediate which is identical for $S_{18}{}^a$, $S_{18}{}^b$, and S_{18}^-.



$S_{18}{}^a$



$S_{18}{}^b$

The possibility that the long-chain acid product was responsible for the accelerative behavior had to also be considered. By remaining noncovalently bound to the polymer after deacylation, the long-chain acid could cause the polymer to become more nonpolar, resulting in an autocatalysis.

Figure 2 shows a series of experiments in which a second aliquot of either S_7^- or S_{12}^- was added to a kinetic run exhibiting the accelerative behavior. The PVIm catalyzed hydrolysis of S_{12}^- in 33 vol % ethanol-water was used as the accelerative case. In case A, the additions were made immediately after the accelerative hydrolysis; in case B, 24 hr after the first reaction. Presumably, complete deacylation of the intermediate should occur during the 24-hr period. For both the hydrolyses of added S_7^- and S_{12}^- in case B, the form and rate of the hydrolyses were *identical* with those observed for the PVIm catalyzed hydrolyses (in case B for S_7^-, $k_{\mathrm{obsd}} = 0.034$ min^{-1}; for PVIm catalysis of S_7^-, $k_{\mathrm{obsd}} = 0.032$ min^{-1}). In case A, however, the rate of hydrolysis of added S_7^- was over twice that observed in case B (0.072 min^{-1} in case A *vs.* 0.034 min^{-1} in case B). These results indicate that the products of the hydrolysis of S_{12}^- do not affect any subsequent hydrolysis. Further, they indicate that the intermediate formed during the hydrolysis (a partially dodecanoylated PVIm) can be responsible for the accelerative behavior.

Because this anomalous kinetic pattern represented a very interesting (and not very common) autocatalytic pattern, we undertook the task of completely defining the cause of the accelerative behavior. Further, it seemed to be intrinsically linked to apolar forces in PVIm catalysis.

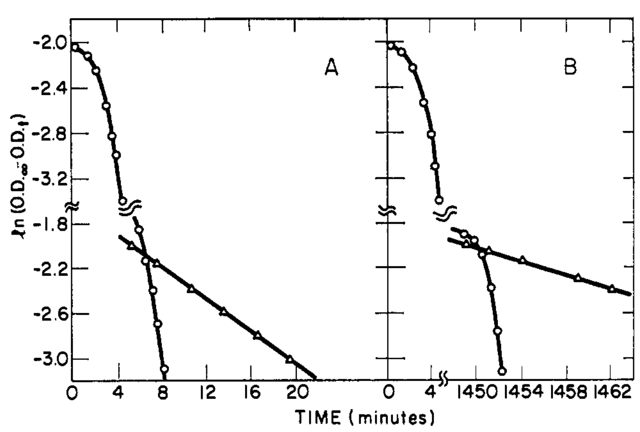


Figure 2. Effect of adding S_{12}^- (○) or S_7^- (△) to typical accelerative case (PVIm catalyzed hydrolysis of S_{12}^- in 33 vol % EtOH-H$_2$O): case A, immediately after reaction; case B, 24 hr after reaction.

Deacylation Rates. To establish unequivocally whether or not the acylated intermediate was accumulating in a particular hydrolytic system, a knowledge of the deacylation behavior of the polymeric intermediate was necessary. In principal, it should be possible to detect the intermediate spectrophotometrically. Bender and Turnquest were able to detect at 245 mμ the acetylimidazole (Im-Ac$_2$) formed during the Im catalyzed hydrolysis of *p*-nitrophenyl acetate (PNPA), and even measured the deacetylation rate.[8] Such a procedure, however, is precluded in the present system by an absorption of the 3-nitro-4-hydroxybenzoic acid product at 260 mμ which overlaps the acylated PVIm absorption at 260 mμ.

A buildup of the acylated PVIm intermediate during the hydrolysis of S_{18}^- in 30 vol % 1-propanol-water (a case exhibiting accelerative kinetics) was suggested while following the hydrolysis reaction at 260 mμ. Figure 3 shows that the optical density *vs.* time plot demonstrated a maximum, which decreased slightly to a constant value. This observation may be interpreted as being due to addition of the acylimidazole and phenolate chromophores to create a maximum. The OD then decreased to a constant value at ∞ time due only to the phenolate. Further, during the PVIm catalyzed hydrolysis of S_2^- in the same solvent system, no maximum was observed indicating that no buildup of the intermediate was occurring in this case.

In order to explain the above results and to aid in elucidating the cause of the anomalous kinetic behavior, the deacylation rates (k_{deacyl}) of various acylated PVIms (PVIm-Ac$_n$) were measured. This represents the first reported direct measurement of the deacylation behavior of a polymeric imidazole species. The literature contains several instances of speculation on the deacylation behavior of polymeric catalyst species,[1-5] but only reports calculated deacylation rates obtained from cases showing saturation kinetics.[3,4] A knowledge of the deacylation rates is necessary information in order to completely define the turnover behavior of this catalytic system.

---

(8) M. L. Bender and B. W. Turnquest, *J. Amer. Chem. Soc.*, 79, 1652, 1656 (1957).

