Imidazole Catalyses in Aqueous Systems. X.
Enzyme-like Catalytic Hydrolyses of Phenyl Esters by Polymers Containing Imidazole and Other Anionic Functions[*]

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ABSTRACT: The catalytic hydrolysis of p-acetoxybenzoic acid (ABA⁻) and 3-acetoxy-N-trimethylanilinium iodide (ANTI⁺) was carried out mainly at 30°C and pH 8.0 in 1.0-M aqueous KCl. The polymer catalysts used were water-soluble terpolymers of acrylamide, N-[p-(4(5)-imidazolyl)benzyl]acrylamide (PI unit), and N-phenyl-methacryl-amide derivatives with anionic functional groups such as sulfonate (BS unit), carboxylate (BA unit), hydroxyl (NP unit), and hydroxamate groups (HA unit). Michaelis—Menten kinetics were observed for all the catalysts; the reaction constants were $K_m = 14.5$—200 mM and $k_{cat} = 0.004$—0.026 min⁻¹ for the imidazole catalysis. The K_m value for ANTI⁺ decreases with increase in the content of BS and BA units in the polymers. On the other hand, the value of K_m for ABA⁻ triples during the same increase: this establishes that the electrostatic interaction contributes to the substrate binding in addition to the hydrophobic interaction. The plot of k_{cat} vs. the BA content in the PI—BA polymers gave a maximum at ca. 30-mol % BA unit, whereas the k_{cat} value for PI—BS polymer increased monotonously. These trends suggest the existence of cooperative action be-tween the imidazole and carboxylate functions, but not between imidazole and sulfonate. The plots of log k_{cat} vs. pK_a of the anionic functional groups gave good linear relation-ships, and the general-basic contribution of the anionic functional groups is proposed. The catalytic behavior of the PI—HA polymer features the burst alkali consumption due to the HA unit. A novel scheme involving the acyl transfer from HA unit to PI unit, which is a model of α-chymotripsin, is suggested.
    KEY WORDS   Polymer Catalyst / Imidazole / Hydrolysis / Michaelis—Menten Kinetics / Multifunctional Catalysis /

Previously we showed that some water-soluble copolymers containing the imidazole unit cata-lyzed the hydrolysis of phenyl esters according to Michaelis—Menten kinetics:[1-6]

$$C + S \underset{K_m}{\rightleftharpoons} C \cdot S \xrightarrow{k_{cat}} C + P \qquad (1)$$
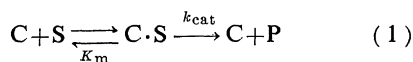
where C, S, and P denote catalyst (neutral imidazole), substrate, and product, respectively. The corresponding reaction rate is expressed by eq 2, which indicates saturation of the reaction rate at high substrate concentrations.

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

With these polymer catalysts, substrates were found to be bound to the catalytic sites of polymers by the hydrophobic force as well as by the electrostatic interaction, and the existence of the carboxylate group as a polymeric neigh-boring group enhanced the nucleophilic activity of the imidazole group in the hydrolysis of a cationic phenyl ester.[5] These results encouraged us to design polymer catalysts in which an imidazole group is densely surrounded by several anionic functions.

It was found in the case of water-soluble co-polymers of acrylamide with some hydrophobic monomers that the pendent hydrophobic groups
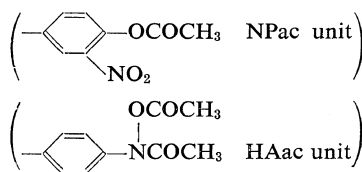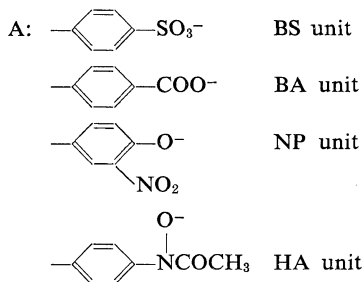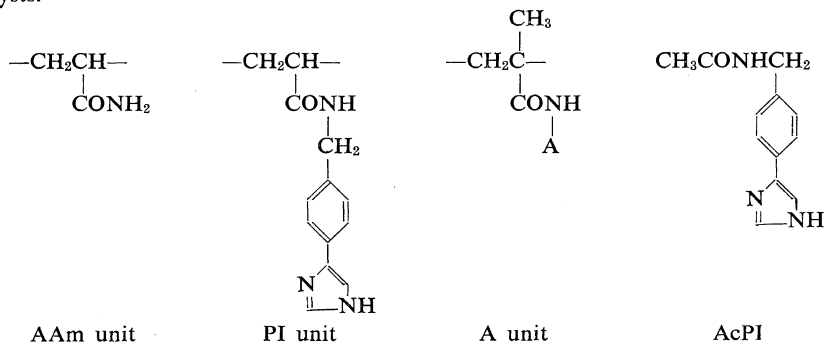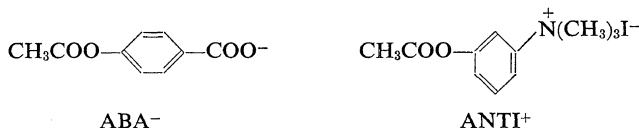
aggregate to form a strengthened binding site.[3] In order to improve the catalytic efficiency we intended to utilize the aggregated conformation through the introduction of imidazole and anionic functions at the tip of the hydrophobic pendent. If the aggregation of such functional groups is actualized, the polymer would possess a hydrophobic binding site and a multifunctional catalytic site as in the active sites of some esterolytic enzymes.[7] Thus, the following terpolymers were synthesized from the corresponding monomers, and their catalytic efficiencies for ANTI$^+$ and ABA$^-$ were assessed. Acrylamide was used as a component to keep the terpolymers water-soluble.

