Behavior of Imidazole on Poly(ethylenimine) Derivatives; Hydrolysis of *p*-Nitrophenyl Caproate

Dorla Mirejovsky

Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois 60201

Received August 23, 1979

The hydrolysis of *p*-nitrophenyl caproate has been measured in the presence of modified poly(ethylenimine) derivatives containing various amounts of imidazole. The second-order rate constant decreases with increased content of imidazole groups on the polymer. Moreover, a large number of imidazoles per catalytic site is found in tightly coiled polymers. This catalytic inefficiency is attributed to the aggregation of imidazoles in the hydrophobic region of the polymer. Imidazoles at the polymer surface are considered to be catalytic species. *Polar* polymer environment in the vicinity of the imidazoles leads to greater efficiency in hydrolysis. Interestingly, the enzymelike pathway does not cause more effective catalysis. The improbability of cooperation between two imidazoles is discussed.

The growing attention given to synthetic polymers is prompted by an endeavor to reproduce enzymelike catalytic behavior.[1,2] Because of the involvement of an imidazole group of a histidyl residue in the catalytic action of many hydrolytic enzymes,[3] imidazole and imidazole derivatives have become widely studied catalysts. However, attempts to create imidazole-containing enzymelike catalysts have been far from successful. The behavior of imidazole in synthetic macromolecular catalysis is still poorly understood.

Enhanced catalytic activity of imidazole in the hydrolysis of neutral activated esters has been well established for several enzymelike models. Three main explanations of the enhancement have been put forward: (a) cooperative action of two imidazole residues,[4-6] (b) hydrophobic interaction between imidazole and substrate,[7-10] (c) electrostatic interaction promoting the formation of imidazole anion.[11,12]

Enhanced activity of imidazole-modified poly(ethylenimine), PEI, toward hydrophobic substrates was previously demonstrated by Klotz et al.[13] The rate enhancement was attributed to enhanced catalysis in apolar domains created by clustering of the attached hydrophobic groups. However, the originally reported rate of hydrolysis was never attained or approached later when other modifications of the polymer were introduced or different imidazole compounds attached. Plainly, more work was needed to get insight into the behavior of modified PEI.

In this paper we present the results of kinetic measurements of hydrolysis of *p*-nitrophenyl caproate, PNPC, by imidazole-containing derivatives of poly(ethylenimine). The influence of acetylation and quaternization of the polymer on the catalytic rate as well as the effect of imidazole content were studied. These results allow us to discuss the following questions: (i) What is the optimal environment for imidazole catalysis? (ii) How probable is cooperation between imidazole species?

Experimental Section

Preparation of Modified Polymers. Poly(ethylenimine), PEI 600, with an average molecular weight of 60000 was obtained from Dow Chemical Co. as a 33% aqueous solution. The polymer solution was first ultrafiltered against water in an Amicon ultrafiltration vessel with a PM 30 membrane and then lyophilized. After removal of the low molecular weight fraction, the PEI contained only 13 ± 2% primary amines (determined spectrophotometrically with sodium trinitrobenzenesulfonate)[14] instead of the 25% found in nonultrafiltered polymer.[14]

Laurylated poly(ethylenimine) was prepared by alkylation of the polymer in absolute ethanol with lauryl bromide and *N,N*-diisopropylethylamine. The reaction occurs preferentially with primary amines. Subsequently, various amounts of 4(5)-imidazolylmethyl chloride[15] were added. This alkylation reaction proceeds preferentially with secondary amines, as originally reported by Turner et al.[15] Polymer composition is reported as L_x-PEI-Im_y, where x and y, respectively, represent the number of lauryl and methyleneimidazole groups per monomer unit.

Acetic anhydride was used to acetylate the polymers. Nearly quantitative acetylation of imidazole-containing polymers was obtained in absolute ethanol with acetic anhydride and triethylamine. Under the same conditions only partial acetylation of nonimidazole-containing polymer was achieved ($L_{0.12}$-PEI-$Ac_{0.42}$). On the other hand, reaction in water with pyridine and acetic anhydride resulted in low acetylation ($L_{0.12}$-PEI-$Ac_{0.07}$).

Reduction of commercially available 3-[4(5)-imidazolyl]-2-propenoic acid (Sigma Chemical Co.)[16] yielded 3-[4(5)-imidazolyl]propionic acid. This was attached to lauryl PEI by using the water soluble DCC, 1-ethyl-3[3-(dimethylamino)-propyl]carbodiimide hydrogen chloride (Sigma Chemical Co.), at pH ~6. Similarly, 4(5)-imidazolylacetic acid (Sigma Chemical Co.) and *N,N*-dimethylhistidine[17] were attached.

The quaternized polymer with free primary amines were prepared by the following three-step procedure: (a) alkylation of PEI by lauryl bromide and *N*-(2-bromoethyl)phthalimide (Aldrich Chemical Co.) in absolute ethanol in the presence of *N,N*-diisopropylethylamine; (b) quaternization with methyl iodide followed by dimethyl sulfate; (c) regeneration of primary amines by cleavage of phthalimide with hydrazine hydrate.

