Multifunctional Hydrolytic Catalyses. 7. Cooperative Catalysis of the Hydrolysis of Phenyl Esters by a Copolymer of *N*-Methylacrylohydroxamic Acid and 4-Vinylimidazole

Toyoki Kunitake* and Yoshio Okahata

Contribution No. 396 from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka, 812, Japan. *Received March 16, 1976*

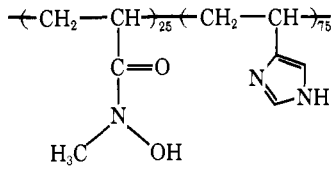
Abstract: A bifunctional polymer catalyst was prepared by radical polymerization of benzyl *N*-methylacrylohydroxamate and 4-vinylimidazole, followed by the removal of the benzyl group, and the hydrolysis of four phenyl ester substrates was examined in 28.9 vol/vol % EtOH–H_2O at 30 °C. The Michaelis–Menten kinetics were observed only for a long-chain, anionic substrate. The major course of catalysis was acylation and deacylation at the hydroxamate group. The acylation process consisted of the simple nucleophilic attack of the hydroxamate anion (k_A–) and the general base (imidazole) catalyzed nucleophilic attack of the hydroxamic acid (k_{HA}). In the case of *p*-nitrophenyl acetate (PNPA), k_{HA} and k_A– were 0.50 and 36 M^{-1} s^{-1}, respectively, and the difference was much smaller for other substrates. The deacylation reaction proceeded efficiently due to the general-base catalysis of the imidazole group. Thus, the imidazole group acts as a general base in both the acylation and deacylation process, in close resemblance to the charge relay system of serine proteases.

Multifunctionality plays important roles in enzymatic catalyses. In previous papers of this series we showed that combinations of hydroxamate and imidazole functions in polymeric[1,2] and small-molecule[3,4] systems produced enhanced catalytic efficiencies toward the hydrolysis of phenyl esters. A similar study was reported very recently by Kitaura and Bender.[5] These systems may be likened to the charge relay system of serine proteases[6] in that the oxygen nucleophile and the imidazole group contribute to the catalysis. However, the concerted bifunctional action characteristic of the enzyme system was not observed in the synthetic systems.

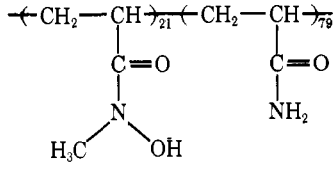
Considerable efforts have been directed to the preparation of multifunctional esterolytic catalysts which act in concerted manners. However, Bruice and co-workers asserted that there were no known examples of intramolecular bifunctional catalysis of ester hydrolysis.[7,8] A recent report by Kirby and Lloyd provides a rare exception in this respect, even in the intramolecular case.[9]

The lack of the concerted action in our previous bifunctional polymers will be, at least partially, attributed to the presence of a third component (acrylamide unit) which was incorporated in order to increase solubility. Thus, a bifunctional polymer without a third component, copoly(*N*-methylacrylohydroxamic acid 4-vinylimidazole) [MHA-VIm (**1**)], was prepared and its catalytic behavior compared with monofunctional polymers, copoly(*N*-methylacrylohydroxamic acid acrylamide) [MHA-AAm (**2**)] and copoly(4-vinylimidazoleacrylamide) [VIm-AAm (**3**)]. Four representative phenyl esters were employed as substrates.

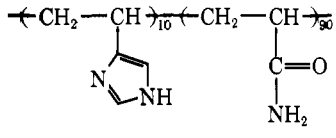
polymers



MHA-VIm (**1**)

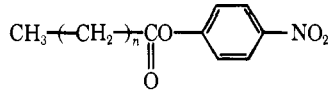


MHA-AAm (**2**)

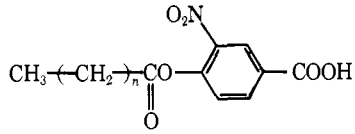


VIm-AAm (**3**)

substrates



PNPA (**4**), $n = 0$
PNPH (**5**), $n = 4$



NABA (**6**), $n = 0$
NNBA (**7**), $n = 7$

Experimental Section

Materials. Phenyl esters were prepared as described previously: *p*-nitrophenyl acetate [PNPA (**4**)],[3] mp 78 °C; 3-nitro-4-acetoxybenzoic acid [NABA (**6**)],[10] mp 154–157 °C; *p*-nitrophenyl hexanoate [PNPH (**5**)],[11] bp 142–147 °C (0.2 mm). 3-Nitro-4-nonanoyloxybenzoic acid [NNBA (**7**)], mp 45 °C, was prepared by application of the preparative procedure of Overberger et al.[12] 4-Vinylimidazole was obtained according to the procedure of Overberger et al.,[12] mp 83 °C. Acrylamide was recrystallized from benzene, mp 83.5–84.5 °C.

Benzyl *N*-methylacrylohydroxamate was synthesized by the following route.

To a carefully dried three-necked flask equipped with a stirrer, a thermometer, and a dropping funnel were added 7.20 g (0.15 mol) of 50% NaH (dispersed in oil) and 50 ml of dry tetrahydrofuran (THF). After purging with dry nitrogen, 36.0 g (0.16 mol) of benzyl benzohydroxamate (mp 101–102 °C)[4,13] in 200 ml of dry THF was dropwise added over 30 min with vigorous stirring at room temperature. Evolution of hydrogen which was very vigorous in the beginning was almost complete in 60 min. Next, 28.4 g (0.20 mol) of methyl iodide in 50 ml of dry THF was added over 30 min at room temperature. The reaction mixture was stirred an additional 4 h at 60–70 °C. After cooling to room temperature, a small amount of methanol was added in order to decompose unreacted NaH, and the mixture was

Table I. Copolymerization[a]