Catalysts:

$-CH_2CH-$ $-CH_2CH-$ $-CH_2C-$ $CH_3CONHCH_2$
 $CONH_2$ $CONH$ $CONH$

AAm unit   PI unit   A unit   AcPI

A: —⟨ ⟩—SO_3^- BS unit

—⟨ ⟩—COO^- BA unit

—⟨ ⟩—O^- NP unit (NO_2)

O^- —⟨ ⟩—$NCOCH_3$ HA unit

(—⟨ ⟩—$OCOCH_3$ NPac unit) (NO_2)

($OCOCH_3$ —⟨ ⟩—$NCOCH_3$ HAac unit)

Substrates:

CH_3COO—⟨ ⟩—COO^-

ABA$^-$

CH_3COO—⟨ ⟩—$\overset{+}{N}(CH_3)_3I^-$

ANTI$^+$

EXPERIMENTAL

Materials

Monomers and catalysts: N-[p-(4(5)-imidazolyl)benzyl]acrylamide(PI monomer) and 4(5)-(p-acetamidomethylphenyl)-imidazole(AcPI) were obtained as described previously.[3,4]

Sodium N-(p-methacryloyl)-aminobenzenesulfonate(BS monomer) was prepared from an equimolar mixture of sodium p-aminobenzenesulfonate and methacryloyl chloride in an ice bath in the presence of excess sodium acetate. The aqueous solution was poured into acetone and the precipitates(NaCl) were filtered. White

crystals were obtained from the concentrated filtrate. N-(p-carboxyphenyl)-methacrylamide (BA monomer), N-(3-nitro-4-hydroxyphenyl)-methacrylamide(NP monomer), and N-(p-nitrophenyl)-methacrylamide were synthesized according to a method similar to that for N-(p-hydroxyphenyl)-acrylamide.[3] Recrystallizations from water—alcohol provided the monomer crystals listed in Table I.

N-(3-nitro-4-acetoxyphenyl)-methacrylamide (NPac monomer) was prepared by the condensation of NP monomer with acetic acid in the presence of N,N'-dicyclohexylcarbodiimide according to the method of Bodanoszky and

Table I. Identification of monomers

Monomer	mp, °C	Yield, %	IR (KBr) νC=O	νC=C cm⁻¹	E.A. Found C	H	N	Calcd C	H	N	for
BS	—	74	1656	1620	45.41	3.81	5.88	45.62	3.83	5.32	$C_{10}H_{10}NO_4SNa$
BA	220—221[a]	78	1690, 1670	1624	63.41	5.48	6.77	63.48	5.40	6.38	$C_{11}H_{11}NO_3$
NP	108—109	66	1658	1615	53.98	4.51	12.52	54.05	4.54	12.61	$C_{10}H_{10}N_2O_4$
NPac	132—133	82			54.54	4.58	10.60	55.88	4.68	10.77	$C_{12}H_{12}N_2O_5$
N-(p-nitrophenyl)-methacrylamide	143—144	84	1670	1612	58.22	4.66	13.51	58.25	4.89	13.59	$C_{10}H_{10}N_2O_3$
HAac	171—174° (dec)	14[b]	1735, 1670	1622	59.56	5.91	10.19	60.86	5.84	10.14	$C_{14}H_{16}N_2O_4$

[a] lit[8] mp 215—216°C

[b] Yield based on N-(p-hydroxylaminophenyl)-methacrylamide.

