

The Michaelis—Menten-Type Hydrolysis of an Anionic Phenyl Ester by Partly Quaternized Poly(1-vinyl-2-methylimidazole) and Its Copolymers*

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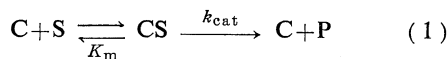
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ABSTRACT: The catalytic hydrolysis of 3-nitro-4-acetoxy-benzoic acid was carried out mainly at 30°C, pH 8.0 in 1.0-*M* aqueous KCl. The polymer catalysts used were prepared by partial quaternization of the homopolymer and copolymers of 1-vinyl-2-methylimidazole with methyl iodide. Michaelis—Menten kinetics were observed for all the catalysts, reaction constants being $K_m=3-12$ mM and $k_{cat}=0.01-0.002$ min⁻¹. Both K_m and k_{cat} values were maximal when the extent of quaternization of MVI polymer was 5—20%. This was probably attributable to the conformational change of the catalytic site in this region. Otherwise, the catalytic activity was explicable in terms of hydrophobic and electrostatic interactions between catalyst and substrate. In the partly quaternized polymers, the binding capacity and the intracomplex reactivity, in general, were increased or decreased simultaneously by varying the polymer composition. However, the binding and intracomplex reaction were compensatory in the case of uncharged polymer catalysts.

KEY WORDS Polymer Catalyst / Imidazole / Michaelis—Menten Kinetics / Hydrolysis / Phenyl Ester /

Previously it had been shown by the authors that several imidazole-containing polymers catalyzed the hydrolysis of phenyl esters according to Michaelis-Menten kinetics as in enzymatic reactions (eq 1 and 2)¹⁻⁴



$$v_{cat} = \frac{k_{cat}[C][S]}{K_m + [S]} \quad (2)$$

where C, S, and P denote catalyst, substrate, and product, respectively.

With these polymer catalysts, substrates were bound to the catalytic site by hydrophobic forces, and substrate binding and intracomplex reaction showed a compensating effect. As a result, the

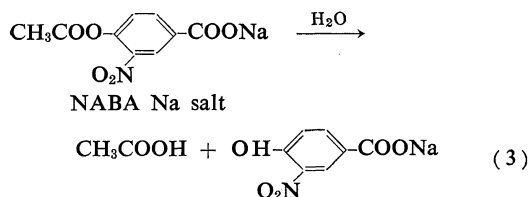
overall efficiency of these catalysts was not particularly good. The compensation effect was not observed when negatively-charged polymer catalysts were employed for hydrolysis of cationic substrates.⁵ In this case, the carboxylate group at the catalytic site presumably enhanced the catalytic efficiency of the imidazole group. Thus, the authors became interested in the catalytic behavior of positively-charged polymer catalysts toward anionic substrates. The catalytic behavior of partly protonated poly(vinylpyridine) and poly(vinylimidazole) toward anionic substrates has been investigated by Letsinger, *et al.*,⁶ and Overberger, *et al.*,⁷ and it was found that the electrostatic attraction between polymer and substrate generally enhanced the catalytic efficiency, although substrate binding was observed only in limited cases.^{8,9}

In this paper, the catalytic hydrolysis (eq 3) of 3-nitro-4-acetoxybenzoic acid (NABA) by partly quaternized homopolymer and copolymers of 1-vinyl-2-methylimidazole is described. The cata-

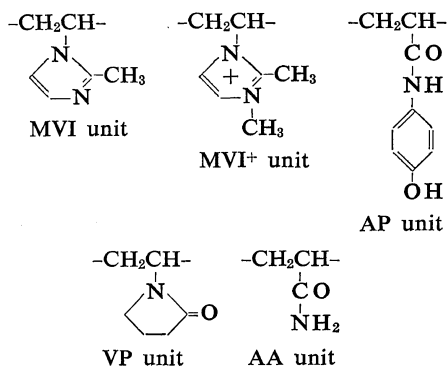
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lytic hydrolysis by unquaternized polymers was also studied for comparison.