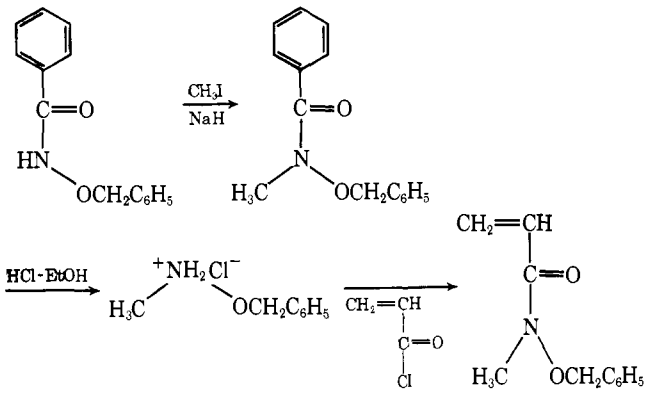
Run	Monomer, M: MHA	VIm	AAm	Time, min	Conversion, wt %	Copolymer composition, mol %: Method[b]	MHA	VIm	AAm
1	0.37	1.50		190	34	PT	25	75	
						NMR	26	74	
						Uv	25	75	
2[c]	1.0			190	87		100		
3	0.45		1.05	170	76	NMR	21		79
4[d]		0.10	0.90	20	40	PT		10	90

[a] 80 °C, methanol solvent; AIBN, 0.5 mol % of total monomer. [b] The copolymer composition was determined by the potentiometric titration (PT), by NMR spectroscopy (NMR), or by phototitration (uv). [c] 80 °C, benzene solvent; AIBN, 0.5 mol % of monomer. [d] From ref 2.

Table II. Titration of Copolymers

Copolymer	Composition, mol %: MHA	VIm	AAm	Functional group	pK_a	n'	pK_{in}[a]
MHA-VIm (**1**)	25	75		MHA[b]	10.5	1.15	10.3
				VIm[c]	5.50	2.15	6.56
MHA-AAm (**2**)	21		79	MHA[b]	10.2	1.66	9.53
VIm-AAm (**3**)[d]		10	90	VIm[c]	5.95	1.46	6.37

[a] pK_{in} is the limiting pK_a value at the zero dissociation for the MHA unit or at the complete dissociation for the VIm unit. [b] Phototitration: 28.9 vol/vol % EtOH–H_2O, 30 °C, μ = 0.1 (KCl), 0.15 M Tris buffer. [c] Potentiometric titration: 30 vol/vol % EtOH–H_2O, 30 °C, μ = 0.1 (KCl). [d] From ref 2.



filtered. The filtrate was evaporated in vacuo. The clear oily residue (45 g) was dissolved in 300 ml of ethanol and heated to reflux for 25 min with 400 ml of concentrated hydrochloric acid. The clear solution was diluted with 500 ml of water and, while still warm, benzoic acid and other by-products were separated by extraction with benzene (200 ml × 3). The aqueous layer was evaporated to dryness under reduced pressure to afford crude *O*-benzyl-*N*-methylhydroxylamine hydrochloride, which was recrystallized from ethanol: yield 24 g (85%); colorless needles; mp 95 °C.

O-Benzyl-*N*-methylhydroxylamine hydrochloride (21 g, 0.12 mol) and 35.0 g (0.33 mol) of triethylamine were dissolved in 300 ml of chloroform. To this solution was added dropwise 12.7 g (0.14 mol) of freshly distilled acryloyl chloride in 30 ml of chloroform with stirring at −5 °C over 40 min, and stirring was continued for an additional 1 h at room temperature. The reaction mixture was washed with 100 ml of water and with three 50-ml portions of a dilute Na_2CO_3 solution and dried over Na_2SO_4. The solvent was evaporated and the brown oily residue was distilled in vacuo: bp 105–108 °C (0.15 mm); yield 60%. Anal. ($C_{11}H_{13}O_2N$) C, H, N.

Polymerizations. The copolymerization of benzyl *N*-methylacrylohydroxamate (MHA monomer) and 4-vinylimidazole (VIm) was conducted in a degassed ampule in methanol with azobis(isobutyronitrile) (AIBN) initiator. The polymerization mixture was then poured into excess ether. The recovered polymer was dissolved in 30% HBr in CH_3COOH, the solution stirred for 100 h at room temperature, and the solvent removed in vacuo. The residual (debenzylated) polymer was reprecipitated from methanol and ether, subjected to extensive dialysis in H_2O (Diaflo Ultra-Filter, UM-2), concentrated, and freeze-dried. Complete cleavage of the benzyl group was confirmed by disappearance of NMR peaks (4.8 and 7.3 ppm) attributable to the benzyl protons. The hydroxamate homopolymer and its copolymer with acrylamide were prepared by similar procedures. The copolymer of vinylimidazole and acrylamide (AAm) was described before.[2]

The composition of the MHA-VIm (**1**) copolymer hydrobromide was determined by three different methods: NMR spectroscopy in D_2O, uv spectroscopy, and potentiometric titration. The NMR method employed the peak areas of the imidazole C-1 (7.7 ppm) and C-2 (9.1 ppm) protons, the *N*-methyl proton (3.5 ppm) of the MHA unit, and the methylene proton (2.4 ppm) of the main chain. In the uv method, the MHA unit content was determined by assuming that the MHA anion possesses an extinction coefficient identical with that of the *N*-methylisobutyrohydroxamate anion: ϵ 1960 at 260 nm in 28.9 vol/vol % EtOH–H_2O. The potentiometric titration was carried out in 30 vol/vol % EtOH–H_2O [30 °C, μ = 0.1 (KCl)] for the protonated VIm unit with 0.1 N NaOH. The copolymer compositions determined by these three methods agreed with each other as shown in Table I. The compositions of other copolymers were similarly determined.