Vigneaud.[9] N-(p-nitrophenyl)-methacrylamide was reduced to N-(p-hydroxylaminophenyl)-methacrylamide by Zn—HCl in NH_4Cl-buffered aqueous THF solution,[10] mp 170—210°C (dec), yield 82%. A broad absorption was observed at 3300—3000 cm⁻¹ in the IR spectrum (KBr). The hydoxylamine derivative was treated with acetic anhydride to provide N-(p-methacryloyl-amidophenyl)-N,O-diacetyl-hydroxylamine (HAac monomer). The yield of the product was low, but no further attempts were made to improve the yield. All analytical results are summarized in Table I.

Substrates: Preparations of p-acetoxybenzoic acid(ABA⁻) and 3-acetoxy-N-trimethylanilinium iodide(ANTI⁺) were described previously.[3,5]

Polymerization and Deacylation

Polymerizations were conducted using azobis-isobutyronitrile (AIBN) as initiator at 70°C. The results are summarized in Table II. Water-soluble polymers were purified by reprecipitation from water to methanol. Some polymers were dissolved by neutralization with aqueous NaOH solution and reprecipitated from water to metha-nol—acetone (1 : 1).

Polymers containing the HAac monomer unit were dissolved in a diluted aqueous NaOH solution and left at room temperature overnight. The solutions were poured into acetone or meth-anol—acetone (1 : 1) and polymers containing N-(p-methacryloylamidophenyl)-acetohydroxamic acid(HA monomer unit) were obtained.

The composition of PI unit was always sup-

Table II. Polymerization[a]

Polymer	Monomer feed, M PI	A	AAM	Poly-merization time, min	Con-version, %
	A = BS[b]				
PI—BS-1	0.05	0.05	2.00	40	30.2
PI—BS-2	0.05	0.30	2.00	40	35.9
PI—BS-3	0.05	0.56	1.50	40	32.5
PI—BS-4	0.05	1.50	0.50	120	29.1
PI—BS-5	0.08	2.00	0	120	18.6
	A = BA[c]				
PI—BA-1	0.05	0.05	2.00	60	38.1
PI—BA-2	0.05	0.30	2.00	60	46.5
PI—BA-3	0.05	0.50	1.50	40	32.1
PI—BA-4	0.05	1.00	1.00	120	32.0
PI—BA-5	0.05	2.00	0	120	22.5
	A = NP[d]				
PI—NP-1	0.05	0.05	2.00	240	22.4
PI—NP-2	0.07	0.10	2.00	600	16.2
NP-1	0	0.05	2.00	240	18.5
	A = NPac				
PI—NPac	0.03	0.10	1.00	180	25.1
NPac-1	0	0.10	1.00	180	30.0
	A = HAac				
PI—HAac	0.05	0.036	2.00	90	36.0
HAac-1	0	0.05	2.00	90	33.5

[a] 70°C; methanol was used as the solvent unless otherwise listed; [AIBN], 1/400 of total monomer concentration.

[b] methanol containg 25-vol % water, BPO as initiator.

[c] methanol containg 25-vol % tetrahydrofuran.

[d] [AIBN], 1/500 of total monomer concentration.

pressed below 4 mol%, in order to make clear the effect of the composition of the A unit.

Characterization of Polymers

The contents of PI, BA, NP, and HA units were determined by titration at 30°C in 1.0-M aqueous KCl. The Visible absorption spectra of NP units and elemental analysis were also useful for this purpose. The polymer compositions were determined, as shown in Table III, by a combination of these techniques.

Some properties of the polymer catalysts are also listed in Table III. The modified Henderson—

Table III. Properties of polymer catalysts

Polymer	PI unit,[d] mol %	A unit,[d] mol %	pK_a PI	pK_a A	n' of PI
		A=BS			
PI—BS-1	3.5	4.1	5.80	—	1.16
PI—BS-2	3.5	26.0	5.82	—	1.39
PI—BS-3	3.4	54.9	5.89	—	1.49
PI—BS-4	3.5	71.0	6.09	—	1.20
PI—BS-5	3.7	96.3	6.44	—	1.04
		A=BA			
PI—BA-1	2.1	2.1	5.78	4.03	1.11
PI—BA-2	2.2	14.5	5.97	4.27	—
PI—BA-3	2.2	33.1	—	—	—
PI—BA-4	2.5	51.0	—	—	—
PI—BA-5	2.9	97.1	—	—	—
		A=NP			
PI—NP-1	1.3	4.1	—	—	—
PI—NP-2[a]	1.6	7.9	—	—	—
PI—NP-3[b]	2.7	9.2	—	—	—
NP-1	0	3.8	—	6.52	—
		A=NPac			
PI—NPac	2.7	9.2	—	—	—
NPac-1	0	9.6	—	—	—
		A=HA			
PI—HA[c]	2.2	1.7	5.87	8.98	1.29
HA-1	0	2.8	—	8.80	—

[a] $[\eta]$, 0.110 dl/g.