The structures of the monomer unit are as follows.



EXPERIMENTAL

Materials

1-Vinyl-2-methylimidazole was kindly provided by Toho Rayon Co. and distilled prior to use: bp 102–103°C (20 mm). The preparations of NABA and *N*-(*p*-hydroxyphenyl)acrylamide and purification of acrylamide were described previously.^{1,3}

Polymerization and Quaternization

Polymerizations were conducted using azobisisobutyronitrile (AIBN) as initiator at 70°C in methanol solvent. The results are summarized in Table I. The copolymers with acrylamide were reprecipitated from water and methanol, and the other polymers were reprecipitated from methanol and acetone or from methanol and acetone–ether.

Partly quaternized polymers, MVI⁺-1–MVI⁺-5, were prepared by the reaction of MVI homopolymer with CH₃I. Methyl iodide was added to MVI homopolymer dissolved in methanol, and the reaction mixture was gently refluxed with stirring. After 24 hr the reaction mixture was poured into acetone–ether, and the polymer recovered. Recoveries were 90–96%. Approximately two thirds of the CH₃I added were consumed.

Polymers MVI⁺–AP-1 and MVI⁺–AP-2, 3 were prepared by reaction of MVI–AP-1 and MVI–AP-2 with CH₃I, respectively. The polymers dissolved in dimethylformamide were treated with CH₃I. The mixture was stirred for 2 hours at room temperature and 20 hours under reflux. When the mixture became too viscous to stir during the reaction period, a small amount of water was added. The polymer was recovered by precipitation in acetone.

Characterization of Polymer

The content of the unquaternized MVI unit was determined by titration at 30°C in 1.0*M* aqueous KCl. The AP unit was determined prior to quaternization from the elemental analytical data and by infrared spectroscopy using phthalimide as an internal standard as described

Table I. Polymerization^a

Polymer	AIBN, <i>M</i> × 10 ³	Monomer, <i>M</i>			Polymerization time, min	Conversion, %
		MVI	AP	AA		
MVI-10	30	5.9	—	—	30	77.0
MVI–AP-2	0.10	2.0	0.05	—	60	46.5
MVI–AP-2	0.10	2.0	0.20	—	60	48.5
MVI–AP–AA-1	0.63	0.02	0.07	0.50	120	28.8
MVI–AP–AA-2	0.63	0.05	0.01	0.50	60	29.2
MVI–AP–AA-3	0.63	0.10	0.015	0.50	50	27.0
MVI–AP–AA-4	0.63	0.50	0.010	0.50	100	30.8

^a 70°C, methanol.

Ester Hydrolysis by Polymer Catalyst

Table II. Properties of unquaternized polymer catalysts

Polymer	MVI unit, mol %	AP unit, ^a mol %	pK _a	n'	pK _{int}
MVI-10 ^b	100	—	5.62	1.75	6.30
MVI-AP-AA-1	1.4	1.2	6.99	1.14	7.13
MVI-AP-AA-2	1.8	6.6	7.02	1.11	7.14
MVI-AP-AA-3	8.7	2.4	7.10	1.30	7.41
MVI-AP-AA-4	48.0	0.5	6.67	1.71	7.33

^a Determination by IR spectroscopy.

^b [η] (dl/g): 0.252 (1.0-M KCl, 30°C); 0.567 (1:1-aqueous ethanol, 30°C).

Table III. Properties of quaternized polymer catalysts

Polymer	Free MVI unit, mol %	AP unit, mol % ^a	pK _a ^b	n'	pK _{int} ^c	[η], dl/g 1-M KCl, 30°C	η _{sp} /c, dl/g (1:1 (vol)-H ₂ O—EtOH, 30°C)
MVI+-1	14.0	—	5.38	1.10	5.65	0.321	—
MVI+-2	54.5	—	5.43	1.22	5.84	0.297	1.61 (c=0.598 g/dl)
MVI+-3	69.4	—	5.42	1.24	5.89	0.315	—
MVI+-4	87.7	—	5.60	1.63	6.24	0.278	—
MVI+-5	96.9	—	5.64	1.65	6.24	0.266	—
MVI+-AP-1	26.3	11.1(10.4)	5.45	1.22	6.0 ^d	0.263	—
MVI+-AP-2	33.2	20.9(19.7)	5.50	1.32	6.2 ^d	0.101	—
MVI+-AP-3	17.9	20.9(19.7)	5.43	1.21	5.8 ^d	0.113	1.35 (c=0.853 g/dl)

^a Determination by IR spectroscopy. The value in parentheses is obtained by elemental analysis.