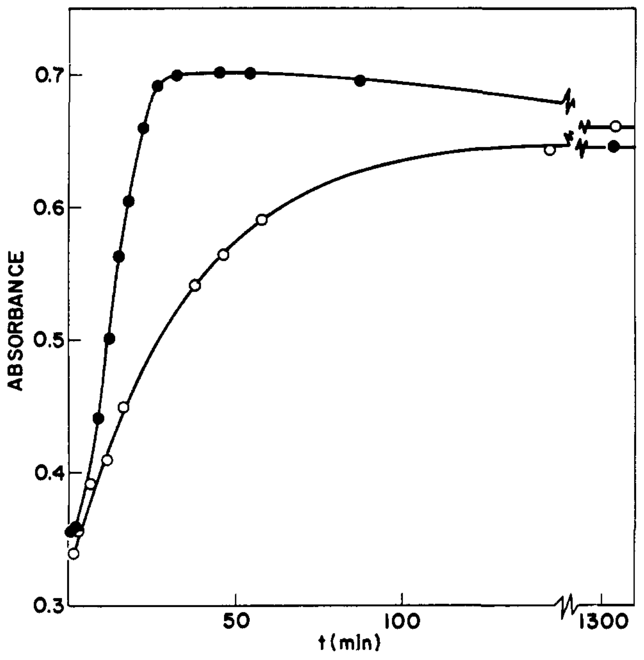
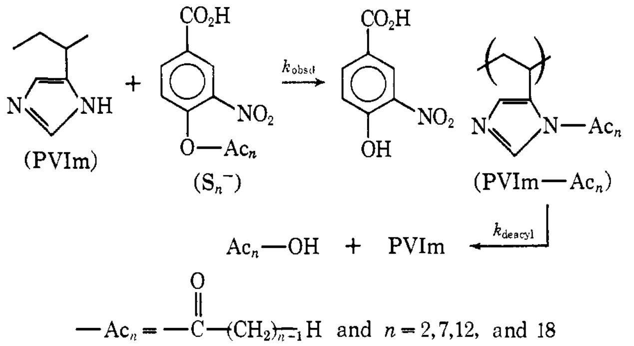


Figure 3. Unusual absorbance behavior observed during the PVIm catalyzed esterolysis of S_{18}^- (—○—) as compared to S_2^- (—●—) (λ 256 mμ): [PVIm] = 5.00×10^{-4} *M*, [S] = 5×10^{-5} *M*, *T* = 26°, μ = 0.02, pH 7.80.

By measurement of the hydrolytic (k_{obsd}) and deacylation (k_{deacyl}) behavior of the PVIm catalyzed systems being discussed here, it was possible to completely define the total reaction scheme for hydrolysis of S_n^- (see Scheme I). The deacylation rates were measured directly by two independent techniques. These two techniques are discussed in detail in the following sections.

Scheme I



$$—Ac_n = —\overset{O}{\overset{\|}{C}}—(CH_2)_{n-1}H \text{ and } n = 2, 7, 12, \text{ and } 18$$

1. Deacylation from Sephadex Isolated Samples. The first technique employed for the measurement of the deacylation rates of the PVIm-Ac_n intermediate (see Scheme II, path A) took advantage of the polymeric nature of the species. It was possible to separate the partially acylated intermediate from the low molecular weight products by utilizing gel permeation chromatography (Sephadex LH-20) on a typical kinetic run (see Experimental Section for details of the procedure used). In this type of chromatography, separation is effected according to size of the molecules passing through the column. Thus, the species with the highest molecular weight will elute first; in this case it was PVIm-Ac_n.

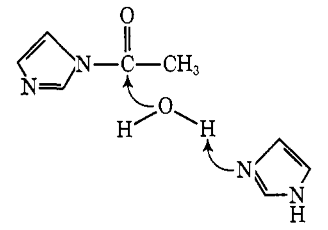
Jencks and later Fife studied the deacylation behavior of various acylimidazoles.[9-11] The hydrolyses were reported to be first-order processes, proceeding principally through the acylimidazolium ions.[12] When the data obtained from the Sephadex-separated, partially acylated PVIm samples (4–38% acylated) were treated as first-order kinetics, a deviation from first-order behavior was obtained. The deviation was accelerative in nature and most apparent from the samples 20% acylated or more. In Table I are reported the deacylation rate constants, k_{deacyl}, determined from the first-order plots at more than 90% deacylation completed. The deacylation of the last 10% of acylimidazoles on the polymer follows essentially first-order kinetics.

Table I. First-Order Rate Constants for Hydrolysis (k_{obsd}) and Deacylation (k_{deacyl}) Reactions[a]

Substrate	k_{obsd}, min^{-1} [b]	Intermediate	k_{deacyl}, min^{-1} [c]
S_2^-	0.022	PVIm-Ac_2	0.250
S_7^-	0.013	PVIm-Ac_7	0.263
S_{12}^-	0.090[d]	PVIm-Ac_{12}	0.041
S_{18}^-	0.500[d]	PVIm-Ac_{18}	0.006

[a] 40 vol % ethanol–water, μ = 0.02, [Tris] = 0.02 *M*, pH 8.0, 26°. [b] [PVIm] = 5.0×10^{-4} *M*, [S_n^-] = 5.0×10^{-5} *M*. [c] Determined for >90% deacylation completed; from Sephadex-isolated samples. [d] Accelerative kinetic behavior.

The accelerative deacylation behavior can be best explained by intramolecular imidazole catalysis of the deacylation. Jencks and Carrioulo reported that a high concentration of imidazole can catalyze the deacetylation of acetylimidazole.[9] The most likely mechanism of the reaction involves proton abstraction by imidazole from a water molecule in the transition state (I).



I

In the present case, as the deacylation proceeds, the local imidazole concentration along the polymer chain is increased, resulting in an increase in the deacylation rate. A possible mechanism is shown for two steps of the deacylation in Scheme III. It must also be considered possible for remote, Im group catalysis of the deacylation to contribute to the observed rates.

Intramolecular catalysis is supported by the observation that the deacylation rate constant is *independent* of the concentration of the polymer, within experimental error. The effect of a catalytically active comonomer group on the reaction of a dissolved chain molecule is well documented.[13a] Classic examples

---

(9) W. P. Jencks and J. Carrioulo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).