***p*K_a Determination.** The pK_a value of the hydroxamate unit was determined by using its absorbance difference at 260 nm: ϵ 1960 in 0.1 N NaOH (complete dissociation) and ϵ 140 at pH 7.2 (neutral species). The pK_a value of the VIm unit was determined by the potentiometric titration. The MHA homopolymer could not be titrated in 28.9 vol/vol % EtOH–H_2O due to its insolubility in the neutral pH region. The titration data were plotted according to the modified Henderson–Hasselbach equation[14]

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

where α is the fraction of the dissociated MHA unit or of the neutral VIm unit. The titration results are summarized in Table II.

Rate Measurement. Hydrolysis reactions were conducted in 28.9 vol/vol % EtOH–H_2O at 30 °C, μ = 0.1 (KCl), and the phenol formation was followed at 401 nm for *p*-nitrophenolate and at 430 nm for 3-nitro-4-oxybenzoate anion. When large excesses (10^{-2}–10^{-3} M) of PNPA (**4**) substrate were employed (burst kinetics), a 0.906-cm spacer was inserted to a 1-cm cell. Otherwise, 1-cm cells were used as such. The kinetic procedures have been described in detail.[1,2] The reaction rates were calculated by using a programmable desk calculator (Compet 364p, Sharp Co., Ltd). The least-squares treatment

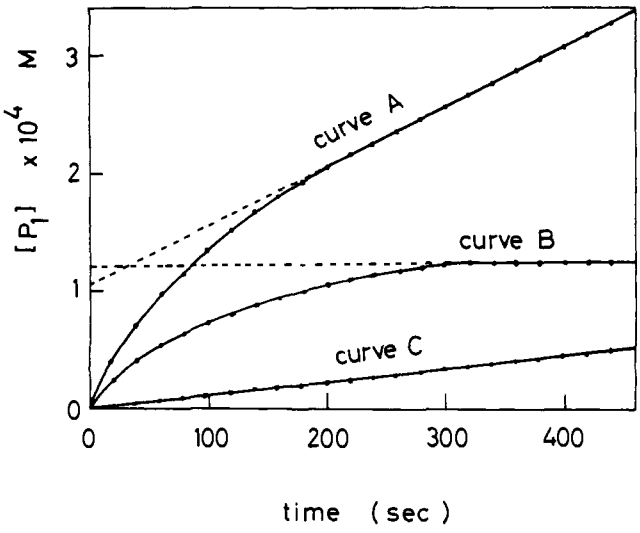


Figure 1. Time course of the *p*-nitrophenol formation in the catalytic hydrolysis of PNPA (**4**): 30 °C, 28.9 vol/vol % EtOH–H_2O, μ = 0.1 (KCl), 0.15 M barbital, pH 8.67 ± 0.03, [PNPA] = 1.55×10^{-2} M. Curve A: MHA-VIm copolymer (**1**), [MHA] = 1.21×10^{-4} M, [VIm] = 3.63×10^{-4} M. Curve B: MHA-AAm copolymer (**2**), [MHA] = 1.23×10^{-4} M. Curve C: VIm-AAm copolymer (**3**), [VIm] = 3.53×10^{-4} M.

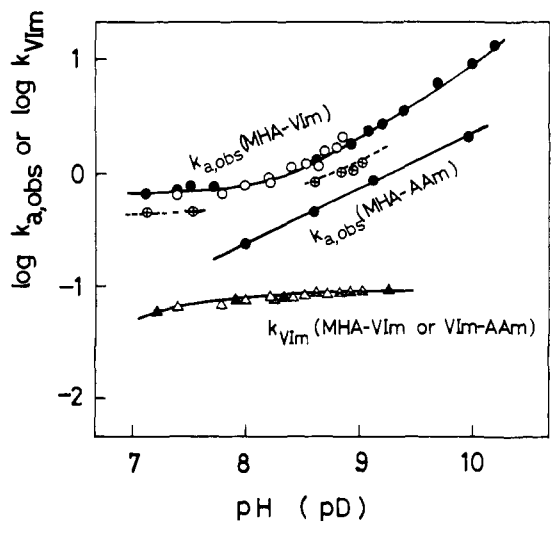


Figure 2. pH (pD) dependence of acylation rates for PNPA (**4**) substrate: in 28.9 vol/vol % EtOH–H_2O, 30 °C, and μ = 0.1 (KCl), (○, △) obtained from the burst kinetics and (●, ▲) obtained from the pseudo-first-order kinetics. In 28.9 vol/vol % EtOD–D_2O, 30 °C, and μ = 0.1 (KCl), (⊕, ▲) obtained from the burst kinetics. Solid curves were calculated from rate constants and α (Tables II and III). The k_{VIm} values were determined using MHA-VIm (**1**) (△, ▲) or VIm-AAm (**3**) (▲) polymers.

was carried out whenever possible. The correlation coefficient was better than 0.99 unless stated to the contrary.

Deuterium Isotope Effect. The deuterated medium (28.9 vol/vol % EtOD–D_2O) was prepared from 99.8% D_2O (Merck) and 99.5% EtOD (Stohler). Buffer solutions were obtained by mixing necessary amounts of dry Tris and 30% DCl in D_2O. The pD values were taken as readings on the pH meter plus 0.4.[15] The pK_a value of *p*-nitrophenol in 28.9 vol/vol % EtOD–D_2O at 30 °C, μ = 0.1 (KCl), was 7.85 ± 0.08, a value higher by 0.52 pD unit than the corresponding value in the protic medium.