After modification, all polymers were ultrafiltered, by using a PM 30 membrane, first against 1 N acetic acid, then against 0.02 M NaCl, and finally against water. After filtration the

(1) Kunitake, T.; Okahata, Y. *Fortschr. Hochpolym.-Forsch.* **1976**, *20*, 159–221.
(2) Manecke, G.; Storck, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 657–70.
(3) Schneider, F. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 583–92.
(4) Overberger, C. G.; St. Pierre, T.; Vorchheimer, N.; Lee, J.; Yaroslavsky, S. *J. Am. Chem. Soc.* **1965**, *87*, 296–301.
(5) Overberger, C. G.; St. Pierre, T.; Yaroslavsky, C.; Yaroslavsky, S. *J. Am. Chem. Soc.* **1966**, *88*, 1184–8.
(6) Overberger, C. G.; Shen, C.-M. *Bioorg. Chem.* **1971**, *1*, 1–12.
(7) Gitler, C.; Ochoa-Solano, A. *J. Am. Chem. Soc.* **1968**, *90*, 5004–9.
(8) Murakami, Y.; Aoyama, Y.; Kida, M.; Nakano, A. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3365–71.
(9) Kunitake, T.; Shinkai, S. *J. Am. Chem. Soc.* **1971**, *93*, 4247–55.
(10) Kunitake, T.; Shinkai, S. *J. Am. Chem. Soc.* **1971**, *93*, 4256–63.
(11) Moss, R. A.; Lukas, T. J.; Nahas, R. C. *Tetrahedron Lett.* **1977**, 3851–4.
(12) Moss, R. A.; Nahas, R. C.; Ramaswami, S.; Sanders, W. J. *Tetrahedron Lett.* **1975**, 3379–82.
(13) Klotz, I. M.; Royer, G. P.; Scarpa, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 263–4.
(14) Johnson, T. W.; Klotz, I. M. *Macromolecules* **1974**, *7*, 149–53.
(15) Turner, R. A.; Huebner, C. F.; Scholz, C. R. *J. Am. Chem. Soc.* **1949**, *71*, 2801–3.
(16) Gamson, E. P. unpublished results.
(17) Reinhold, V. N.; Ishikawa, Y.; Melville, D. B. *J. Med. Chem.* **1968**, *11*, 258–60.

0022-3263/79/1944-4881$01.00/0 © 1979 American Chemical Society

Table I. Second-Order Rate Constants for Hydrolysis of *p*-Nitrophenyl Caproate by Laurylated Poly(ethylenimine) Containing Various Amounts of Imidazole[a]

polymer	k_{corr}[b]/(M^{-1} min^{-1})	polymer	k_{corr}[b]/(M^{-1} min^{-1})
$L_{0.12}$-PEI-$(CH_2Im)_{0.14}$	3300	$L_{0.12}$-PEI-$(CH_2Im)_{0.28}$	1510
$L_{0.12}$-PEI-$(CH_2Im)_{0.42}$	2700	$L_{0.12}$-PEI-$(CH_2Im)_{0.56}$	810
$L_{0.12}$-PEI-$(CH_2Im)_{0.71}$	1890		

[a] [PNPC] = 1×10^{-5} M; [Im] = $(1-4) \times 10^{-4}$ M; pH 7.3; 0.05 M BisTris·HCl; 25 °C. [b] Second-order rate constant corrected for the contribution of the polymer backbone.

polymers were lyophilized. The extent of modification was determined by ^1H NMR and C, N elemental analysis.

Kinetics. Hydrolysis of PNPC at pH 7.3 in 0.05 M [bis(2-hydroxyethyl)imino]tris(hydroxymethyl)methane (BisTris) chloride at 25 °C was followed by measuring the absorbance of *p*-nitrophenolate ion at 400 nm. The stock solution of 0.02 M PNPC (Sigma Chemical Co.) was prepared in acetonitrile.

Under conditions of excess polymer, pseudo-first-order kinetics were observed for at least 70% of the reaction. When PNPC was in excess, the kinetics obeyed first-order requirements for at least 50% of the reaction with the acetylated polymers and 60% of the reaction with $L_{0.21}$-PEI-$Im_{0.09}$. Thereafter, an accelerative deviation from first-order kinetics was observed. The reactions of quaternized polymers with excess PNPC proceeded according to pseudo-first-order kinetics up to 80% of the conversion. No accelerative deviation was discerned.[18]

^1H NMR Titration of Imidazole. All ^1H NMR spectra were recorded on a Varian CFT-20 NMR spectrometer at 27 ± 2 °C. The residue molar concentration of each polymer was approximately 0.5 M in D_2O. All pH measurements were uncorrected for D_2O. As is well-known, the isotope effect at the glass electrode appears to be approximately equal and opposite in sign to the isotope effects on the histidine pK_a value.[19-21]

Results

Variable Amounts of Methyleneimidazole on PEI. The rate of hydrolysis of PNPC was determined for a series of polymers containing variable amounts of methyleneimidazole. The second-order rate constants are given in Table I. The same residue molar polymer concentration was used for every polymer measured. The concentration of imidazole groups was 10–40-fold greater than the substrate concentration. All rates were corrected for the contribution of the polymer backbone. The background hydrolysis was measured in presence of laurylated poly(ethylenimine) containing a low number of acetyl groups, $L_{0.12}$-PEI-$Ac_{0.07}$, at an equimolar residue molar polymer concentration. The result was subtracted from the observed pseudo-first-order rate of the imidazole-containing polymer (approximately 10% of the rate).