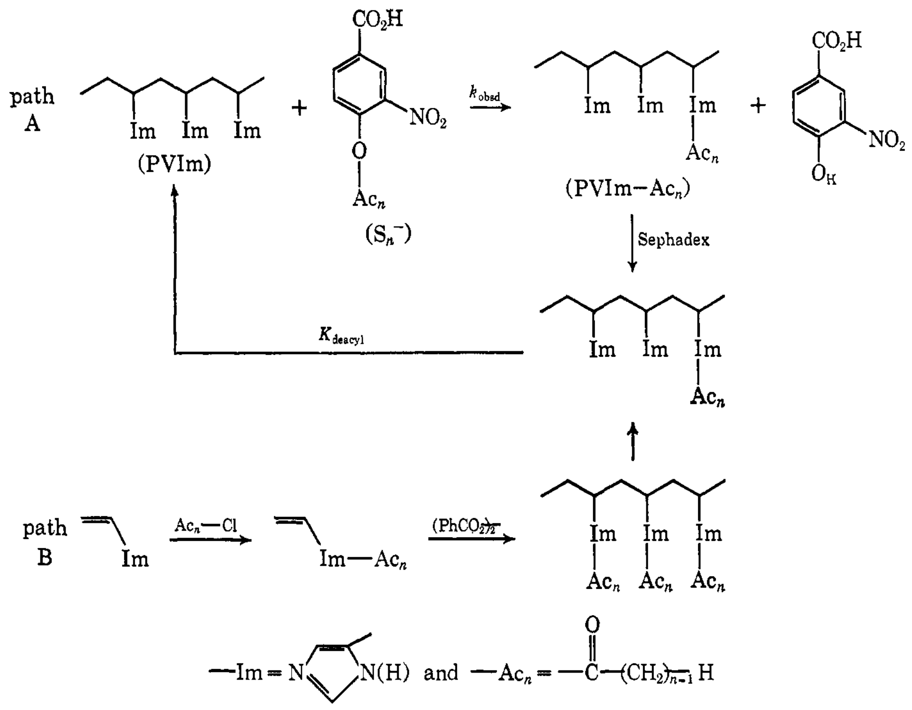
(10) T. H. Fife, *J. Amer. Chem. Soc.*, **87**, 4597 (1965).

(11) J. A. Fee and T. H. Fife, *ibid.*, **88**, 2343 (1966).

(12) R. Wolfden and W. P. Jencks, *ibid.*, **83**, 4390 (1961); D. G. Oakenfull and W. P. Jencks, *ibid.*, **93**, 178, 188 (1971).

(13) (a) See H. Morawetz, "Macromolecules in Solution," Interscience, New York, N. Y., 1965, pp 422–434, for a discussion of intramolecular catalysis in polymeric systems. (b) N. Goodman and H. Morawetz, *J. Polym. Sci., Part C*, **31**, 177 (1971); *J. Polym. Sci., Part A-2*, **9**, 1657 (1971).

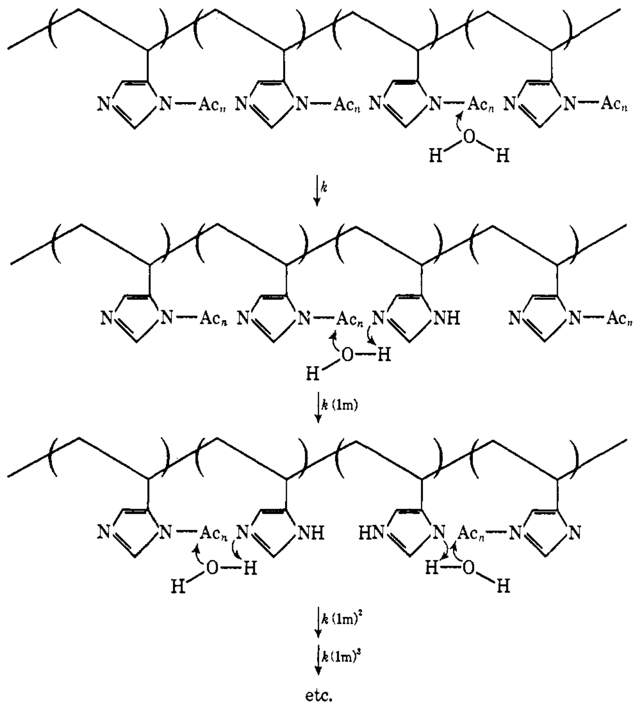
Scheme II



include the hydrolysis of poly(vinyl acetate) to poly(vinyl alcohol) and the hydrolysis of acrylamide–acrylic acid copolymers to give poly(acrylic acid). Further, a recent example describes the internal catalysis of a small number of reactive nitrophenyl ester groups by catalytic pyridine residues attached to a vinyl polymer backbone.[13b]

The deacylation rates showed an unusually large dependence on the chain length of the acyl group. The rate decreased by a factor of approximately 40 as a result of increasing the chain length of the acyl group from 2 to 18 carbon atoms. This observation must be due to factors other than increased steric hindrance to deacylation. Fife showed that alkaline or Im catalyzed hydrolysis of a series of *N*-acylimidazoles was not affected by branching at the α carbon of the acyl group, and only slightly retarded due to branching at the β carbon.[10] One possible explanation for the results observed in this study is a coiling of the long-chain, acyl group causing exclusion of a water molecule from the vicinity of the carbonyl. A second similar explanation pictures the long-chain, acylimidazole group becoming buried in the hydrocarbon coil of the polymer backbone.

Scheme III



2. Deacylation from the Authentic Sample. The second technique employed for the measurement of the deacylation rates was unequivocal synthesis of 1-acylpoly[4(5)-vinylimidazole] (see Scheme II, path B). This route permitted confirmation of the data obtained from the Sephadex-separated samples (path A). Also, the *N*-acylated-PVIm sample obtained from the synthetic route was totally acylated, permitting a closer look at the accelerative deacylation behavior.