Results

Hydrolysis of PNPA (4) under Burst Conditions. The catalytic hydrolysis of large excesses of PNPA (**4**) (10^{-2}–10^{-3} M) with the bifunctional polymer proceeds according to the typical burst kinetics, the initial rapid release of *p*-nitrophenol followed by the slower, steady release. An example is given in Figure 1. These curves were obtained by subtracting the phenol formation due to spontaneous hydrolysis from the overall formation. These experiments with and without the polymer catalyst were always conducted consecutively, in order to minimize the experimental error. Curve A is in sharp contrast with the time course of the reaction of the MHA-AAm (**2**) (curve B) and VIm-AAm (**3**) (curve C) polymers with PNPA (**4**) which is carried out under the same conditions. In the case of the MHA-AAm (**2**) polymer, the secondary phenol formation was too slow to determine the reaction rate. The catalytic hydrolysis of PNPA (**4**) by the VIm-AAm (**3**) copolymer followed the first-order kinetics.

It has been established that Bender and Marshall's analysis of the burst kinetics[16] was applicable to synthetic polymer catalysts.[1,2] The catalytic hydrolysis by hydroxamate imidazole copolymers includes the following two pathways

$$C_{MHA} + S \underset{\text{fast}}{\overset{k_a}{\rightarrow}} \underset{+}{\text{Ac-}C_{MHA}} \underset{\text{slow}}{\overset{k_d}{\rightarrow}} C_{MHA} + P_2 \qquad (2)$$
$$P_1$$

$$C_{VIm} + S \underset{\text{slow}}{\overset{k_{VIm}}{\rightarrow}} \underset{+}{\text{Ac-}C_{VIm}} \underset{\text{fast}}{\rightarrow} C_{VIm} + P_2 \qquad (3)$$
$$P_1$$

where C_{MHA} and C_{VIm} denote the MHA and VIm group, respectively, S is the substrate, Ac-C_{MHA} and Ac-C_{VIm} are the acylated polymer intermediates, and P_1 and P_2 are *p*-nitrophenol and acetic acid, respectively. The acetyl intermediate was not detectably accumulated in the imidazole-catalyzed hydrolysis of PNPA (**4**) in the present system, since the catalytic hydrolysis by the VIm-AAm copolymer (**3**) followed the first-order kinetics as shown in Figure 1 (curve C).

Then, the rate of the *p*-nitrophenol formation is given by

$$[P_1] = A't + B(1 - e^{-bt}) \qquad (4)$$

where

$$A' = \frac{k_a \cdot k_d[S]_0[MHA]_0}{k_a[S]_0 + k_d} + k_{VIm}[S]_0[VIm]_0 \qquad (5)$$

$$B = \frac{k_a^2[S]_0^2[MHA]_0}{(k_a[S]_0 + k_d)^2} \qquad (6)$$

$$b = k_a[S]_0 + k_d \qquad (7)$$

The rate constants are therefore given by

$$k_a = \frac{b\sqrt{B}}{[S]_0\sqrt{[MHA]_0}} \qquad (8)$$

$$k_d = b - k_a[S]_0 \qquad (9)$$

$$k_{VIm} = \frac{1}{[VIm]_0[S]_0}\left(A' - \frac{k_a \cdot k_d[MHA]_0[S]_0}{k_a[S]_0 + k_d}\right) \qquad (10)$$

The burst-type hydrolysis was carried out at several pH's, and the resulting pH–rate profiles are given in Figures 2 and 3. The burst experiments in the deuterated medium were similarly conducted.

Hydrolysis of PNPA (4) under Pseudo-First-Order Conditions. When the hydrolysis of PNPA (**4**) was carried out with excess catalysts ([MHA] = $\sim 2 \times 10^{-3}$ M, [PNPA] = 4–10 $\times 10^{-5}$ M), the reaction followed the pseudo-first-order kinetics up to 90% completion

$$k_{total} = k_{a,obsd}[MHA] + k_{VIm}[VIm] + k_{spont} \qquad (11)$$

where k_{spont} is the rate constant of the uncatalyzed (spontaneous) hydrolysis. Since $k_{a,obsd}$ and k_{VIm} in eq 11 cannot be

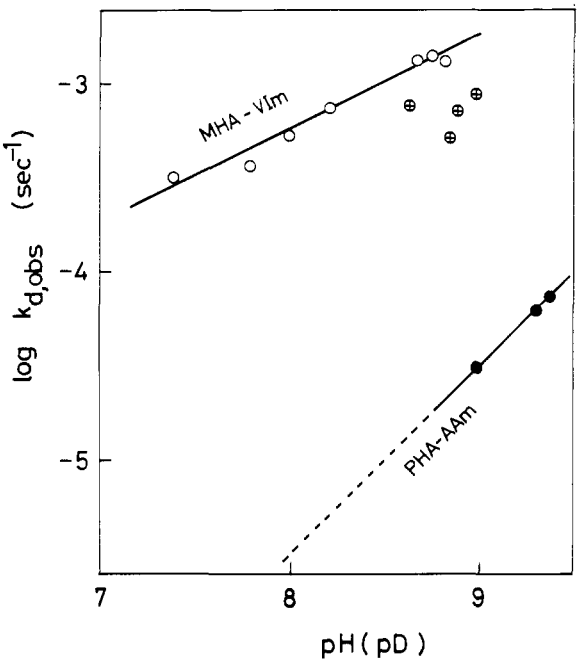


Figure 3. pH (pD) dependence of deacylation rates for PNPA (4) substrate: in 28.9 vol/vol % EtOH-H_2O, 30 °C, and μ = 0.1 (KCl), (○) 0.15 M Tris buffer and (●) extrapolated to the zero buffer concentration (ref 2). In 28.9 vol/vol % EtOD-D_2O, 30 °C, and μ = 0.1 (KCl), (⊕) 0.15 M Tris buffer.