^b Titration condition: 30°C, 1.0-M KCl.

^c Obtained from eq 5 by extrapolation to α=1.

^d Linearity of the pK_{app}—α plot of eq 5 was not very good.

previously.³

Some properties of the polymer catalyst are summarized in Tables II and III. In these tables, the pK_a values correspond to the pH values at half neutralization (α=0.5). The modified Henderson—Hasselbach equation

$$pK_a = pH + n' \log [(1-\alpha)/\alpha] \quad (4)$$

was applied to the titration data. In this equation, (1-α) represents the degree of protonation of the unquaternized imidazole unit, and n', if greater than unity, indicates the presence of electrostatic repulsion between the protonated imidazole groups.

The pK_{int} value is obtained by plotting pK_{app} against the degree of protonation, 1-α, and extrapolating to α=1.

$$pK_{int} = \lim_{\alpha \rightarrow 1} pK_{app} = \lim_{\alpha \rightarrow 1} \left(pH + \log \frac{1-\alpha}{\alpha} \right) \quad (5)$$

Thus pK_{int} values correspond to the pK_a value of the polymer of which unquaternized imidazole unit is not protonated at all. The electrostatic effect persists for pK_{int} of quaternized polymers since the influence of the quaternized imidazole unit cannot be removed by this procedure.

Kinetics

The hydrolysis was investigated by using a pH-stat system (TOA Electronics Ltd., model HS-IB) mainly at pH 8.0, 30°C in 1.0-M aqueous KCl. The observed rate v_{obsd} is the sum of the rates of spontaneous and catalytic hydrolyses. Details of the procedure is given elsewhere.¹

Viscosity

The viscosities of the polymer catalysts were measured at 30°C using a modified Ubbelohde viscometer. The results are included in Tables II and III. Linear plots of η_{sp}/c vs. c were

observed for all the polymer samples in 1.0-*M* KCl. The high ionic strength used (1.0-*M* KCl) is therefore, sufficient to suppress the electroviscous effect even for a highly charged polymer like MVI⁺-1 (86% quaternized). In 1:1(vol)-aqueous ethanol the electroviscous effect was observed for all the samples used except for MVI-10.

RESULT OF CATALYTIC HYDROLYSIS

Quaternized Polymer Catalyst

The catalytic hydrolysis of NABA with positively-charged polymer catalyst proceeded according to Michaelis—Menten kinetics (eq 1). An example is shown in Figure 1. It is clear that the reaction rate reaches a saturation value at high substrate concentrations. The reaction parameters were calculated from the linear relationship in the Lineweaver—Burk plot¹⁰

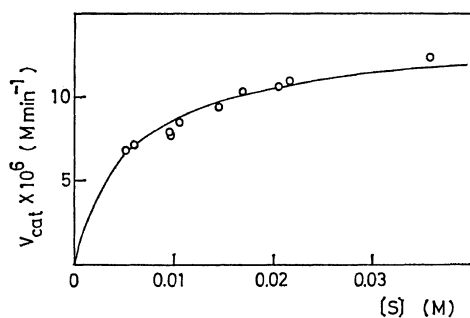


Figure 1. Substrate saturation curve; catalyst, MVI⁺-3, 6.16×10^{-3} *M*; 30°C; 1.0-*M* KCl; pH 8.0.