The synthetic route used involved preparation of the appropriate *N*-acyl-4(5)-vinylimidazole (VIm-Ac_n) monomers. The acylated monomers were found to be unexpectedly resistant to free-radical solution polymerization. Under conditions from which the precursor, 4(5)-vinylimidazole (VIm), was almost quantitatively polymerized, no polymer was obtained from VIm-Ac_n, where n = 2, 7, 12, or 18. Further, attempted copolymerization of VIm and VIm-Ac_{18} (equimolar quantities) produced only a homopolymer of VIm.

Polymerization of VIm-Ac_7 was successfully accomplished by treatment of the melted monomer with aliquots of benzoyl peroxide over a period of 6 days (20% yield, see Experimental Section for details). From a knowledge of the factors affecting free-radical polymerizations,[14] and the expected inductive or resonance effects on the reactivity of the vinyl group from

---

(14) R. Lenz, "Organic Chemistry of High Polymer," Interscience, New York, N. Y., 1967.

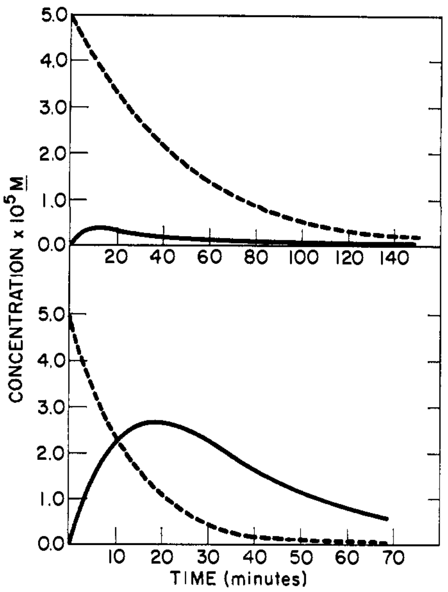


Figure 4. Variation in concentration of substrate and acylated intermediate with time: 40 vol % EtOH-H_2O, [PVIm] = 5.0 × 10^{-4} *M*. Top: $[S_2^-]_t$ (- - - - -), $[\text{PVIm-Ac}_2]_t$ (—). Bottom: $[S_{12}^-]_t$ (- - - - -), $[\text{PVIm-Ac}_{12}]_t$ (—); solid lines are calculated.

adding an *N*-acyl group to VIm, no explanation for the low reactivity of VIm-Ac$_n$ can be offered.

The deacylation rate of *N*-heptanoylpoly[4(5)-vinylimidazole] was continuously accelerative as previously discussed. The final rate of deacylation (k_{deacyl} = 0.251 min^{-1} for >90% deacylation completed) was about six times faster than the initial rate (k_{deacyl} = 0.038 min^{-1} for <10% deacylation completed) in 40 vol % ethanol-water. This result was independent of concentration of polymer which supports the intramolecular, Im catalyzed deacylation mechanism.

Table II summarizes the deheptanoylation data in varying vol % ethanol-water. The value in 40% (k_{deacyl} = 0.251 min^{-1}) agrees within experimental error with the deheptanoylation value obtained from the Sephadex-separated sample (k_{deacyl} = 0.263 min^{-1}). The deacetylation rate constant for acetylimidazole in 28.5 vol % ethanol-water at pH 7.1 is 0.017 min^{-1}. Thus, the intramolecular Im catalysis of the deacylation represents a rather significant (~tenfold) rate enhancement.

Table II. First-Order Rate Constants for Hydrolysis (k_{obsd}) and Deacylation (k_{deacyl}) in Varying Volume Per Cent Ethanol-Water

Vol % EtOH/ H_2O[a]	k_{obsd}, min^{-1} [b]	k_{deacyl}, min^{-1} [c]
20	0.696[d]	0.137
25	0.183[d]	0.166
40	0.013	0.251

[a] μ = 0.02, [Tris] = 0.02 *M*, pH 8.0, 26°, contains 7% THF (v/v). [b] [PVIm] = 5.0 × 10^{-4} *M*, $[S_7^-]$ = 5.0 × 10^{-5} *M*. [c] Determined for >80% deacylation completed, from authentic sample. [d] Accelerative kinetic behavior.

Acylated Poly(4-vinylimidazole) during the Hydrolysis of S_n^-. The measurement of the deacylation rates permits an overall view of the PVIm catalyzed hydrolysis of S_n^-. Scheme I shows that two steps are involved in the total catalytic mechanism. The first step involves an acyl transfer from the substrate to a pendant imidazole unit on the polymer chain; the second step is the deacylation of the intermediate to regenerate the catalyst.

Table I compares the pseudo-first-order rate constants for the disappearance of S_n^- (k_{obsd}) with the first-order hydrolysis of the acyl PVIm intermediate (k_{deacyl}) in 40 vol % ethanol-water. These data show that k_{obsd} is enhanced markedly for S_{12}^- and S_{18}^- as discussed in the previous paper.[6] However, the effect on the deacylation rate of the corresponding intermediates is in the opposite direction. The rates of deacylation are retarded as the chain length of the acyl group increases. Possible reasons for this observation have also previously been discussed.

The net result of these opposing effects is a change in the rate-determining step of the process. In 40 vol % ethanol-water, k_{obsd} is the rate-determining step for the hydrolyses of S_2^- and S_7^-. However, for the cases of S_{12}^- and S_{18}^-, k_{deacyl} is the slower process and is rate determining.

The data in Table II show that during the PVIm catalyzed hydrolytic process for S_7^- in varying vol % ethanol-water, a change in rate-determining step also results. In 40 vol %, k_{obsd} is rate determining; however, in 25 and 20 vol % ethanol-water k_{deacyl} becomes rate determining. This is indicative of the fact that by lowering the ethanol content of the solvent, k_{obsd} is enhanced due to the appearance of significant apolar forces. The effect on k_{deacyl} is the reverse, although not as dramatic.