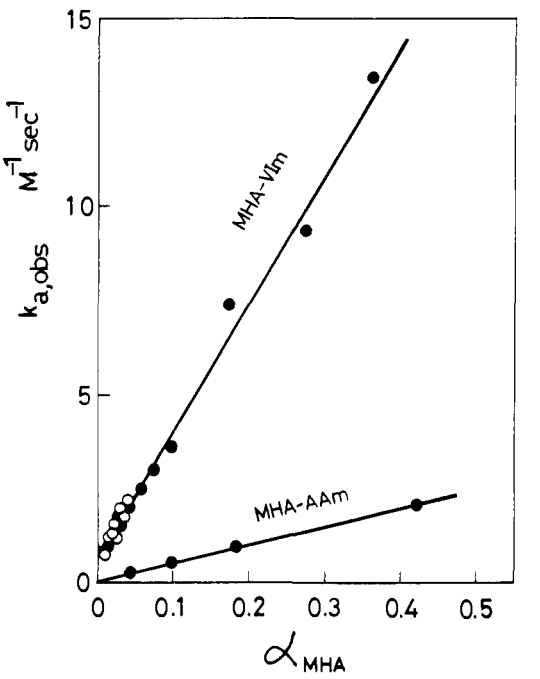


Figure 4. Acylation of the hydroxamic acid unit with PNPA (4) substrate: in 28.9 vol/vol % EtOH-H_2O, 30 °C, and μ = 0.1 (KCl), (○) obtained by the burst kinetics and (●) obtained by the pseudo-first-order kinetics.

estimated separately, $k_{a,obsd}$ was calculated by assuming that k_{VIm} is identical with that obtained in the hydrolysis of PNPA (4) by VIm-AAm copolymer (3). The pH dependence of $k_{a,obsd}$ is shown in Figure 2. The $k_{a,obsd}$ values estimated by these kinetic procedures agreed with each other.

The catalytic hydrolysis with the MHA-AAm copolymer (2) was also performed under the pseudo-first-order condition ([MHA] = 1.52×10^{-3} M and [PNPA] = 9.05×10^{-5} M), and $k_{a,obsd}$ obtained is similarly shown in Figure 2.

In the MHA-VIm copolymer (1), the rate of acyl transfer to the MHA unit is 10–100 times larger than that to the VIm unit. Therefore, the initial acylation of the bifunctional polymer occurs almost exclusively at the MHA unit.

The plots of $k_{a,obsd}$ values against the fraction of the dissociated MHA unit (α_{MHA}) give linear relations, as shown in Figure 4. In the case of the monofunctional polymer [MHA-AAm (2)], the line of the plot passes through the origin, indicating that only the hydroxamate anion is reactive. On the other hand, the line of the plot for the bifunctional polymer (1) possesses an intercept. This suggests that the undissociated MHA unit is involved in acylation. Therefore, the rate constant for acylation consists of two terms, one due to the dissociated form and the other to the undissociated form of MHA, as follows.

$$k_{a,obsd} = k_{A^-} \cdot \alpha_{MHA} + k_{HA} \cdot (1 - \alpha_{MHA}) \qquad (12)$$

Hydrolysis of Other Phenyl Esters [PNPH (5), NABA (6), and NNBA (7)]. When the catalytic hydrolysis by the bifunctional copolymer (1) was carried out with large excesses of these substrates, the burst-type time course was observed for the phenol release. However, the true steady state was never obtained, and the complete kinetic analysis was impossible. The product inhibition is its probable cause. Therefore, only the acylation rates were determined from the initial rate, and $k_{a,obsd}$ and k_{VIm} were separated according to eq 11, in the same way as was done for PNPA (4).

The catalytic rates were proportional to the substrate concentration for 10^{-2}–10^{-3} M PNPH (5) and NABA (6). When the rate constant of initial acylation was plotted against α_{MHA}, linear relations were obtained in accordance to eq 12. The k_{HA} and k_{A^-} values thus obtained are summarized in Table III, together with those for PNPA (4).

In the hydrolysis of NNBA (7) with the MHA-VIm copolymer (1), the initial rate showed a saturation tendency at higher substrate concentrations (Figure 5). This is explained by the occurrence of the Michaelis-Menten kinetics.

$$C + S \underset{}{\overset{K_m}{\rightleftharpoons}} C{\cdot}S \xrightarrow{k_{cat.}} CS' \longrightarrow C + P_2 \qquad (13)$$
$$\downarrow P_1$$

The initial rate is given by eq 14, and kinetic constants are obtained from the Lineweaver-Burk plot (eq 15) and given in Table IV.

$$v_{cat.} = v_{total} - v_{spont} = \frac{k_{cat.} \cdot [S]_0}{K_m + [S]_0} [C]_0 \qquad (14)$$

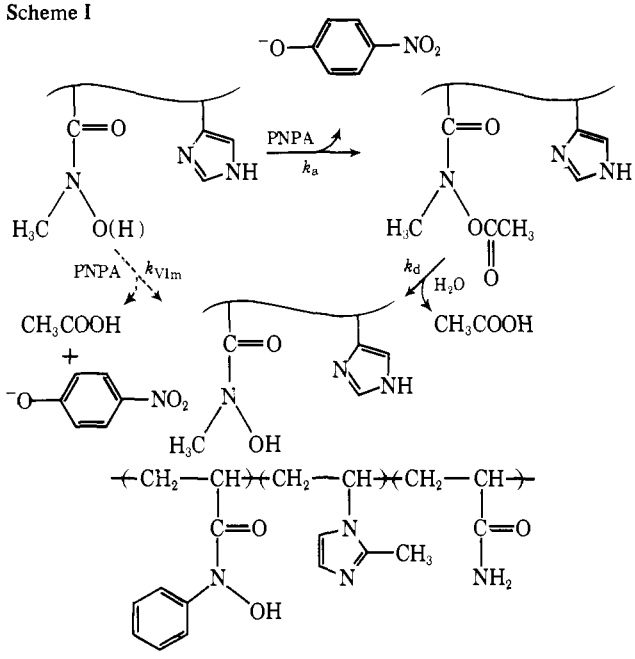
$$\frac{1}{v_{cat.}} = \frac{K_m}{k_{cat.} \cdot [C]_0} \cdot \frac{1}{[S]_0} + \frac{1}{k_{cat.} \cdot [C]_0} \qquad (15)$$

The $k_{cat.}$ value may be attributed, in large part, to acylation at the MHA unit, as in the hydrolysis of the other three substrates. It is interesting that substrate saturation was not observed with the monofunctional polymer, MHA-AAm (2) (see Figure 5 and Table IV).