As can be seen from Table I, the second-order rate constant decreases as the imidazole content increases.

Acetylated PEI with Various Amounts of Methyleneimidazole. The rate of hydrolysis of PNPC was measured for polymers with highly acetylated secondary amines. Unlike nonacetylated polymers, the acetylated polymers exhibit saturation behavior; the pseudo-first-order rate constants reach a plateau as imidazole concentration is increased.

Table II. Kinetic Parameters for Hydrolysis of *p*-Nitrophenyl Caproate by Acetylated PEI Derivatives[a]

polymer	k_2[b]/min^{-1}	nk_2[c]/(K_M min^{-1} M^{-1})	$1/n$[d]	$10^5 K_M$[e]/M
$L_{0.12}$-PEI-$(CH_2Im)_{0.14}$-$Ac_{0.4}$				
[Im] > [S][f]	2.5	1670		1.1
[S] > [Im][g]		1250	125	1.6
$L_{0.12}$-PEI-$(CH_2Im)_{0.28}$-$Ac_{0.58}$				
[Im] > [S][h]	1.7	620		
[S] > [Im][i]		620	77	3.5
$L_{0.12}$-PEI-$(CH_2Im)_{0.28}$-$Ac_{0.58}$				
[Im] > [S][j]	0.6	240		
[S] > [Im][k]		240	45	5.4

[a] pH 7.3; 0.05 M BisTris·HCl; 25 °C. [b] First-order rate constant. [c] Second-order rate constant. [d] Number of imidazoles per catalytic site. [e] Association constant of substrate is given by K_M^{-1}. [f] [PNPC] = 1×10^{-5} M; [Im] = $(0.9-2.2) \times 10^{-4}$ M. [g] [Im] = 0.55×10^{-5} M; [PNPC] = $(3-12) \times 10^{-5}$ M. [h] [PNPC] = 1×10^{-5} M; [Im] = $(1.2-4.2) \times 10^{-4}$ M. [i] [Im] = 0.9×10^{-5} M; [PNPC] = $(3-12) \times 10^{-5}$ M. [j] [PNPC] = 5×10^{-5} M; [Im] = $(2-28) \times 10^{-4}$ M. [k] [Im] = 1.4×10^{-5} M; [PNPC] = $(3-12) \times 10^{-5}$ M.

Saturation behavior is characteristic of enzymelike catalysis

$$C + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CS \xrightarrow{k_2} C + \text{product}$$

where C represents a catalytic site on the polymer and S is the substrate. The method of kinetic analysis described in detail for poly(ethylenimine) catalysis by Suh et al.[22] was followed. When the concentration of imidazole is in excess over that of substrate, [Im] > [S], then

$$k_{obsd} = nk_2[Im]/(K_M + n[Im])$$

where k_{obsd} is the observed first-order rate constant, [Im] is the molar concentration of imidazole, n is the number of catalytic sites per imidazole, and $K_M = (k_{-1} + k_2)/k_1$. Alternatively, under conditions of excess of substrate, [S] > [Im] and

$$k_{obsd} = nk_2[Im]/(K_M + [S])$$

A linear transformation of these equations provides the parameters n, k_2, and K_M (Table II). Every rate measured was corrected for the background hydrolysis determined in presence of acetylated polymer, $L_{0.12}$-PEI-$Ac_{0.42}$.

Again, the second-order rate constant decreases with an increase of imidazole groups on the polymer. Moreover, the large number of imidazole residues per catalytic site deserves notice.

Different Imidazole Compounds on PEI. The consequence of replacement of 4(5)-imidazolylmethyl by 3-[4(5)-imidazolyl]propionyl can be derived from a comparison of the data in Table III with those in Table I and II. The most striking rate lowering was observed when 4(5)-imidazolylacetic acid and *N,N*-dimethylhistidine were attached to PEI. The second-order rate constants obtained for polymer $L_{0.12}$-PEI-$(COCH_2Im)_{0.14}$-$Ac_{0.58}$ and $L_{0.12}$-PEI-$[COCH(N(CH_3)_2)CH_2Im]_{0.12}$-$Ac_{0.71}$ (25 and 12 M^{-1}

(18) We attribute this accelerative deviation to the effect of *p*-nitrophenolate ion released during the hydrolysis and bound to the tightly coiled polymers. A hypsochromic shift of its UV absorption (about 6–8 nm) confirms the presence of this ion in the less polar environment.

(19) Markley, J. L. *Acc. Chem. Res.* **1975**, *8*, 70–80.

(20) Tanokura, M.; Tasumi, M.; Miyazawa, T. *Biopolymers* **1976**, *15*, 393–401.

(21) Markley, J. L. *Biochemistry* **1973**, *12*, 2245–50.

(22) Suh, J.; Scarpa, I. S.; Klotz, I. M. *J. Am. Chem. Soc.* **1976**, *98*, 7060–7064.