A critical result of the above discussed change in rate-determining step is the effect on the concentration of the particular intermediate during the hydrolysis. Considering Scheme I under the conditions of the hydrolysis experiments, it is possible to calculate the variation in concentration of intermediate during the hydrolysis reaction.[15] If Scheme I is treated as a series of first-order reactions, only k_{obsd} and k_{deacyl} are necessary to obtain a description of the of the intermediate's concentration profile. Solution of the total rate expression for the intermediate, PVIm-Ac$_n$, during the hydrolysis, gave eq 1.

$$[\text{PVIm-Ac}_n]_t = \frac{[S_n^-]_0 k_{\text{obsd}}}{k_{\text{deacyl}} - k_{\text{obsd}}}(e^{-k_{\text{obsd}}t} - e^{-k_{\text{deacyl}}t}) \quad (1)$$

It is very significant that only in those cases in which k_{deacyl} is the rate-determining step is the accelerative behavior observed. In these cases, a relatively high concentration of the intermediate is present compared to the cases in which k_{obsd} is rate determining. The effect of the change in rate-determining step on the amount of the intermediate is shown graphically for two cases in Figure 4. The top graph shows that the intermediate during the hydrolysis of S_2^- is present in a small, steady-state concentration. No accelerative kinetic behavior is observed for this case. In the bottom graph of Figure 4, a case which does demonstrate an accelerative behavior, the intermediate is present in a significant concentration during the hydrolysis.

Catalysis by Partially Dodecanoylated Poly[4(5)-

---

[15] A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," Wiley, New York, N. Y., 1953, p 153.

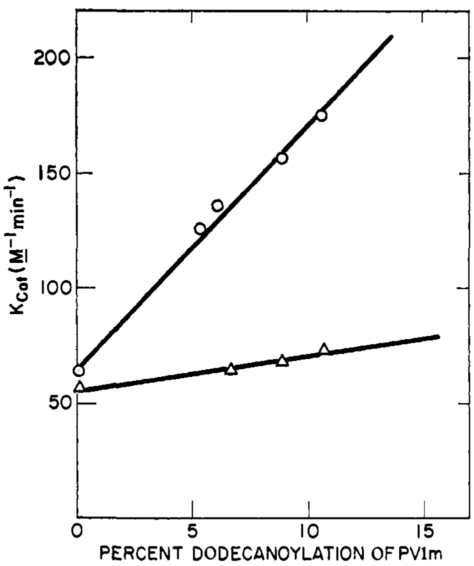


Figure 5. Catalysis by PVIm-Ac_{12} of S_7^- (○) and S_2^- (△): 33 vol % EtOH-H_2O, μ = 0.02, [Tris] = 0.02 *M*, pH 8.0, 26°.

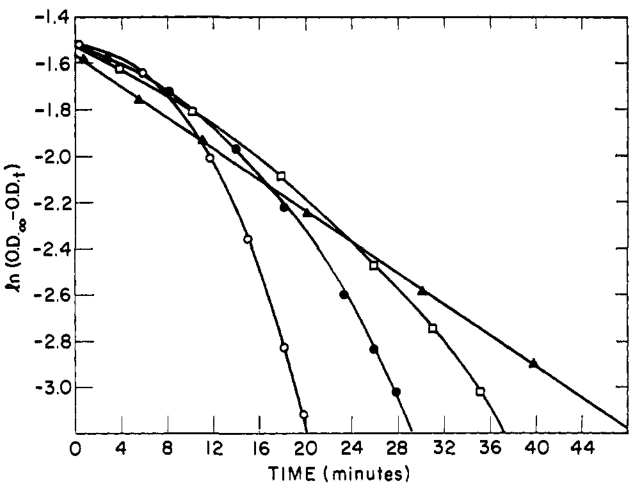


Figure 6. Effect of increasing concentration of hydroxylamine on accelerative behavior: 40 vol % EtOH-H_2O, [PVIm] = 5.0 × 10^{-4} *M*, [S_{12}^-] = 5.0 × 10^{-5} *M* (○); with [NH_2OH] = 3.0 × 10^{-4} *M* (●), 4.5 × 10^{-4} *M* (△), 11.1 × 10^{-4} *M* (▲).

vinylimidazole]. The coincidence of a buildup of long-chain, acylated PVIm intermediate in the cases exhibiting the accelerative behavior suggests that the intermediate may be responsible for the acceleration. However, this postulate demands that the intermediate be, in some way, a more effective catalyst for the hydrolysis of the substrate than unacylated PVIm. The acylimidazole moiety itself cannot be responsible for the enhancement occurring during the hydrolysis. This leaves the possibility that the long-chain, acyl intermediate is providing some physical role in the hydrolysis process.

Klotz and coworkers have demonstrated that derivatives of polyethylenimine which were acylated with butyryl, hexanoyl, or dodecanoyl groups showed increased complexation with methyl orange due to apolar forces. Also, the 10% dodecanoylated polyethylenimine was about 10^3 times more effective in catalyzing the hydrolysis of *p*-nitrophenyl laurate as polyethylenimine itself.[5] Also, Kabanov and coworkers demonstrated rate enhancements with poly(4-vinylpyridines) which had been over 90% alkylated with various hydrocarbon moieties.[4]

The above results suggest that it is very probable that increasing acylation of PVIm during the solvolysis of, *e.g.*, S_{12} could lead to the accelerative effect, if the intermediate is relatively stable. Earlier it was mentioned that when S_7^- was immediately hydrolyzed in the same cuvette in which S_{12}^- was first hydrolyzed (see Figure 2), the rate of hydrolysis of S_7^- was over two times faster than that of the normal PVIm catalyzed hydrolysis. If, however, 24 hr elapsed before the addition of S_7^-, the rate was the same as the normal PVIm catalyzed rate. With a quantitative knowledge about the presence of the intermediate in certain systems, this type of experiment could be exploited to test the catalytic efficiency of PVIm which was acylated to varying degrees.