Table IV. Catalytic hydrolysis with quaternized polymer^a

Polymer	[C] ^b , <i>mM</i>	<i>K_m</i> , <i>mM</i>	10 ³ <i>k_{cat}</i> , min ⁻¹
MVI ⁺ -1	6.09	6.67	2.98
MVI ⁺ -2	6.12	7.14	2.63
MVI ⁺ -3	6.16	6.90	2.32
MVI ⁺ -4	6.60	9.71	1.92
MVI ⁺ -5	3.18	12.3	6.26
MVI ⁺ -AP-1	2.60	4.55	5.19
MVI ⁺ -AP-2	4.35	4.00	5.61
MVI ⁺ -AP-3	3.06	3.47	10.7

^a Hydrolysis condition: 30°C, pH 8.0, 1.0-*M* KCl.

^b Concentration of the free MVI unit in the reaction mixture.

$$\frac{1}{v_{\text{cat}}} = \frac{K_m}{k_{\text{cat}}[C]} \frac{1}{[S]} + \frac{1}{k_{\text{cat}}[C]} \quad (6)$$

The results with quaternized polymer catalysts are summarized in Table IV. The substrate binding capacity of these polymers increases somewhat with increasing extent of quaternization. This means that, in addition to the hydrophobic interaction, the electrostatic interaction with the anionic substrate contributes to the substrate binding.

Introduction of the AP unit enhanced substrate binding (*K_m* term) and catalytic activity (*k_{cat}* term) simultaneously.

Unquaternized Polymer Catalysts

In Table V are shown results of catalytic hydrolysis with the MVI homopolymer and

Table V. Catalytic hydrolysis with unquaternized polymer^a

Polymer	[C] ^b , <i>mM</i>	<i>K_m</i> , <i>mM</i>	10 ³ <i>k_{cat}</i> , min ⁻¹
MVI-10	4.42	7.81	8.42
MVI-10 ^c	6.87	6.45	2.05
MVI-AP-AA-1	7.69	20	97
MVI-AP-AA-2	5.70	15	88
MVI-AP-AA-3	2.02	22	79
MVI-AP-AA-4	8.28	55	64
MVI-AA ^d	—	52	109

^a Hydrolysis condition: 30°C, pH 8.0, 1.0-*M* KCl unless stated otherwise.

^b Concentration of the free MVI unit in the reaction mixture.

^c Hydrolysis condition: 30°C, pH 6.0, 1.0-*M* KCl. *pK_{app}* at pH 6 is 5.78, and 37.8% of the MVI unit is protonated under the condition used.

^d Data from ref 1.

MVI-AP-AA terpolymers. Michaelis—Menten kinetics were observed for all cases. The reaction constants were derived from the Lineweaver—Burk plot as mentioned above. The hydrolysis result with MVI-AA copolymers is included for comparison.

The *k_{cat}* values of the unquaternized polymers (MVI-10) were greater than that of the partly quaternized counterpart given in Table IV. This may be related to the decreased nucleophilicity of the MVI unit in the latter polymers, as reflected in lower *pK_{int}* values. This problem is

further discussed below.

The MVI homopolymer has 37.8% of the MVI unit protonated at pH 6.0. When the catalytic properties at pH 8.0 and 6.0 are compared, it is clear that substrate binding slightly increases with increasing protonation but that k_{cat} decreases appreciably. The catalytic properties at pH 6.0 resemble closely those of MVI⁺-3 polymer which has 30.6% of the monomer unit quaternized. The effects of protonation and of quaternization on the catalytic property are therefore very similar. In other words, it is not significant whether the cationic unit is formed by protonation or by quaternization.

From a comparison of the data for MVI-AA and MVI-AP-AA copolymers, it is apparent that the presence of the AP unit contributed appreciably to substrate binding through hydrophobic forces. At the same time, k_{cat} values for the terpolymer were smaller than those for MVI-AA copolymers.

Influence of Some Hydrolysis Conditions on the Overall Rate

Figures 2 and 3 show influences of ionic strength and solvent composition on the overall rate of catalytic hydrolysis.