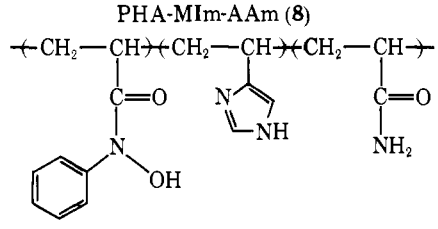
Discussion

Catalytic Scheme. The catalytic hydrolysis of PNPA (4) by the bifunctional copolymer (1) proceeds according to Scheme I. In this scheme, the acetyl group is transferred from PNPA (4) to the MHA unit of the catalytic polymer, and the acetyl intermediate formed is subsequently hydrolyzed with the help of the neighboring VIm unit. This overall picture of catalysis is the same as those observed in the hydrolysis of PNPA (4) by related bifunctional polymers: PHA-MIm-AAm (8)[1] [copoly(*N*-phenylacrylohydroxamic acid 2-methyl-*N*-vinylimidazoleacrylamide)] and PHA-VIm-AAm (9)[2] [copoly(*N*-phenylacrylohydroxamic acid 4-vinylimidazoleacrylamide)].

Scheme I



PHA-MIm-AAm (8)

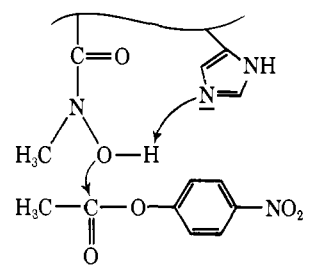


PHA-VIm-AAm (9)

Apart from substrate binding, the same scheme would apply to hydrolyses of other phenyl esters, although only the initial rate was measured in the latter cases.

Acylation Mechanism. Probably the most important finding in the present study is the concerted action of the MHA and VIm groups in the acylation process. In the previously investigated bifunctional polymers (**8** and **9**), the nucleophilic behavior of the PHA anion toward phenyl esters was intrinsically the same in the presence and absence of the neighboring imidazole group. The lack of the concerted action in these cases may be partly attributed to the dilution of the catalytic group by the acrylamide unit which was introduced in order to increase the solubility of the catalytic polymers in aqueous systems. The MHA-VIm copolymer (**1**) is soluble in 28.9 vol/vol % EtOH-H_2O without introducing a third component, thus the probability of the concerted action being enhanced.

The undissociated MHA unit in the MHA-VIm copolymer (**1**) is reactive toward PNPA (**4**). Since this is not the case with the MHA-AAm copolymer (**2**) ($k_{HA} = 0$ in eq 12), the k_{HA} term in the bifunctional polymer must have come from the cooperative action of the two functional groups. The most plausible mechanism is the nucleophilic attack of the undissociated hydroxamic acid group assisted by the general-base



I

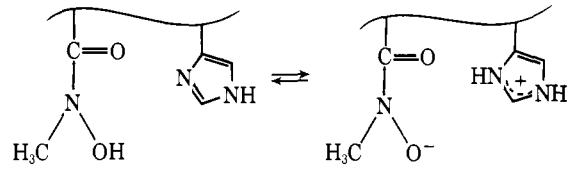
Table III. Rate Constants of Acylation[a]

Substrate	Polymer catalyst	k_{A^-}, $M^{-1}\,s^{-1}$	k_{HA}, $M^{-1}\,s^{-1}$	k_{VIm}, $M^{-1}\,s^{-1}$
PNPA (**4**)	MHA-VIm (**1**)	36[c]	0.50[c]	0.10[c]
	MHA-VIm (**2**)	4.8	0	
	VIm-AAm (**3**)			0.10
PNPH (**5**)[b]	MHA-VIm (**1**)	13	1.2	0.04[d]
	MHA-AAm (**2**)	1.6	0	
NABA (**6**)[b]	MHA-VIm (**1**)	15	1.3	0.10[d]
	MHA-AAm (**2**)	5.9	0	

[a] 30 °C, 28.9 vol/vol % EtOH-H_2O, μ = 0.1 (KCl). [b] Rate constants obtained from the initial rate. [c] Rate constants obtained from the burst kinetics. [d] Assumed to be equal to those observed with VIm-AAm (**3**) catalyst.

catalysis of the neighboring imidazole group (I). This mechanism is supported by the kinetic solvent isotope effect observed. As shown in Figure 2, $k_{a,obsd}$ values in the deuterated medium were always smaller than those in the nondeuterated medium. Since the $k_{a,obsd}$ value above pH 8 is composed of the k_{HA} and k_{A^-} terms and since the electrostatic effect of the polymer on $k_{a,obsd}$ would be different in the nondeuterated and deuterated media, the isotope effect observed in this region may not be interpreted in a straightforward fashion. On the other hand, in the region of pH <8, $k_{a,obsd}$ is composed solely of the k_{HA} term and the polymer is little charged. Therefore, the observed isotope effect ($k^H/k^D = 1.6$) at pH 7-8 is associated with the k_{HA} process. It is to be noted that the kinetic isotope effect was not observed for the k_{VIm} term, consistent with the simple nucleophilic mechanism of the VIm unit.

This concerted mechanism cannot be kinetically distinguished from the nucleophilic attack by the hydroxamate imidazolium ion pair.