Table III. Kinetic Parameters for Hydrolysis of *p*-Nitrophenyl Caproate by PEI Derivatives Modified with 3-[4(5)-Imidazolyl]propionic Acid[a]

polymer	k_2[b]/min^{-1}	nk_2[c]/(K_M min^{-1} M^{-1})	$1/n$[d]	$10^5 K_M$[e]/M
$L_{0.12}$-PEI-$(COCH_2CH_2Im)_{0.15}$		1280[f]		
$L_{0.12}$-PEI-$(COCH_2CH_2Im)_{0.15}$-$Ac_{0.50}$				
[Im] > [S][g]	0.29	110		
[S] > [Im][h]		110	67	3.9
$L_{0.22}$-PEI-$(COCH_2CH_2Im)_{0.10}$, $CH_{3\ 1.35}$; Cl				
[Im] > [S][i]	0.08	130		21.6
[S] > [Im][j]		180	3	15.8
$L_{0.23}$-PEI-$(COCH_2CH_2Im)_{0.10}$, $CH_{3\ 1.65}$; Cl				
[Im] > [S][k]	0.53	910		
[S] > [Im][l]		910	7	8.7

[a] pH 7.3; 0.05 M BisTris·HCl; 25 °C. [b] First-order rate constant. [c] Second-order rate constant. [d] Number of imidazoles per catalytic site. [e] Association constant of substrate is given by K_M^{-1}. [f] Second-order rate kinetics; [PNPC] = 1×10^{-5} M; [Im] = $1 \times (10\text{-}25) \times 10^{-5}$ M. [g] [PNPC] = 5×10^{-5} M; [Im] = $(2\text{-}18) \times 10^{-4}$ M. [h] [Im] = 0.75×10^{-5} M; [PNPC] = $(2\text{-}12) \times 10^{-5}$ M. [i] [PNPC] = 1×10^{-5} M; [Im] = $(8\text{-}25) \times 10^{-5}$ M. [j] [Im] = 0.5×10^{-5} M; [PNPC] = $(3\text{-}12) \times 10^{-5}$ M. [k] [PNPC] = 1×10^{-5} M; [Im] = $(5\text{-}30) \times 10^{-5}$ M. [l] [Im] = 1×10^{-5} M; [PNPC] = $(3\text{-}13) \times 10^{-5}$ M.

Table IV. Effect of Increased Content of Lauryl Groups in Imidazole PEI on Hydrolysis of *p*-Nitrophenyl Caproate[a]

polymer	k_2[b]/ min^{-1}	nk_2[c]/(K_M min^{-1} M^{-1})	$1/n$[d]	$10^5 K_M$[e]/ M
$L_{0.12}$-PEI-$(CH_2Im)_{0.11}$		3300[f]		
$L_{0.21}$-PEI-$(CH_2Im)_{0.09}$				
[Im] > [S][g]	2.8	9000		
[S] > [Im][h]		8300	16	2.1

[a] pH 7.3; 0.05 M BisTris·HCl; 25 °C. [b] First-order rate constant. [c] Second-order rate constant. [d] Number of imidazoles per catalytic site. [e] Association constant of substrate is given by K_M^{-1}. [f] Table I. [g] [PNPC] = 1×10^{-5} M; [Im] = $(0.75\text{-}1.5) \times 10^{-4}$ M. [h] [Im] = 0.45×10^{-5} M; [PNPC] = $(3\text{-}10) \times 10^{-5}$ M.

min^{-1}, respectively) were no higher than the rate constant reported for imidazole molecule itself (25 M^{-1} min^{-1} [23]). So far, we are unable to account for this effect.

Lauryl-Quaternized PEI. As expected, no reaction occurred between the free primary amines of the quaternized polymer and 4(5)-imidazolylmethyl chloride.[15] Therefore, the behavior of quaternized polymer was assessed in the series of polymers obtained by attachment of 3-[4(5)-imidazolyl]propionic acid. Saturation kinetics were observed as in the case of acetylated polymers (Table III). The background rate hydrolysis was measured in the presence of quaternized polymers with acetylated primary amines: $L_{0.23}$-PEI-$(CH_2CH_2NHCOCH_3)_{0.10}$,$(CH_3)_{1.65}$,$Cl^-$ and $L_{0.12}$-PEI-$(CH_2CH_2NHCOCH_3)_{0.10}$,$(CH_3)_{1.35}$,$Cl^-$.

It was found that the second-order rate constant was only slightly higher than that of the acetylated polymer. On the other hand, the number of imidazole molecules per catalytic site in quaternized polymer is considerably lower.

Different Amounts of Lauryl Groups. Two series of polymers with different contents of lauryl groups were studied. Table III gives the results for quaternized polymers. Table IV shows the results for methyleneimidazole on two PEI derivatives, one with 12% and the other with 21% of the residues laurylated. Saturation behavior was observed for the 21% laurylated derivative.

Note that in both series a higher content of lauryl groups results in higher catalytic activity.

^{1}H NMR Titration of Imidazole. Titrations of the ring protons of imidazole were performed for polymers $L_{0.12}$-PEI-$(CH_2Im)_{0.28}$, $L_{0.12}$-PEI-$(CH_2Im)_{0.28}$-$Ac_{0.58}$, and $L_{0.22}$-PEI-$(COCH_2CH_2Im)_{0.10}$,$(CH_3)_{1.35}$,Cl^- in D_2O by using ^{1}H NMR spectroscopy. Titration curves for the imidazole C^2 ring proton of these three polymers are shown in Figure 1.