The relative stability of PVIm-Ac_{12} in 33 vol % ethanol–water permitted the *in situ* preparation of PVIm samples which were dodecanoylated to various degrees; in this system $k_{\text{obsd}}/k_{\text{deacyl}}$ was about 20. The per cent dodecanoylation was varied simply by varying the initial [PVIm] to [S_{12}^-] ratio. Immediately after the S_{12}^- hydrolysis, an aliquot of S_7^- was added. Its hydrolysis rate, now catalyzed by PVIm-Ac_{12}, was measured. The same type of experiment was also performed with added S_2^-.

The results of these experiments are shown in Figure 5. It is evident that increasing dodecanoylation of the polymer leads to increasing catalytic efficiency for the hydrolyses of S_7^- and S_2^-. The effect is much more pronounced for S_7^-, indicating that apolar forces are responsible. When PVIm was greater than 25% dodecanoylated in 33 vol % ethanol–water, a small amount of turbidity was noticed by following the reaction at 700 mμ. At this wavelength none of the species in the reaction system absorb, so that any turbidity could be observed by an increase in OD due to the light scattering effect. This observation may be indicative of a coiling-up of the macromolecule as it takes on a polysoap structure with increasing dodecanoylation.

The accelerative kinetic behavior may now be confidently attributed to the formation of a long-chain, acylated PVIm intermediate. As the polymer becomes increasingly acylated (and increasingly nonpolar), the apolar forces attracting the substrate to the polymer domain become stronger. The result of this process is the observance of an autocatalytic behavior.

Effect of Hydroxylamine on the Accelerative Kinetic Behavior. If the accelerative kinetic behavior is due to the acylated PVIm intermediate formed through hydrolysis of a long-chain ester substrate, then elimination of the intermediate should eliminate the acceleration. Jencks and Carrioulo have reported that hydroxylamine is 10^5 times more effective than water as an acyl acceptor from acetylimidazole.[9] These results prompted us to observe the effect of added hydroxylamine on a hydrolysis reaction showing accelerative kinetics.

Figure 6 shows the effect of increasing hydroxylamine content on the PVIm catalyzed, accelerative hydrolysis of S_{12}^- in 40 vol % ethanol–water. The accelerative behavior, as evidenced by a curved pseudo-first-order rate plot, diminished as the concentration

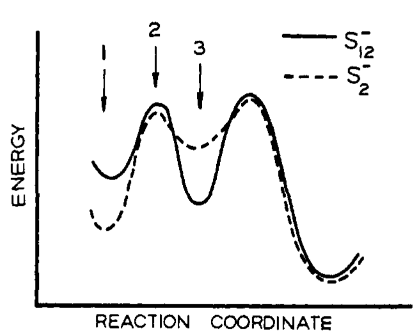
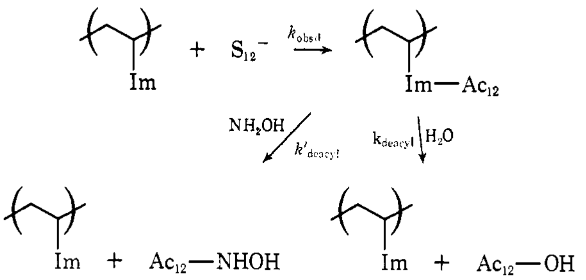


Figure 7. Reaction coordinate diagram for PVIm catalyzed hydrolyses of S_2^- and S_{12}^- in low vol % EtOH-H_2O.

of hydroxylamine was increased. Also, as a result, the total reaction times were considerably lengthened. At a hydroxylamine concentration of 1.1×10^{-3} *M*, a straight line, pseudo-first-order rate plot was obtained, *i.e.*, the accelerative behavior was completely destroyed! The reduction of the accelerative tendency can be explained by a decrease in the concentration of the dodecanoylated PVIm intermediate with increasing amounts of added hydroxylamine. Eventually, the accelerative behavior is completely eliminated indicating that the intermediate is being destroyed at a faster rate than the substrate is being hydrolyzed. Thus, in Scheme IV ($k'_{\text{deacyl}} + k_{\text{deacyl}}$) $> k_{\text{obsd}}$ at high hydroxylamine concentrations.

Scheme IV



Hydroxylamine also catalyzes the hydrolysis of the substrate. Table III shows the blank rate of hydrolyses of the substrate at the hydroxylamine concentrations employed. For the case employing the highest concentration of hydroxylamine, the blank rate accounts for about 30% of the hydrolysis of the substrate. Also shown is the effect of added hydroxylamine on the initial (k_i) and final (k_f) rates.

Table III. Effect of Added Hydroxylamine on the PVIm Catalyzed Hydrolysis of S_{12}^- in 40 Vol % Ethanol-Water[a]

[$NH_2OH \cdot$ HCl] $\times 10^4$ *M*	k_{blank}, min^{-1}	k_i, min^{-1} (to 15%)	k_f, min^{-1} (~75%)	k_f/k_i
11.1	0.0123	0.0339	0.0339	1.0
7.4	0.0081	0.0267	0.0355	1.3
6.0	0.0064	0.0254	0.0400	1.6
4.5	0.0057	0.0252	0.0544	2.1
3.0	0.0038	0.0236	0.0825	3.5
1.5	0.0020	0.0216	0.1304	6.0
0.0	0.0000	0.0190	0.1475	7.8

[a] [PVIm] = 6.10×10^{-4} *M*, [S_{12}^-] = 5.92×10^{-5} *M*, $\mu = 0.02$, [Tris] = 0.02 *M*, 26°.