[b] Hydrolysis product of PI—NPac; $[\eta]$, 0.191 dl/g.

[c] Hydrolysis products of PI—HAac and HAac-1, respectively.

[d] The compositions of terpolymers were evaluated by two independent results as follows: PI—BS-1—5, titration of PI and elemental analysis; PI—BA-3—5, titration of PI+BA and elemental analysis; PI—NP-1—3, titration of PI+NP and visible absorption of NP; PI—HA, PI—BA-1—2 titration (titration curves for each functional group were separated).

Hasselbach equation was applied to the titration curve, in which $(1-\alpha)$ represents the degree of protonation of the dissociable functions, and n', if greater than unity, indicates the presence of electrostatic repulsion between the protonated phenylimidazole units.

$$pK_a = pH + n' \log[(1-\alpha)/\alpha] \qquad (3)$$

Viscosities of the polymer catalysts were measured at 30°C by using a modified Ubbelohde viscometer. Linear plots of η_{sp}/C *vs.* C were observed for all the polymer samples in 1.0-M KCl, which was the same condition as that of the kinetic measurement. It is seen, therefore, that the high ionic strength employed here is sufficient to suppress the electroviscous effect.

Kinetics

The catalytic hydrolysis was carried out by using a pH-stat system (TOA Electronics Ltd., model HS-1B) mainly at pH 8.0 and 30°C in 0.1-M aqueous KCl. The observed rate (v_{obs}) is the sum of the spontaneous rate (v_{sp}) and the catalytic rate (v_{cat}). The details of the procedure for determination of v_{cat} values have been described previously.[1]

The hydrolysis of NPac unit in polymer was conducted in aqueous solutions buffered by Tris (0.02 M) at 28°C. The ionic strength was adjusted to 0.1 with KCl. The hydrolysis rates were determined by monitoring the absorption of nitrophenolate unit (λ_{max} 432 nm, log ε=3.58, as determined by using NP-1 polymer).

RESULTS

Catalytic Hydrolysis with PI—BS and PI—BA Terpolymers

The catalytic hydrolysis of ANTI⁺ and ABA⁻ with PI—BS and PI—BA terpolymer catalysts proceeded according to Michaelis—Menten kinetics (eq 2), in which the reaction rate reaches a saturation value ($v_{max}=k_{cat}[C]$) at high substrate concentrations. The reaction parameters, k_{cat} and K_m, were calculated from the linear relationship in the Lineweaver—Burk plot (eq 4):

$$\frac{1}{v_{cat}} = \frac{K_m}{k_{cat}[C]} \cdot \frac{1}{[S]} + \frac{1}{k_{cat}[C]} \qquad (4)$$

where [C] denotes the concentration of unprotonated imidazole. The results were sum-

Table IV. Catalytic hydrolysis by the phenyl-imidazole function in the terpolymers[a]

Polymer	A unit, mol %	Substrate = ANTI[+] $k_{cat} \times 10^3$, min^{-1}	K_m, mM	Substrate = ABA[−] $k_{cat} \times 10^3$, min^{-1}	K_m, mM
PI—BS-1	4.1	16.4	21	4.2	8.7
PI—BS-2	26.0	20.5	30	5.0	9.3
PI—BS-3	54.9	38.3	32	14	17.5
PI—BS-4	71.0	38	24	19	19
PI—BS-5	96.3	43	14.5	22	26
PI—BA-1	2.1	41	83		
PI—BA-2	14.5	51	83	11.9	19
PI—BA-3	33.1	74	38	25.7	17
PI—BA-4	51.0	53	25	13.1	50
PI—BA-5[b]	97.1	48	18	10.5	55
PI—NP-1	4.1	92	143		
PI—NP-2	7.9	109	200	13.6	14
PI—NP-3	9.2	95	41	18	20
PI—HA	1.7	33[c]	56		

[a] 30°C; 1.0-M KCl; pH 8.0; [PI unit], 0.52—1.71 × 10^{-3}M; [Substrate], 0.005—0.10M.
[b] 4-vol % acetonitrile.
[c] k_{cat} for the PI unit; k_{cat} for the HA unit was 870 min^{-1}.

marized in Table IV. The K_m value for ANTI[+] decreased with increase in the content of BS and BA units in polymers. In contrast, the valve of K_m for ABA[−] tripled during the same increase. It is evident, therefore, that ANTI[+], the cationic substrate, is favorably bound to the anionic polymer catalysts, and that the electrostatic interaction contributes to the formation of C·S complex in addition to the hydrophobic interaction.