The overall rate with partly quaternized catalysts, MVI⁺ and MVI⁺-AP, was hardly affected by the variation of ionic strength (Figure 2). In mixed solvents of acetonitrile and water, $v_{cat}/[C]$ increased, probably because of increased electrostatic forces between polymer and substrate. The overall rate with MVI⁺-AP-3 in 40% aqueous acetonitrile decreased remarkably with the addi-

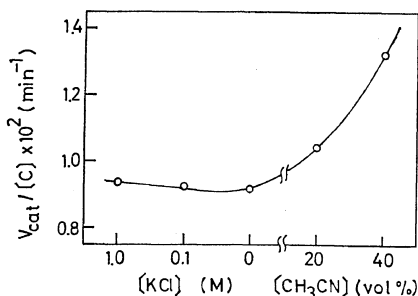


Figure 2. Influence of ionic strength and solvent composition on the catalytic rate: catalyst, MVI⁺-AP-3, 3.06×10^{-3} M; substrate, 0.020 M; 30°C; pH 8.0.

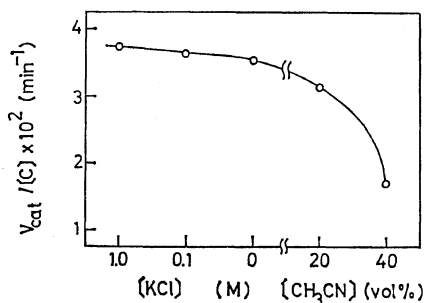


Figure 3. Influence of ionic strength and solvent composition on the catalytic rate: catalyst, MVI-AP-AA-3 2.02×10^{-3} M; substrate, 0.020 M; 30°C; pH 8.0.

tion of 0.5-M KCl. It appears that both the hydrophobic and electrostatic interactions are considerably reduced in this system. The influence on the intracomplex process is not clear.

On the other hand, overall rates $v_{cat}/[C]$ of neutral catalyst MVI-10 and MVI-AP-AA-3, decreased with decrease in ionic strength and with addition of acetonitrile (Figure 3). These results can probably be ascribed to weakening of hydrophobic forces and the corresponding decrease in substrate binding.

DISCUSSION

Substrate Binding

In previous publications it was pointed out that the conformation of the polymer catalyst correlates with its catalytic behavior.³ Figure 4 shows the relation between the viscometric and titration behavior of the quaternized MVI polymer and the degree of quaternization. The intrinsic viscosity increased with increasing quaternization, suggesting the expansion of the polymer coil due to intramolecular electrostatic repulsion. The decrease of the n' value with the extent of quaternization indicates that the electrostatic repulsion among unquaternized MVI units decreases due to the increasing screening effect of the quaternized unit.

The variation of K_m with the degree of quaternization is more complex as shown in Figure 5. It is interesting that the Michaelis constant increased by 70% by introducing only 3% of the quaternized unit (MVI⁺-5). The K_m value

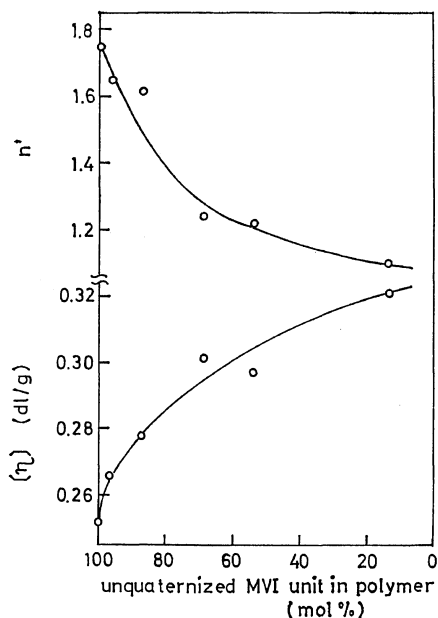


Figure 4. Viscometric and titration behavior of partly quaternized polymers: 30°C; 1.0-*M* KCl.