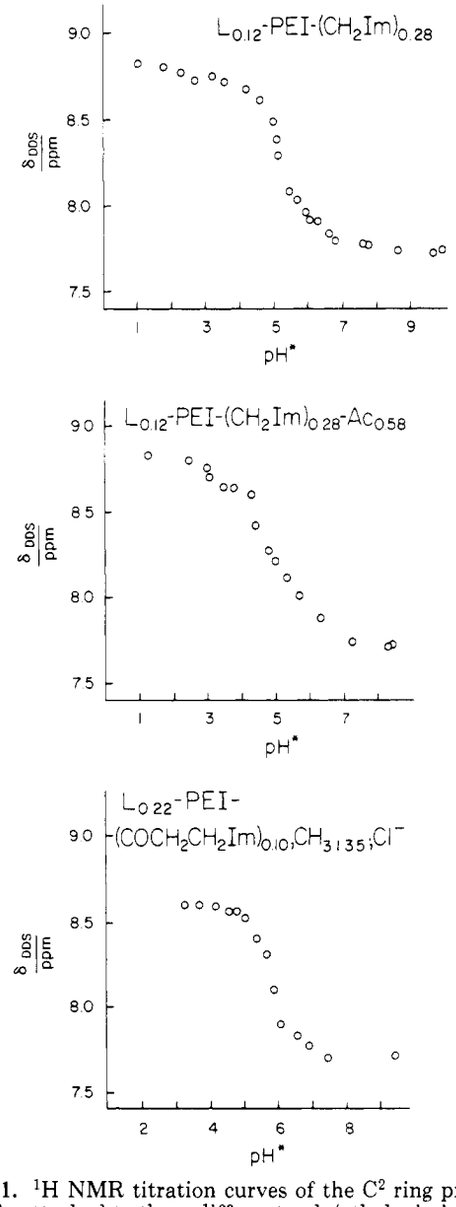


Figure 1. ^{1}H NMR titration curves of the C^2 ring protons of imidazole attached to three different poly(ethylenimine) derivatives. The symbol pH* indicates an uncorrected glass-electrode pH meter reading of a D_2O solution.

(23) Bruice, T. C.; Schmir, G. L. *J. Am. Chem. Soc.* **1958**, *80*, 148-156.

The curve of the quaternized polymer follows, more or less, the symmetric function expected for a simple proton-association equilibrium. An apparent pK of 5.9 was calculated from the linear transformation of a modified form of the Hill equation[19] (Hill coefficient n = 1.15; R = 0.994).

On the other hand, the curves for acetylated and nonacetylated polymers do not follow the symmetric function expected for a simple proton-association equilibrium. Three inflections can be seen in the C^2-proton titration curves. Moreover, in the middle of the titration the C^2 resonances of these polymers are broadened into a series of small peaks. Raising the temperature to 68 °C did not influence this effect. The chemical shift plotted in Figure 1 is an average of this broad series.

In the acetylated and nonacetylated polymers the multiple inflections can be attributed to titration of secondary and tertiary amines in the imidazole titration region. Because there are many interacting sites on each molecule, the specific acid dissociation constants of individual sites cannot be deduced. It is worthwhile to note that the shape of the titration curves is similar to that of L-histidine methyl ester obtained by Shrager et al.,[24] in which the interaction of an amino group (pK = 7.55) with imidazole (pK = 5.52) gave rise to asymmetry. Similarly in our case, the amino groups of the polymer backbone compete with imidazole for protonation. Approximate values of 4.5 and 5.0 for the pK_a of imidazole in acetylated and nonacetylated polymer, respectively, can be estimated from these curves.

Discussion

Position of Imidazole on the Polymer. There are two striking features coming out from our kinetic results: (1) the large number of imidazoles per catalytic site and (2) the rate constant decrease with increase of imidazole concentration. These results suggest that not every single imidazole participates in hydrolytic catalysis. We tend to attribute this catalytic inefficiency to the association of imidazoles in the hydrophobic part of the polymer, resulting in an exclusion of these imidazoles from the reaction. This would imply that only imidazoles at the polymer surface are responsible for the reaction.

Recent work of Wertz and Scheraga on the position of amino acids in proteins suggests that histidine prefers the hydrophobic interior of proteins over the more hydrophilic protein surface.[25] Correspondingly, a preference of imidazole for the more hydrophobic part of the polymer can be expected. The presence of imidazole in the hydrophobic region of the polymer implies that imidazole behaves in a way similar to that observed in nonpolar solvents. An association of imidazoles can therefore be expected.[26]

A strong binding of imidazole to the hydrophobic part was suggested by our ^{1}H NMR titration. The broadening of the resonances was particularly pronounced in the acetylated polymer. The asymmetry of the titration curves of acetylated and nonacetylated polymer caused by interfering titration of the backbone sites indicates the proximity of imidazoles to the main polymer chain. A lowering of pK_a due to the electrostatic inhibition of protonation can be expected. However, a low pK_a value can also have its origin in the more hydrophobic environment. It is obvious that ease of protonation is determined by the local dielectric constant and the proximity of positive or negative charges. This is particularly valid in the case of the acetylated polymer where a majority of secondary amines are acetylated and only a few tertiary amines are available for protonation. The broadening of the absorption near the pK_a of imidazole supports the view that imidazole is present in the hydrophobic region. In accord with the literature[19,21,27] we attribute this effect to a slow exchange between different conformational environments having different chemical shifts and different pK_a values. Protonation of an imidazole which is more accessible to ionization would cause a loosening of the association of the imidazoles in the hydrophobic part of the polymer. The next imidazole, which is nearer to the inside of the polymer and has a slightly lower pK_a would then become more prone to protonation.