Hydrolysis of NPac Unit

In order to assess the catalytic activity of the intrapolymeric PI unit toward the *o*-nitrophenyl ester unit(NPac), hydrolysis was conducted in three different manners: (A) spontaneous hydrolysis of the NPac unit in the polymer (NPac-1); (B) catalytic hydrolysis of the NPac unit in NPac-1 by a monomeric imidazole, AcPI; (C) catalytic hydrolysis of the NPac unit in PI—NPac by the intra-polymeric PI unit. The reaction rates were determined by the measurement of the absorption based on the released *o*-nitrophenolate anion in visible spectroscopy (Table V). The addition of AcPI slightly increased the apparent reaction rate. On the other hand, the NPac unit in the PI—NPac terpolymer decayed rapidly due to the intra-polymeric catalysis of

Table V. Hydrolysis of NPac unit in polymer[a]

Method	Polymer	Imidazole, M	k_{app}, min^{-1}	k_{cat},[b] M^{-1}min^{-1}
A	NPac-1	—	0.0050	
B	NPac-1	2.90 × 10^{-4} (AcPI)	0.0068	4.8
C	PI—NPac	1.46 × 10^{-4} (PI unit)	0.0236	127

[a] Tris buffer 0.02M; pH 8.0; 28.0°C; μ, 0.10 with KCl.
[b] $k_{cat} = (k_{app} - k_{spont})/$[imidazole].

the PI unit. These results suggest the preference of the intra-polymeric catalysis to that of the intermolecular AcPI catalysis. In fact, the second-order rate constant for process C is 26 times faster than process B. Therefore, the introduction of a catalytic group into a polymeric substrate greatly improves the intramolecular catalytic efficiency.

Catalytic Hydrolysis with PI—NP Terpolymers

The catalytic hydrolysis with PI—NP terpolymer catalysts also satisfied Michaelis—Menten kinetics and showed increased k_{cat} values relative to those with PI—BS and PI—BA catalysts (Table IV). Since the intra-polymeric catalysis of *o*-nitrophenyl ester unit by the PI-unit is efficient, it becomes important to assess whether the nitrophenolate function in PI—NP polymers serve as a nucleophile toward the substrates. Once the acetyl group is transferred from the substrate to the *o*-nitrophenolate unit, the acetyl intermediate would be decomposed rapidly by the intrapolymeric catalysis of phenylimidazole (Scheme I).
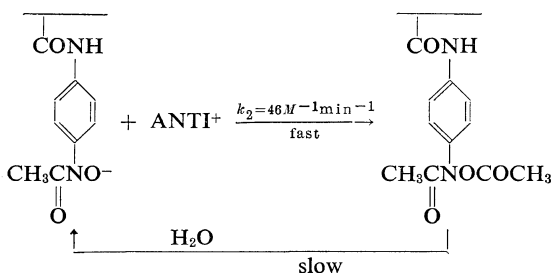

Scheme I
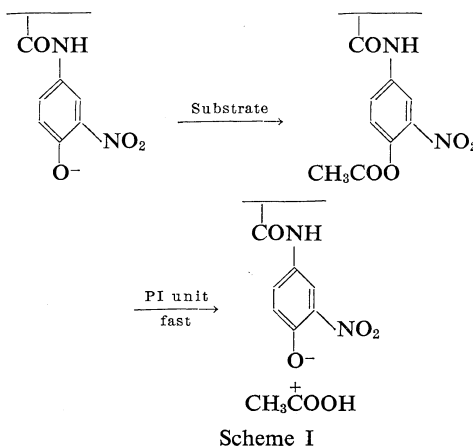
The NP-1 polymer, having no PI unit, was added to an $ANTI^+$ solution ([NP unit]$=3.80\times10^{-4}M$, [$ANTI^+$]$=2.0\times10^{-4}M$, pH 8.0) and then the time-course of the nitrophenolate absorption (432 nm) and the alkali consumption due to the hydrolysis were examined. The absorption at 432 nm was constant and the rate of alkali consumption decreased by *ca.* 5% compared to the spontaneous rate. These observations indicate that the nitrophenolate function in the polymer does not act as a nucleophile, but rather retards the spontaneous decay of $ANTI^+$. The k_{cat} values in the catalytic hydrolysis with PI—NP polymers were larger than those with PI—BS and PI—BA polymers, so that it is concluded that the nitrophenolate function enhances the nucleophilicity of the PI group.