Summary and Conclusions

The work described herein lends credence to the postulate that apolar forces can be a significant factor in enhancing the rate of a chemical transformation. Indeed, it supports the recent suggestion that apolar forces are responsible for the proximity effect in enzymic reactions.[16] Rate enhancements of up to 400-fold were observed for the PVIm catalyzed hydrolysis of a long-chain substrate, relative to monomeric Im catalyzed hydrolysis of the same substrate. The apolar forces responsible for this acceleration were found to be critically dependent on both the alkyl chain length of the substrate and the composition of the ethanol–water solvent system. The measurement of the deacylation behavior of the intermediates formed during the hydrolysis reaction indicated that a reversal in rate-determining step was occurring as a result of increasing chain length of the substrate. The net result of this observation is that although the hydrolysis of the substrate is being markedly enhanced, the actual catalytic process involving regeneration of the catalyst is markedly retarded.

Figure 7 shows the corresponding reaction coordinate diagrams for the PVIm catalyzed hydrolyses of S_2^- and S_{12}^-. The apolar approximation of reactants enhances the rate by in effect *raising* the ground state free energy of the reactants in the case of the long-chain substrate. This is shown by point 1 in Figure 7 and results in an effective decrease in the free energy of activation for hydrolysis (*i.e.*, k_{obsd} for $S_{12}^- > k_{\text{obsd}}$ for S_2^-). Point 2 indicates that there is some steric hindrance in the transition state during the nucleophilic attack of a pendant Im group on S_{12}^-, but the effect is small. The next step in the catalytic process is deacylation of the corresponding acyl intermediates to regenerate PVIm and give the final products of the hydrolysis. Point 3 indicates that for the case of the deacylation of the long-chain, acylated-PVIm an unusual stability of the intermediate was observed (*i.e.*, k_{deacyl} for PVIm-Ac_{12} < k_{deacyl} for PVIm-Ac_2). This is postulated to be a result of intramolecular micellarization of the polymer as it assumes a polysoap structure. Thus, the free energy of the "ground state" of the intermediate is considerably lowered, representing the fact that an attack of a water molecule at the carbonyl carbon of the acyl group is hindered in some way.

During the study of the PVIm catalyzed hydrolysis of the long-chain substrates, an autocatalytic or accelerative behavior was observed. It was confirmed that this accelerative kinetic pattern was due to the increasing degree of acylation of the polymer during the esterolysis. As the per cent acylation increases, the apolar character of the catalyst species increases and the hydrolytic process is enhanced.

Experimental Section

Measurement of Deacylation Rates (k_{deacyl}). The rate of deacylation was determined by following the decrease in optical density (OD) at 260 mμ (or 257 mμ) as a function of time. The data so obtained were treated according to first-order kinetics by plotting ln ($OD_t - OD_\infty$) *vs.* time. The deacylation data for the authentic sample of completely heptanoylated PVIm were obtained by adding 0.25 ml of PVIm-Ac_7 (in dry THF) to 3.0 ml of

---

[16] B. H. J. Hafster, *Nature (London)*, **213**, 42 (1967).

the appropriate buffer in a quartz cuvette. A turbid solution resulted which cleared approximately 5 min after mixing in the cuvette. The deacylation rates for all the polymeric samples measured were found to be continuously accelerative. The value for the deacylation rate constant was taken from the slope of the first-order rate plot at greater than 80% deacylation completed in all cases.

Measurement of Hydrolysis Rates (k_{obsd}). The procedure used was identical with that described in the accompanying paper.[6]

Isolation of Partially Acylated Poly[4(5)-vinylimidazole]. The column (12 cm × 4 cm) was packed with Sephadex LH-20 (50 g swollen in ethanol–water buffer for 24 hr) and the reservoir flask charged with the appropriate vol % ethanol–water buffer. A typical kinetic run was prepared and placed on the thermostated column. The eluent flow, *ca.* 1.5 ml/min, was begun and after the collection of 30–32 ml of eluent (yellow, phenolate band traveled ~4 cm) a 3.0–3.5-ml fraction was collected in a 1-cm quartz cuvette. A uv scan was then immediately taken on a Perkin-Elmer 402 recording spectrophotometer; then the cuvette was rapidly transferred to a Beckman DU-2 spectrophotometer for measurement of the deacylation rate.

The per cent acylation of the polymer as it came off the column was calculated from the following relation

$$\%\ \text{acylation} = (\text{OD}_t^{260} - \text{OD}_\infty^{260})/(\text{OD}_0^{260} - \text{OD}_\infty^{260}) \quad (2)$$

where OD_∞^{260} is the OD at 260 mμ after complete deacylation, OD_t^{260} is the OD as the polymer came off the column, and OD_0^{260} is the OD of the totally acylated polymer. The value of OD_0^{260} is unknown by direct measurement from the nature of the experiment. However, the extinction coefficient of PVIm is known ($\epsilon^{213}{}_{\text{PVIm}}$ 4.7×10^3) as well as the extinction of the totally acylated polymer ($\epsilon^{260}{}_{\text{PVIm-Ac}_7}$ 3.48×10^3). For a partially acylated sample, it is possible to express the following relation

$$\frac{\epsilon^{213}{}_{\text{PVIm}}}{\epsilon^{260}{}_{\text{PVIm-Ac}_n}} = \frac{\text{OD}_\infty^{213}}{\text{OD}_0^{260}} = \frac{4.70 \times 10^3}{3.48 \times 10^3} \quad (3)$$

where OD_∞^{213} refers to the OD of the sample after it is totally deacylated, *i.e.*, PVIm. Rearranging (3) and substituting the expression for OD_∞^{260} into (2) gives eq 4.[17]

$$\%\ \text{acylation} = 1.35\,(\text{OD}_t^{260} - \text{OD}_\infty^{260})/\text{OD}_\infty^{213} \quad (4)$$

For cases involving dodecanoylation ($n = 12$) and octadecanoylation ($n = 18$), it was not possible to obtain a highly acylated sample (*i.e.*, >10%) for measurement of the deacylation rate. The reason for this was turbidity formation for S_{12}^- and S_{18}^- when the ratio of $[S_n^-]/[\text{PVIm}]$ was over 0.1, at the concentrations used. It was only possible to use excess substrate in the case of S_2^-.