The symmetry of the titration curve of quaternized polymer indicates that the imidazole does not interact with the amino groups of the polymer backbone. Furthermore, it is reasonable to assume that the ionizations of imidazole residues were more or less independent (n = 1.15). The absence of broadening in this polymer reflects a weaker binding of imidazole to the hydrophobic part of the polymer. A decrease in pK_a can be ascribed solely to the electrostatic effect of the positively charged polymer.

These behavior differences can be explained by the dissimilarity of polymer conformations. One would expect that the positively charged polymer is extended. Nevertheless, the high concentration of imidazole should enhance the tendency to associate. Low imidazole concentration, on the other hand, would make association difficult. Finally, the conformation of acetylated polymer would be relatively compact, with imidazole in a more or less buried position. As shown by kinetic measurements, such position does not favor catalysis.

Effect of Different Imidazole Environments. In nonmodified polymer, second-order rate constants decrease approximately fourfold when imidazole concentration increases threefold (Table I). The differences between the values of k_{obsd} were not substantial at the same residue molar polymer concentration. This fact indicates that when imidazole content is high, only some of the imidazoles participate in catalysis.

The second-order rate constant given originally by Klotz et al.[13] for $L_{0.10}$-PEI-$(CH_2Im)_{0.15}$ is of a magnitude similar to that obtained here.

The activity of acetylated polymer is considerably lower (Table II) despite the fact that saturation behavior was detected. Analysis of the kinetic parameters shows a large number of imidazoles per catalytic site. This is in accordance with our previous statement that some of the imidazoles are associated in a hydrophobic environment and do not react. However, an interesting feature can be stressed, i.e., the magnitude of the first-order catalytic constant k_2. We compare our value of k_2 (1.7 min^{-1}) with the result for hydrolysis of PNPC in the presence of $L_{0.10}$-PEI-$(CH_2Im)_{0.21}$-$(C_3H_7)_{0.33}$ (k_2 = 0.15 min^{-1}) reported by Nango and Klotz.[28] The fact that the content of imidazole and of lauryl groups was similar in both cases makes a comparison possible. It seems that the *more* polar environment created by the N-acetyl group leads to greater efficiency in catalysis as compared to that created by reductive isopropylation.

Nango and Klotz attributed the enhancement in k_2 that occurred when both N,N-dimethylglycine ethyl ester and

(24) Shrager, R. I.; Cohen, J. S.; Heller, S. R.; Sachs, D. H.; Schechter, A. N. *Biochemistry* **1972**, *11*, 541–7.
(25) Wertz, D. H.; Scheraga, H. A. *Macromolecules* **1978**, *11*, 9–15.
(26) Hofmann, K. "The Chemistry of Heterocyclic Compounds"; Interscience: New York, 1953; Part I, pp 5–11.
(27) Edwards, B. F. P.; Sykes, B. D. *Biochemistry* **1978**, *17*, 684–9.
(28) Nango, M.; Klotz, I. M. *J. Polym. Sci., Polym. Chem. Ed.* **1978**, *16*, 1265–73.

imidazole were attached to the polymer to a cooperative effect of these two groups. However, the question arises, in light of our observations, as to whether this enhancement is not due to the change of microenvironment in the vicinity of the polymer chain.

The creation of a permanent charge in quaternized polymer should lead similarly to a more polar microenvironment. However, this polymer has a more loose conformation than the acetylated polymer. Moreover, in the quaternized polymer the imidazole is attached to the primary amines whereas it is attached to the secondary amines in all other polymers. Therefore, it is not possible to conclude which modification produces a more polar environment.

The density of the polymer plays an important role. The second-order rate constant obtained with highly laurylated PEI, $L_{0.21}$-PEI-$(CH_2Im)_{0.09}$ (Table IV), is the highest ever reached with an imidazole polymer. We are convinced that this may be about the highest attainable rate. Further increase of lauryl groups would not only reduce polymer solubility but would also prevent a larger number of imidazoles from participating in the reaction.

Correlation of rate increase with an increased content of lauryl groups has been observed previously.[22,29] The effect was attributed to the enhanced apolar environment at the catalytic site. This contradicts our interpretation that the apolar environment augments the natural tendency of imidazole to associate and therefore to be excluded from the reaction. However, one could argue that in a hydrophobic environment the activity of imidazole is raised by desolvation. On the other hand, it is clear that a more polar microenvironment in the vicinity of the polymer provides a greater stabilization of the highly polar transition state.