The pK_a difference between the hydroxamate and imidazole functions amounts to 5 pK units (Table II). Therefore, the concentration of the ion pair would be about $1/10^5$ of the neutral species, and the reactivity of the hydroxamate anion would have to be improbably large ($\sim 5 \times 10^4\ M^{-1}\,s^{-1}$) in order to explain the k_{HA} value observed. Furthermore, the presence of the deuterium isotope effect is consistent with the latter mechanism only if the general-acid (imidazolium) catalysis of the nucleophilic attack is operating. This is not likely for the combination of a good nucleophile (hydroxamate) and a substrate with good leaving group (PNPA).

The advent of the concerted nucleophilic attack appears to be related to the pK_a data of the polymer. Thus, pK_{inl} of the MHA unit in the MHA-VIm copolymer (**1**) is higher than that in the MHA-AAm copolymer (**2**) (see Table II). It is possible that the pK_a value is enhanced due to hydrogen bonding to the imidazole group. In contrast, lowering of pK_a in the presence of the VIm unit was observed for the bifunctional polymer (**9**) containing the N-phenylhydroxamate (PHA) group,[2] which does not show concerted nucleophilic behavior.

To our knowledge, the only other example of the concerted (intermolecular) nucleophilic attack is the reaction of *o*-carboxy-*N*-methylbenzohydroxamic acid with PNPA (II).[17] In this case, the undissociated hydroxamic acid group is again

Table IV. Catalytic Hydrolysis of NNBA (7) by Polymer Catalysts[a]

MHA-VIm (1)				MHA-AAm (2)	
pH	K_m, mM	$10^2 k_{cat.}$, s^{-1}	$k_{cat.}/K_m$, $M^{-1} s^{-1}$	pH	$k_{a,obsd}$, $M^{-1} s^{-1}$
7.58[b]	0.668	1.28	19.2	7.85[b]	0.018
7.98[b]	2.53	1.90	7.50	8.35[b]	0.031
8.74[b]	2.48	1.86	7.50	9.02[b]	0.055
9.10[c]	3.63	2.14	5.90	9.98[c]	0.183
9.58[d]	4.75	3.71	7.80		
9.88[d]	9.86	8.40	8.68		

[a] 30 °C, 28.9 vol/vol % EtOH-H_2O, μ = 0.1 (KCl); [MHA] = 7.57×10^{-5} M, [VIm] = 2.27×10^{-4} M, [NNBA] = $0.12\text{-}6.32 \times 10^{-3}$ M. [b] 0.15 M Tris buffer. [c] 0.15 M barbital buffer. [d] 0.15 M borate buffer.

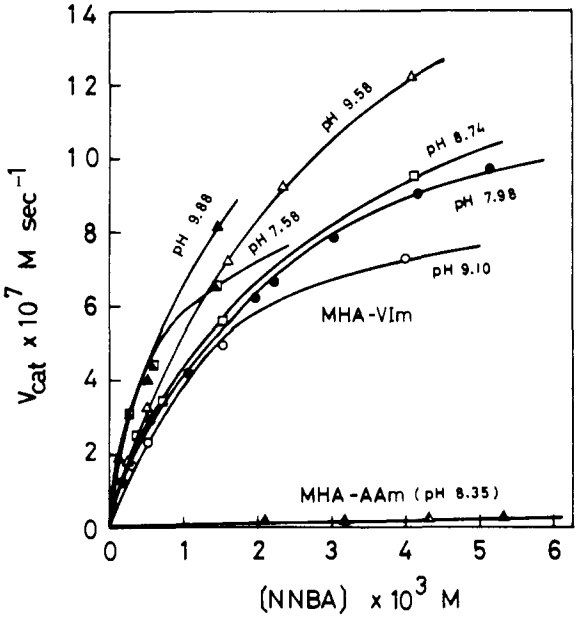
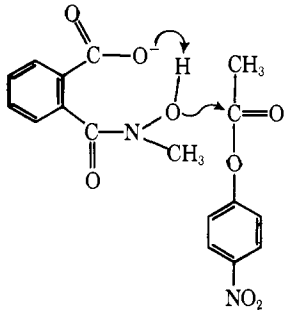
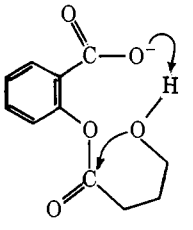


Figure 5. Acylation of copolymers by NNBA (7) substrate: 28.9 vol/vol % EtOH-H_2O, 30 °C, μ = 0.1 (KCl), [MHA] = 7.57×10^{-5} M.

involved in the acyl transfer reaction, along with the hydroxamate anion. The general-base assistance of the *o*-carboxylate anion was confirmed by the solvent kinetic isotope effect of k^H/k^D = 2.5. An intramolecular version of the concerted nucleophilic attack (k^H/k^D = 2.28) was proposed by Kirby and Lloyd (III).[9]