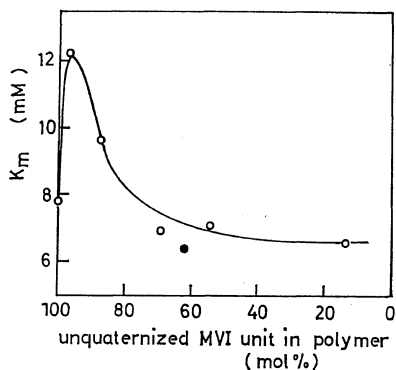


Figure 5. Variation of K_m with the degree of quaternization of MVI polymers: ○, 30°C, 1.0-*M* KCl, pH 8.0; ●, catalyst MVI-10, 30°C, 1.0-*M* KCl, pH 6.0.

then decreases to a constant value at about 30% quaternization. The substrate binding of MVI-10 catalyst is ascribable mainly to hydrophobic forces. The increased K_m value of MVI⁺-5 relative to MVI-10 is attributed, at least in part, to decreased hydrophobicity of the former catalyst. By further increasing the amount of the charged unit, K_m decreases probably because of the increased electrostatic attraction between

polymer and substrate. It is possible that the increasing electrostatic attraction is compensated by decreasing hydrophobic forces when the content of the charged unit is greater than 30%.

Intracomplex Process

Figure 6 shows variations of pK_{int} and $\log k_{cat}$ with the composition of the neutral MVI

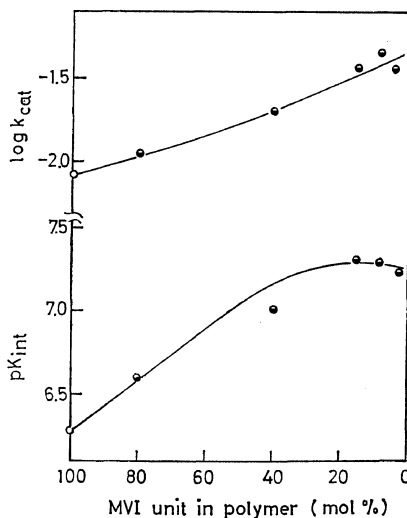


Figure 6. Variation of pK_{int} and $\log k_{cat}$ with the composition of unquaternized polymers: data taken from ref 1.

copolymer. pK_{int} and $\log k_{cat}$ show parallel trends. The corresponding relation is more complex for the partly quaternized MVI polymer, as shown in Figure 7. The pK_{int} value decreases approximately linearly with increasing quaternization. On the other hand, $\log k_{cat}$ decreases rather abruptly with quaternizations of up to 20%, and gradually increases with further quaternization. The abnormal change of k_{cat} in the region of low MVI⁺ contents has an interesting correspondence with the variation of K_m in the same region (cf. Figure 5). Thus both the substrate binding ($1/K_m$) and the intracomplex reaction rate (k_{cat}) of the partly quaternized polymer are minimal when the extent of quaternization is 5–20%. The introduction of relatively small amounts of the quaternized unit into the MVI polymer seems, therefore, to cause an appreciable change in the nature of the catalytic site.

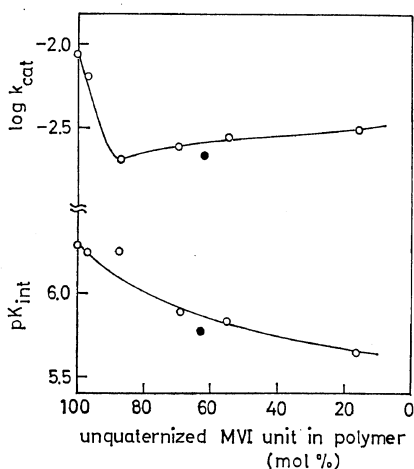


Figure 7. Variation of pK_{int} and $\log k_{cat}$ with the degree of quaternization of polymer catalysts: ○, MVI⁺ polymers 30°C, 1.0-M KCl, pH 8.8; ●, MVI-10 polymer at pH 6.0, 30°C, 1.0-M KCl. pK_{app} of MVI-10 polymer is 5.78 at pH 6.0.