Catalytic Hydrolysis with Polymers Containing the Hydroxamate Function

Hydroxamate groups have been shown to display excellent nucleophilic activity due to the α-effect.[11-14] High esterolytic activities were observed for the monomeric hydroxamates[15,16] as well as for the polymeric hydroxamates.[17] The N-phenylacetohydroxamate unit in the acrylamide-base polymer (HA-1) also gave rise to rapid alkali consumption which concluded within 15 min (Figure 1). The rate of the subsequent alkali consumption was identical with that of spontaneous hydrolysis of $ANTI^+$, suggesting that the decomposition of the acetyl hydroxamate intermediate is too slow to be discriminated from the spontaneous hydrolytic rate (Scheme

II). The apparent second-order rate constant, $k_2(=v_{obs}/[\text{hydroxamate}][ANTI^+])$, was computed to be 46 $M^{-1}\,min^{-1}$ at 30°C, 1.0-M KCl, whereas the k_2 value for the hydrolysis of $ANTI^+$ catalyzed by AcPI was 0.75 $M^{-1}\,min^{-1}$ under the same reaction conditions.
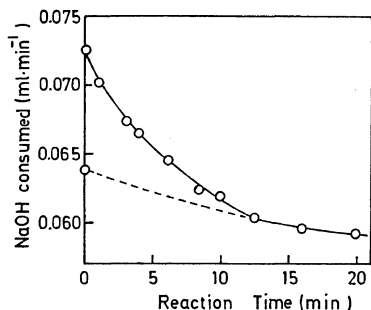
Scheme II

These results imply that the hydroxamate function in the PI—HA polymer attacks the substrate more readily than the imidazole function does.

The catalytic hydrolysis of $ANTI^+$ with PI—HA polymer also was in accord with the Michaelis—Menten equation (eq 2). The hydrolysis was conducted at two pH values, 8.0 and 9.1, where the K_m values were found to be identical. The observed rate is composed of the PI and HA terms as follows:

$$v_{cat}=k_{cat}[C]=k_{cat,PI}[PI]+k_{cat,HA}[HA] \quad (5)$$

The $k_{cat,PI}$ and $k_{cat,HA}$ values were calculated to be 33 and 870 min^{-1}, respectively, from the pK_a of the HA unit and the overall rates observed.

DISCUSSION

Substrate Binding

The catalytic hydrolysis by AcPI, which corresponds to the monomeric unit of the polymeric PI residue, followed the second-order kinetics, whereas all the polymers in Table III showed saturation of the reaction rate at high substrate concentrations. It is obvious, therefore, that this difference is caused by the strengthened hydrophobic binding sites brought about by the aggregation of polymer residues. In previous publications we have also substantiated the intrapolymeric aggregation of hydrophobic pendent residues in aqueous systems through the titration, viscometric, and catalytic behavior.[3-5]
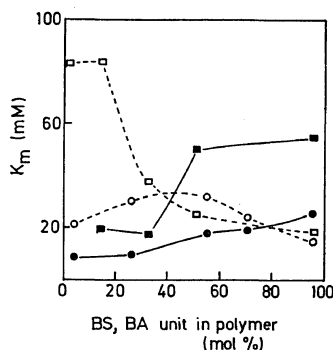
Figure 1. Hydrolysis rate of $ANTI^+$ with the hydroxamate-containing polymer (HA-1). The dotted curve denotes the spontaneous hydrolysis rate. pH 9.0; 30°C; μ, 1.0 with KCl; [S], 0.010-M $ANTI^+$, [HA unit], $3.20\times10^{-3}M$; NaOH used is 0.0935 N.

Figure 2. Variation of K_m with the degree of anionic functions: ○, ANTI$^+$ with PI—BS polymers; □, ANTI$^+$ with PI—BA polymers; ●, ABA$^-$ with PI—BS polymers; ■, ABA$^-$ with PI—BA polymers.

The effect of the content of the second component (A unit) on the substrate binding is shown in Figure 2. One would anticipate that the anionic polymer would favor the cationic substrate in preference to the anionic counterpart, and this is the case. The increase of BA content brought forth the decrease of K_m in the binding of ANTI$^+$ and the increase in the ABA$^-$ binding. This result means that some selectivity could be achieved by the combination of the charged polymer and substrates. On the other hand, the effect of the BS content on the K_m value is rather slight. This difference between BS- and BA-containing polymers may be caused by the conformation change, which would bring about a difference in the charge density around the PI unit.