Preparation of *In Situ* Dodecanoylated Poly[4(5)-vinylimidazole]. The stability of the acylated PVIm intermediate during the hydrolysis of a long-chain ester substrate could be used to prepare *in situ* samples of varying per cent dodecanoylation. The PVIm catalyzed hydrolysis of S_{12}^- was followed in 33 vol % ethanol–water. The concentration of S_{12}^- was held at 5.0×10^{-5} *M* and the concentration of PVIm varied. The procedure used was the same as outlined above, *i.e.*, 0.2 ml of substrate was added to 3.0 ml of the catalyst. After the hydrolysis of S_{12}^- was ~95% completed or more, a second aliquot (0.2 ml in 33 vol % ethanol–water buffer) of S_7^- or S_2^- was added to the cuvette (4.7×10^{-5} *M*). The concentration of imidazole units on the polymer was calculated based on 3.4 ml; also, a correction was made for the per cent of the imidazole units which were acylated. The hydrolysis of the added S_2^- or S_7^- gave a straight line, first-order rate plot. The k_{cat} value was calculated as discussed above, *i.e.*, $k_{\text{cat}} = k_{\text{obsd}}/[\text{PVIm}]_{3.4}$.

Substrates. The synthesis of the substrates S_2^-, S_7^-, S_{12}^-, and S_{18}^- is described in the accompanying paper.[6]

2,4-Dinitrophenyl Stearate ($S_{18}{}^{a}$) and 4-Nitro-3-Bromophenyl Stearate ($S_{18}{}^{b}$). These substrates were obtained by reaction of stearoyl chloride with 2,4-dinitrophenol and 4-bromo-3-nitrophenol, respectively, according to the method described for S_{12}^-. $S_{18}{}^{a}$ was recrystallized from heptane, then methanol, mp 59.5–61°. *Anal.* Calcd for $C_{24}H_{38}N_2O_6$: C, 63.97; H, 8.50; N, 6.22. Found: C, 63.93; H, 8.44; N, 6.36.

$S_{18}{}^{b}$ was recrystallized from methanol, mp 47–48°. *Anal.* Calcd for $C_{24}H_{38}BrNO_4$: C, 59.50; H, 7.91; Br, 16.49; N, 2.89. Found: C, 59.66; H, 7.82; Br, 16.48; N, 2.89.

Poly[4(5)-vinylimidazole] (PVIm). The synthesis of the sample used is described in the accompanying paper.[6]

***N*-Heptanoylpoly[4(5)-vinylimidazole] (PVIm-Ac$_7$).** VIm-Ac$_7$ (1.34 g, 6.5 mmol) was placed in a 10 cm × 3 cm glass tube and tightly capped with a two-hole bottle cap with a neoprene insert. The tube was flushed with dry nitrogen for 6 hr *via* needles through the neoprene cap, then placed in an 85–90° oil bath. After the solid had melted, 0.25 ml of a benzoyl peroxide (BPO) solution, [BPO] = 2.6×10^{-2} *M* in benzene, was added by means of a gas-tight syringe and needle. The tube was left in the oil bath for 200 hr, during which time aliquots of the above BPO solution were injected, totaling 2.10 ml (0.9 mol % of monomer). After removal from the oil bath, the sample was dried in a vacuum dessicator (0.05 mm) for 24 hr, then transferred to a microsublimation apparatus. Some of the unreacted monomer was recovered by sublimation (60°, (0.1 mm)) for 24 hr; however, trapping of the monomer under the polymer glass was evident. The polymer could be further purified by several reprecipitations from chloroform (3–4 ml) into hexane (300 ml), 18% yield: $\eta_{\text{sp}} = 0.098$ (at 0.5 g/dl, $CHCl_3$, 25.0°); nmr ($CDCl_3$) showed no protons in the vinyl region; ir (film from $CHCl_3$) showed a strong carbonyl absorption (1730 cm^{-1}); uv max (40 vol % ethanol–water buffer) 220 mμ (ϵ 6.45×10^3) and 260 mμ (ϵ 3.48×10^{-3}), after complete deacylation, *i.e.*, for PVIm, 213 mμ (ϵ 4.70×10^3).

Anal. Calcd for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.79; N, 13.58. Found: C, 68.72; H, 8.40; N, 14.36.

Hydrolysis of PVIm-Ac$_7$ in 25 vol % methanol–water gave PVIm as determined by ir, $\eta_{\text{sp}} = 0.16$ (at 0.5 g/dl, MeOH, 25.0°).

***N*-Heptanoyl-4(5)-vinylimidazole (VIm-Ac$_7$).** To 4(5)-vinylimidazole (0.94 g, 0.01 mol in 75 ml of benzene) was added triethylamine (1.10 g, 0.01 mol). To this well-stirred solution was slowly added heptanoyl chloride (1.48 g, 0.01 mol) and the stirring continued for 30 min at room temperature. The white mixture was filtered to remove the triethylamine hydrochloride and the filtrate evaporated under reduced pressure on a rotary evaporator to leave a white solid (95% yield). It could be purified by recrystallization from hexane or by sublimation (55° (0.05 mm), mp 69° (95% yield).

Anal. Calcd for $C_{12}H_{18}N_2O$: C, 69.90; H, 8.34; N, 13.59. Found: C, 70.05; H, 8.66; N, 13.53.

Acknowledgment. The authors are grateful for support from the National Institutes of Health under Grant No. GM15256. The authors are also grateful to Dr. Keith N. Sannes for his helpful discussions leading to the experiments with hydroxylamine, and his assistance in the preparation of this manuscript.

---

[17] It is assumed that the extinction for the acyl group from PVIm-Ac$_7$ is identical for -Ac$_2$, -Ac$_{12}$, and -Ac$_{18}$.