It is conceivable that the variation of the nature of the catalytic site involves the conformational change. As shown in Figure 7, pK_{int} decreases with the extent of quaternization, indicating that the basicity of the MVI unit gradually decreases due to the electrostatic effect of the charged unit. Therefore, if the simple electrostatic effect of the MVI⁺ unit is the sole factor in determining the catalytic properties, $\log k_{cat}$ should change monotonously with the content of the MVI⁺ unit.

The observed variation of K_m and k_{cat} at low MVI⁺ contents, therefore, cannot be explained simply in terms of the electrostatic effect of the MVI⁺ unit. It is possible that an appreciable change occurs in the nature and structure of the catalyst-substrate complex in this region. The mode of substrate binding may change considerably due to the introduction of small amounts of the charged unit into the neutral MVI homopolymer. Considering the limited solubility of the neutral MVI polymer in aqueous solutions, the MVI polymer will be fairly compact in 1.0-M aqueous KCl. Quaternization of some of the MVI unit should cause expansion of the compact polymer coil. Previously it was concluded that a catalytic loop was formed upon binding of substrated with vinylpyrrolidone

polymers.¹ A catalytic loop may also be formed in the present case, its structure being sensitive to the presence of the quaternized unit. At present, further delineation of the catalytic site is not possible.

It has been reported that polyvinylpyridine and polyvinylimidazole showed maximum catalytic activities on the phenyl ester hydrolysis at appropriate pH regions where neutral and protonated base groups coexisted.^{6,7} In these cases the results were discussed on the assumption that the reactivity of a basic group is the same, irrespective of varying extents of protonation. However, the present data shows that k_{cat} is dependent on the degree of quaternization and/or protonation, apart from the possible influence of the conformation of the catalytic site. Although the k_{cat} value refers to the rate of intracomplex reaction, a similar situation probably arises in the ordinary second-order reaction, and the reactivity of a given catalytic group in polymer catalysts such as polyvinylpyridine and polyvinylimidazole may as well vary with the degree of protonation. In fact, pK_{int} values, which are a measure of the nucleophilic reactivity, were dependent on the extent of quaternization in the present catalytic system. It is particularly important to know the reactivity of a catalytic group in a polymer under the given reaction condition.

Correlation of Substrate Binding and Intracomplex Reaction

The compensation between substrate binding and intracomplex reaction was observed previously for some uncharged polymer catalysts.⁴ A similar relation can be found for the uncharged polymer catalysts containing the MVI unit. In Figure 8 are plotted the unitary free-energy changes of substrate binding ΔG_u against the free energy of activation ΔG^\ddagger . These free-energy changes were calculated from the following equations.⁴

$$\Delta G_u = RT \ln K_m - 7.98 T \quad (7)$$

$$\Delta G^\ddagger = -RT \ln \frac{k_{cat} h}{kT} \quad (8)$$

It is apparent that ΔG^\ddagger increases with increasing substrate binding ($-\Delta G_u$ increases). Therefore, the intracomplex reaction becomes more

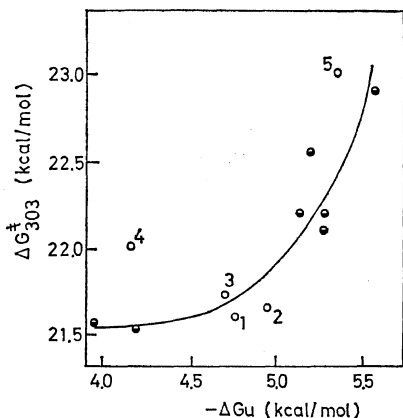


Figure 8. Relation between the free energy change of substrate binding and the activation free-energy of intracomplex reaction for unquaternized polymer catalysts; 30°C, 1.0-*M* KCl, pH 8.0. Catalyst: 1, MVI-AP-AA-1; 2, MVI-AP-AA-2; 3, MVI-AP-AA-3; 4, MVI-AP-AA-4; 5, MVI-10; ●, data from ref 1.