We previously examined the esterolytic efficiency of the PI unit in the copolymers with acrylic acid and methacrylic acid.[5] Interestingly, the rate of catalytic hydrolysis of ABA$^-$ with the PI—acrylic acid copolymer was proportional to the substrate concentration and the second-order rate constant ($0.163\ M^{-1}\ min^{-1}$) was smaller than that obtained with AcPI catalyst ($0.272\ M^{-1}\ min^{-1}$). This smaller catalytic activity of the polymer is probably due to the repulsion between the like charges of the polymer and the substrate. In contrast, the anionic polymer of Table IV showed substrate binding for ABA$^-$, in spite of the expected electrostatic repulsion. Observation of the Michaelis—Menten kinetics

in the present system proves that the hydrophobic attraction overcomes the electrostatic repulsion in substrate binding.

Intracomplex Catalysis

It is expected in the intracomplex hydrolysis of bound substrates that the second functional groups cooperate with the PI unit to result in the enhanced catalytic efficiency. A basic functional group will be preferred for this purpose. The pK_a value of sulfonic acids is estimated to be around 0.6^{18}, and the nucleophilic and/or general-basic catalytic contribution of the BS unit appears negligible. The pK_a value of the PI unit in the PI—BS terpolymer rose with increase in the BS content and k_{cat} showed an apparent parallel behavior (Figure 3). One may consider that the BS unit simply provides negatively charged environments where pK_a (and therefore the nucleophilicity) of the PI unit is enhanced. It is to be noted that the n' value of the PI unit in the PI—BS polymer is maximal at *ca.* 50-mol% BS content (Table III). This indicates that the electrostatic interaction of the PI unit is greatest when *ca.* 50-mol% BS unit is contained. Conceivably, this effect is superimposed on the environment effect of the BS unit mentioned above.

Carboxylate groups play an important role in some hydrolytic enzymes.[7] This is also true in synthetic systems, as shown clearly in the
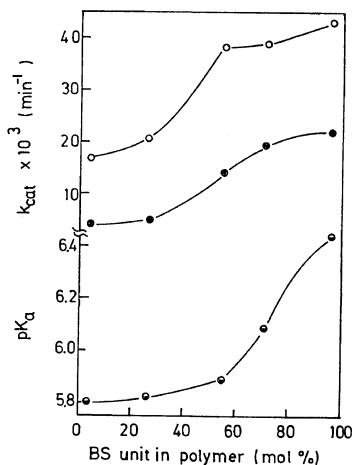
Figure 3. Variation of k_{cat} with the degree of BS unit and correlation with pK_a: ○, ANTI$^+$ with PI—BS polymers; ●, ABA$^-$ with PI—BS polymers.

hydrolysis of acrylic acid—nitrophenyl acetate copolymer[19] and of aspirin.[20] The importance of the imidazole—carboxylate combination in the polymer catalysis has been reported by Overberger, *et al.*,[21] and Shimidzu, *et al.*,[22] Overberger, *et al.*, observed that the apparent second-order rate constant was maximal at 47-mol% imidazole content for the hydrolysis of ANTI[+] catalyzed by copolymers of 4(5)-vinyl-imidazole and acrylic acid. In contrast, the activity of the vinylimidazole—vinylsulfonic acid copolymer increased monotonously with increase in the content of the vinylsulfonic acid unit. Subsequently, Shimidzu and coworkers established that the catalytic hydrolysis by vinylimidazole—acrylic acid copolymers was of the Michaelis—Menten type, and found that the intracomplex rate constant k_{cat} was minimal at 20—30-mol% imidazole content. The maximum of the apparent rate in this region was brought about, instead, by the favorable binding.

In the present study, the plot of k_{cat} *vs.* the BA content in the PI—BA terpolymer gave a maximum at *ca.* 30-mol% BA unit (Figure 4), whereas the k_{cat} value for the PI—BS terpolymer increased monotonously with increase in the BS content (Figure 3). These trends are contrary to those observed in the above-mentioned system, and suggest that the PI and BA units act cooperatively in the catalytic process. Apparently, this cooperative action becomes particularly favorable at a BA content of *ca.*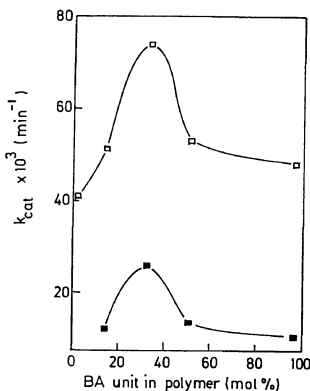 30 mol%. The flexibility of the polymer chain, which is controlled by the electrostatic repulsion and the hydrophobic attraction of side chains, can have a dominating influence on the cooperativity.