This statement is in line with known reports about the activity of imidazole in micelles. Analysis of the kinetic data has shown the favorable effect due to increasing the concentration of the reagents in the micelles is almost fully compensated by the unfavorable effect of the hydrophobic micellar environment.[30-32]

Possibility of Cooperation between Imidazole Species. The enhanced catalytic activity of poly[4(5)-vinylimidazole] as compared to imidazole in the hydrolysis of *p*-nitrophenyl acetate (PNPA) has been attributed by Overberger et al. to a bifunctional interaction between neighboring imidazoles in the polymer.[4-6] The validity of this explanation is still taken for granted.[33,34]

The existence of bifunctional imidazole catalysis was previously observed in the hydrolysis of substrates with poorer leaving groups.[35-37] It has never been detected in the hydrolysis of PNPA in water. On the other hand, imidazole–imidazole dimers have been postulated as catalytic species in the hydrolysis of PNPA in acetonitrile.[38] Similarly, associated imidazoles are thought to be responsible for catalysis of ester cleavage in benzene.[39,40]

Our opinion is that the reaction should occur at the polymer surface and not in the hydrophobic region of the polymer. As proposed above, imidazoles associated in the hydrophobic part do not participate in the reaction. Consequently, the probability that two imidazoles will be at the right reaction distance at the polymer surface is very low. Therefore, the assumption that the rate enhancement in the catalysis of imidazole containing polymer is brought about by cooperation of two imidazole residues seems to be rather farfetched.

Moreover, the existence of the cooperation would imply that the poly[4(5)-vinylimidazole] exhibits a higher catalytic activity than copolymers or our polymers. The very opposite is the case. An *inefficient* association of imidazoles instead of the cooperative effect is strongly suggested by the results of many workers. However, this fact was never clearly understood and pointed out.

Kopple, commenting on the distribution of imidazoles in copoly[*N*-vinylpyrrolidinone–4(5)-vinylimidazole] as compared to poly[4(5)-vinylimidazole], concluded that the imidazoles in the homopolymers are bunched.[41] The rate found was slightly higher for copolymers than for homopolymers.

The results of Kunitake and Shinkai have shown that a low concentration of *N*-(5-benzimidazolyl)acrylamide in the copolymer with vinylpyrrolidone does not give rise to aggregation. On the other hand, an increase of content of benzimidazole groups in the copolymer with acrylamide causes an aggregation of benzimidazole units. Consequently, the second-order rate constant given by k_{cat}/K_M decreases with an increase in benzimidazole.[9]

Kunitake et al. concentrated exclusively on the binding capacity of the polymers. They concluded that if hydrophobic interaction is seen as the cause of substrate binding, then a further raising of binding capacity does not necessarily enhance the k_{cat}.[9,10] A lowering of second-order rate constants was never discussed. The following aspects were not considered: (1) unproductive aggregation of imidazole-containing compounds whenever their content increases and (2) unproductive binding of these compounds with other hydrophobic groups present. Schematic representations of the mode of intramolecular aggregation and substrate binding proposed by Kunitake et al. are therefore meaningless.[1,9,42]

Finally, we return to the behavior of poly[4(5)-vinylimidazole]. Overberger and Morimoto have studied the influence of various ethanol–water compositions on this polymer.[43] They rightly concluded that the polymer in low as well as in high ethanol content is tightly coiled due to the nonpolar or H-bonding interaction, respectively. Similarly, an increase in neutral imidazole groups gives rise to a more compact conformation. A rate increase was observed. This rate enhancement was attributed to bifunctional catalysis brought about by the shrinkage of the polymer. The following aspects were, however, neglected. (1) The imidazoles with a tendency to aggregate are exclusively responsible for the compact conformation. They function in a similar way as the lauryl groups in the PEI or the hydrophobic components in copolymers. (2) Only

(29) Birk, Y.; Klotz, I. M. *Bioorg. Chem.* **1971**, *1*, 275–81.
(30) Martinek, K.; Osipov, A. P.; Yatsimirski, A. K.; Dadali, V. A.; Berezin, I. V. *Tetrahedron Lett.* **1975**, 1279–82.
(31) Martinek, K.; Osipov, A. P.; Yatsimirsky, A. K.; Berezin, I. V. *Tetrahedron* **1975**, *31*, 709–18.
(32) Heitmann, P.; Husung-Bublitz, R.; Zunft, H. J. *Tetrahedron* **1974**, *30*, 4137–40.
(33) Overberger, C. G.; Dixon, K. W. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 1863–8.
(34) Overberger, C. G.; Kawakami, Y. *J. Polym. Sci., Polym. Chem. Ed.* **1978**, *16*, 1237–48.
(35) Bruice, T. C.; Benkovic, S. J. *J. Am. Chem. Soc.* **1964**, *86*, 418–26.
(36) Kirsch, J. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 833–7, 837–46.
(37) Caplow, M.; Jencks, W. P. *Biochemistry* **1962**, *1*, 883–93.
(38) Hogg, J. L.; Moris, R., III; Durrant, N. A. *J. Am. Chem. Soc.* **1978**, *100*, 1590–4.
(39) Tonellato, U. *J. Chem. Soc., Perkin Trans. 2* **1978**, 307–12.
(40) Rivetti, F.; Tonellato, U. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1176–9.
(41) Kopple, K. D. *Biopolymers* **1968**, *6*, 1417–24.
(42) Kunitake, T.; Shimada, F.; Aso, C. *J. Am. Chem. Soc.* **1969**, *91*, 2716–23.
(43) Overberger, C. G.; Morimoto, M. *J. Am. Chem. Soc.* **1971**, *93*, 3222–8.

the nonaggregated imidazoles can act as catalysts. The probability of a bifunctional catalysis therefore seems extremely low.