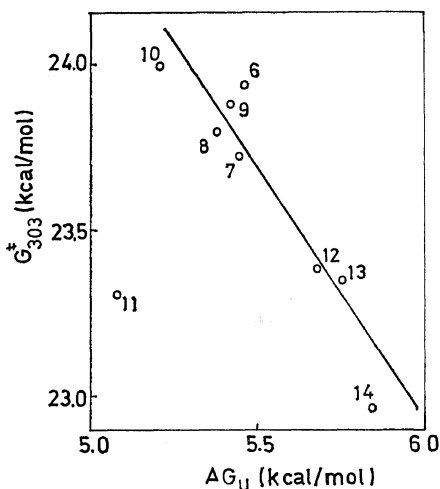


Figure 9. Relation between the free-energy change of substrate binding and the activation free-energy of intracomplex reaction for partly quaternized polymer catalysts; 30°C; 1.0-*M* KCl; pH 8.0 unless noted otherwise. Catalyst: 6, MVI-10 (pH 6.0); 7, MVI⁺-1; 8, MVI⁺-2; 9, MVI⁺-3; 10, MVI⁺-4; 11, MVI⁺-5; 12, MVI⁺-AP-1; 13, MVI⁺-AP-2; 14, MVI⁺-AP-3.

difficult as the hydrophobic nature of the catalytic site increases, as in the previous system.⁴

Figure 9 shows the relation of ΔG_u and ΔG^\ddagger

for the quaternized polymer catalysts. The data for MVI⁺-AP terpolymers (No. 12, 13, and 14) are included. Excluding one plot, ΔG^\ddagger is approximately inversely proportional to $-\Delta G_u$. The plot for MVI-10 catalyst at pH 6 (No. 6) falls near the same line. This relation indicates that the substrate binding and intracomplex reaction rate are simultaneously enhanced or depressed. For instance, the increase in substrate binding due to the introduction of the hydrophobic AP unit (MVI⁺-AP catalysts) lowered the ΔG^\ddagger of the intracomplex reaction.

Although the plots in Figure 9 give a linear relationship, the linearity is not very satisfactory. The plot for MVI⁺-5 (No. 11) deviates significantly. The deviation, as also reflected in the plots of Figures 5 and 7, is apparently due to conformational changes in the polymer chain as discussed above.

As shown in Figure 8, the hydrophobic nature, *per se*, of the catalytic site is not advantageous for the intracomplex hydrolysis. On the other hand, when the charged group exist at the predominantly hydrophobic catalytic site, the enhanced substrate binding due to additional hydrophobic interaction appears to result in better stabilization of the transition state of the intracomplex reaction. This tendency was observed also in the catalytic hydrolysis of positively-charged phenyl esters by anionic polymer catalysts.⁵

Somewhat similar results were reported for a micellar system by Gitler and Ochoa-Solano.¹¹ They noted in a mixed micelle system of *N*-myristoyl-L-histidine and cetyltriethylammonium bromide that the increased hydrophobicity of phenyl esters was involved at the micellar surface in the stabilization of the activated complex in the presence of the micellar positive charge. Bruice, *et al.*,¹² studied the aminolysis of positively- and negatively-charged phenyl esters by anionic micellar systems containing secondary amine groups, and found that the rate was affected by the combination of the hydrophobic and electrostatic interactions.

CONCLUSION

Partly quaternized poly(1-vinyl-2-methylimidazole) and its copolymer catalyzed the hydrolysis

of 3-nitro-4-acetoxybenzoic acid according to Michaelis—Menten kinetics. That the positively-charged unit at the catalytic site lowered the free energy of activation of the intracomplex reaction is of particular interest. Since the quaternized imidazole unit should not show specific catalytic properties, its influence is due to the enhancement of the polar nature of the catalytic site, which leads to stabilization of the ionic transition state. The contribution of the quaternized unit to the overall catalytic efficiency is not great, and one has to look for better combinations of catalytic groups, in order to attain much higher catalytic activities.

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