In the catalytic hydrolysis with the PI—NP polymer, we could not obtain any evidence for the nitrophenolate anion acting as a nucleophile. Therefore, the higher activity of the PI—NP polymer (*ca.* 2 times as reactive as PI—BA) should be attributed to the general-basic contribution of the nitrophenolate anion, which can be efficient due to its high pK_a value. Unfortunately, the values of pK_a for the PI unit in PI—BA and PI—NP polymers could not be determined because of the overlapping of their titration curves with those of the BA and NP groups. Therefore, the possible enhancement of catalysis due to increased pK_a (*i.e.*, nucleophilicity) of the PI unit cannot be separated from the general-basic contribution of the second functional groups.

$$(6)$$
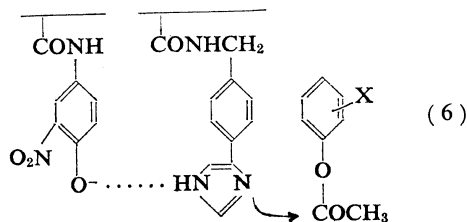
The plots of log k_{cat} of A unit give good linear relationships for copolymers containing less than 30 mol% of A unit (Figure 5). The observed linearity may dictate that the second functional groups (BS, BA, and NP) contribute
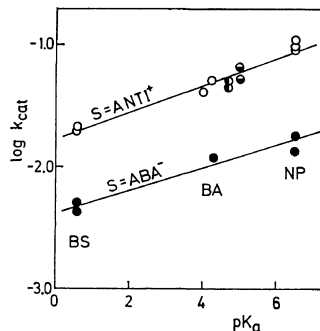
Figure 4. Variation of k_{cat} with the degree of BA unit: □, ANTI[+] with PI—BA polymers; ■, ABA[−] with PI—BA polymers.

Figure 5. Relation between log k_{cat} and pK_a of anionic functions. The previous results for the following systems[5] are also recorded: ◖, PI—acrylic acid copolymers; ◗, PI—methacrylic acid copolymers.

to the catalytic efficiency by the same mechanism (cf. eq 6).

The catalytic behavior of the PI—HA polymer is greatly different from that of other polymers. The k_{cat} values of PI and HA units were determined to be 33 and 870 min^{-1}, respectively, from the pH dependence of the overall k_{cat} value. Since k_{cat} for the HA unit is much greater than that of the PI unit, the hydrolysis may be preferentially catalyzed by the HA unit at the initial stage of the reaction. As established for PI—NPac polymer, the intra-polymeric acetyl transfer from nitrophenolate ester to imidazole is efficient. Since the alkaline hydrolysis of acetohydroxamate ester proceeds more easily than that of p-nitrophenyl ester,[24] the ester of the HA unit would be also decomposed readily by the PI unit. Application of this scheme to the PI—HA polymer enables us to depict a novel scheme of the bifunctional catalysis (Scheme III):
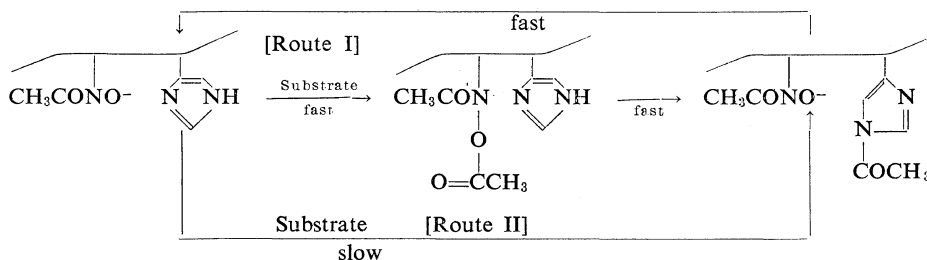
Scheme III

Although the deacylation of acetylimidazole is very fast, the nucleophilicity of the imidazole group is not so excellent (Route II). The hydroxamate anion shows contrasting behavior in the acylation—deacylation relationship (Scheme II).[23,24] Hence, the combination of these two groups would cancel out the slower rate determining steps unless the intra-molecular acyl transfer, newly required for Route I, becomes rate-determining. One should note that the process of Route I is quite similar to the catalytic process of α-chymotrypsin. Unfortunately, no clear evidence for the recycling of the catalyst groups shown in Route I could be obtained from the PI—HA polymer at hand, because it was difficult to discriminate the catalytic turn-over from the spontanous hydrolysis. It is expected that the polymers containing higher compositions of the hydroxamate unit would follow Route I. In fact, recent studies in this laboratory have established that this route is taken when the copolymer of 4(5)-vinylimidazole and N-phenylacrylohydroxamic acid[25,26] is employed.

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