Conclusion

Some of our results can help to clarify several aspects of imidazole catalysis as well as of polymer behavior. The following features deserve attention.

(1) A compact conformation of polymer maintained by hydrophobic forces of apolar groups gives rise to rate enhancement. We attribute this effect mainly to an increase in concentration of the substrate in the polymer domain. However, our results show that a tightly coiled conformation does not favor imidazole catalysis. The position on the polymer preferred by different catalytic species as determined by their polar character should be kept in mind. The implication for imidazole is that we are confronting two factors working in opposite directions. This effect therefore sets a limit to our capability to attain a higher acceleration of the hydrolysis of neutral esters.

(2) Hydrophobic binding is getting continual attention because of its supposed crucial role in enzymatic reactions. However, it has been shown above that the enzymelike pathway does not necessarily cause a more efficient catalysis.

(3) Modifications of remaining amino groups in the PEI do not raise the catalytic activity of imidazole. This effect has been already analyzed above. On the other hand, it seems quite possible that an introduction of polar or apolar groups would produce in the environment of the polymer conditions favorable to a higher rate, provided that the catalytic species would not be attracted into the hydrophobic part of the polymer. The modification chosen would depend on the kind of reaction being studied and on the character of its transition state.

(4) We would like to stress the necessity of having a low imidazole concentration on the polymer. The strikingly high activity of PEI-containing imidazole suggests that modified polymers rather than imidazole polymers should be used in the future.

Acknowledgment. This investigation was supported in part by a grant (No. DMR77-24152) from the Polymers Program, Division of Materials Research, National Science Foundation. The author is grateful to Professor I. M. Klotz for financial support and for the opportunity to perform this research. Also, I am indebted to Ms. Elizabeth W. Fischer for the CFT-20 NMR measurements and thankful to Dr. Susan M. Freier for helpful and refreshing discussions.

Registry No. *p*-Nitrophenyl caproate, 956-75-2.

Functionalized (C_s)-C_{17}-Heptaquinane Derivatives. Chemical Transformations along the Fluted Perimeter of a Topologically Spherical Molecule

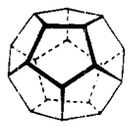
Ronald L. Sobczak, Morey E. Osborn, and Leo A. Paquette*

Evans Chemical Laboratories, The Ohio State University, Columbus, Ohio 43210

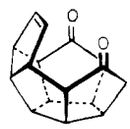
Received July 9, 1979

The previously described hexacyclic enedione **2** is shown to be capable of conversion to the functionalized C_{17}-heptaquinane derivative **4** by reaction with ethyl formate and potassium *tert*-butoxide. The hydroxyl group in **4** can be readily functionalized, but a variety of basic reagents induce ready loss of these groups by E_2 elimination. The conversion of **4** to dienedione **9** is smoothly achieved in two steps (tosylation, treatment with tertiary amine), despite the obvious strain associated with its twisted α,β-unsaturated carbonyl moiety. The conjugated double bond in **9** is a good Michael acceptor. Indeed, diethyl sodiomalonate adds with high stereospecificity to give **12**. **12** was subjected to phenylselenation and oxidative elimination of the selenium substituent to orient the malonate group into an endo position. Subsequent catalytic hydrogenation proceeded with delivery of hydrogen from the exterior surface of the molecule to give **15**. The role that the latter compound might play in the ultimate construction of the pentagonal dodecahedrane molecule is discussed.

As the most elaborate of the Platonic solids, the dodecahedron has held a preeminent position in solid geometry for centuries. In recent years, the many rapid advances in synthetic methodology have prompted organic chemists to attempt the construction of **1** from carbon and



1



2

hydrogen. Although the bond angles in this highly symmetric array of 12 five-membered rings approximate ideality,[1-3] access to this topologically unique hydrocarbon remains a significant challenge. Nonetheless, imaginatively different approaches are being reported with increasing frequency.[4-8] One major obstacle is the need to control

(1) (a) Schultz, H. P. *J. Org. Chem.* **1965**, *30*, 1361. (b) Engler, E. M.; Andose, J. D.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1973**, *95*, 8005. (c) Clark, T.; Knox, T. McO.; Mackle, H.; McKervey, M. A. *J. Chem. Soc., Chem. Commun.* **1975**, 666.

(2) Schulman, J. M.; Venanzi, T.; Disch, R. L. *J. Am. Chem. Soc.* **1975**, *97*, 5335.

(3) Ermer, O. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 411; *Angew. Chem.* **1977**, *89*, 431.

(4) Woodward, R. B.; Fukunaga, T.; Kelley, R. C. *J. Am. Chem. Soc.* **1964**, *86*, 3162.

(5) Jacobson, I. T. *Acta Chem. Scand.* **1967**, *21*, 2235.

0022-3263/79/1944-4886$01.00/0 © 1979 American Chemical Society