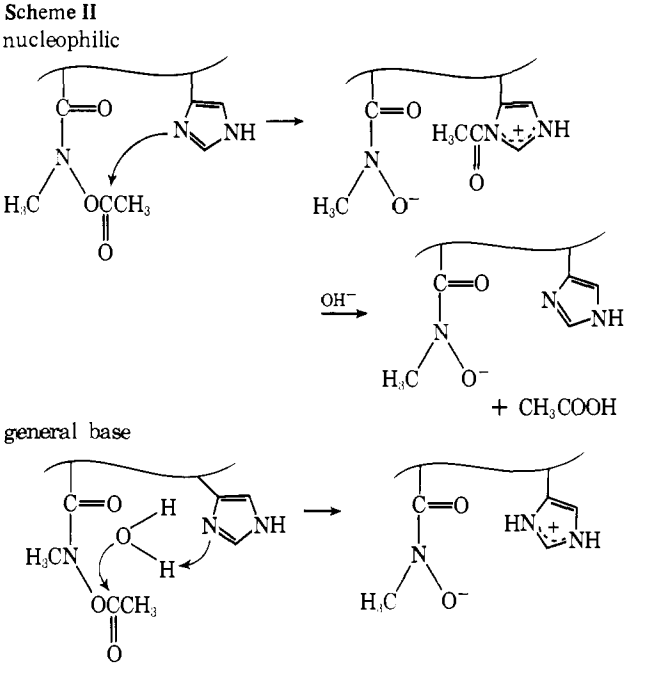
II



III

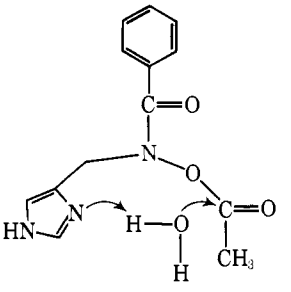
The concerted acylation was possible for various phenyl ester substrates. The efficiency of the k_{HA} term relative to that of the k_{A^-} term varies with substrates as shown in Table III. Also noteworthy is the fact that k_{HA} is invariably larger (5-30-fold) than k_{VIm}. This indicates that the direct nucleophilic attack of the imidazole group is less efficient than its general-base assistance of the nucleophilic attack of the hydroxamic acid group.

Scheme II

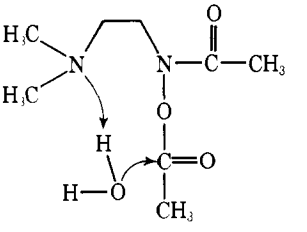


Deacylation Mechanism. The hydrolysis of the acyl hydroxamate intermediate is greatly accelerated by the neighboring imidazole group, as already shown for polymeric and small-molecule bifunctional catalysts.[1-5] This is true with the present system and the $k_{d,obsd}$ value was enhanced by ~100 times by the intrapolymeric imidazole (Figure 3, pH 8). There are two probable mechanisms for deacylation. One is the acyl transfer to the imidazole group and the subsequent hydrolysis and the other is general-base catalysis by the imidazole group (Scheme II).

The solvent kinetic isotope effect for $k_{d,obsd}$ was in the range of 1.4-3.2 at 8.5-9.0. Therefore, the general-base mechanism appears to prevail in the deacylation reaction. Examples of general-base-catalyzed hydrolysis of the acyl hydroxamate intermediate have been recently reported in simpler systems (IV[4] and V[18]).



IV, k^H/k^D = 2.0



V, k^H/k^D = 2.05

The log $k_{d,obsd}$ value increased linearly with pH. This cannot be explained by the increase in the neutral VIm unit because the VIm unit is almost completely dissociated in this pH range ($\alpha_{VIm} > 0.85$ above pH 7). When plotted against α_{VIm}, $k_{d,obsd}$ shows a steep rise as α_{VIm} approaches unity. A similar result was found previously for bifunctional polymer **8**.[4] Probably, the polymer molecule forms a tighter coil as the charge density decreases ($\alpha_{VIm} \rightarrow 1$), thus enhancing the catalytic efficiency of the intramolecular VIm group.

Substrate Binding. The hydrolysis of NNBA (**7**) by the MHA-VIm copolymer (**1**) proceeds according to the Michaelis-Menten kinetics. On the other hand, no substrate binding was observed for the other three substrates. Therefore, both coulombic and hydrophobic attractions are required for efficient binding. The VIm unit must make an important contribution to substrate binding, since the MHA-AAm catalyst (**2**) did not bind NNBA (**7**). The binding tendency as given by $1/K_m$ decreases with increasing pH, indicating the coulombic contribution of the protonated VIm unit to substrate binding. The apparent rate of the intracomplex catalysis, $k_{cat.}$, increases with increasing pH. This is attributed to the dissociation of the MHA unit. Detailed examination of the $k_{cat.}$ term cannot be done, though multiple acylation processes may be present. The apparent catalytic efficiency of the MHA-VIm copolymer, $k_{cat.}/K_m$, is greater by factors of 10–200 than that of the MHA-AAm copolymer (**2**) due to substrate binding.

Conclusion

In the charge relay system at the active site of serine proteases, the acylation and deacylation reactions at the seryl hydroxyl group are assisted by the general-base catalysis of the histidylimidazole group. Since the presence of the charge relay system was reported, considerable efforts have been directed to the preparation of model charge relay systems, with partial or little success. To our knowledge, the MHA-VIm copolymer (**1**) is the first hydrolytic catalyst in which both nucleophilic activation of the OH group and the accelerated hydrolysis of the acyl intermediate are made possible by the general-base assistance of the neighboring imidazole group. This catalytic scheme is very similar to that of the charge relay system in serine proteases,[6] except that the aspartic carboxylate anion is involved in the activation of the imidazole group in the enzyme system. The occurrence of substrate binding in the case of NNBA (**7**) renders the present system still more suitable as a proteolytic enzyme model. It is amazing that a simple vinyl copolymer such as MHA-VIm (**1**) can mimic several important characteristics of the serine protease action.

Acknowledgment. The authors appreciate the capable experimental assistance of Miss R. Ando. This investigation was supported by a grant-in-aid from the Ministry of Education.

References and Notes

(1) T. Kunitake and Y. Okahata, *Bioorg. Chem.*, **4**, 236–148 (1975).
(2) T. Kunitake and Y. Okahata, *Macromolecules*, **9**, 15 (1976).
(3) T. Kunitake and S. Horie, *Bull. Chem. Soc. Jpn.*, **48**, 1304–1309 (1975).
(4) T. Kunitake, Y. Okahata, and T. Tahara, *Bioorg. Chem.*, **5**, 155–167 (1976).
(5) Y. Kitaura and M. L. Bender, *Bioorg. Chem.*, **4**, 237–249 (1975).
(6) D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature (London)*, **221**, 337–340 (1969).
(7) T. Maugh II and T. C. Bruice, *J. Am. Chem. Soc.*, **93**, 3237–3248 (1971).
(8) T. C. Bruice and I. Oka, *J. Am. Chem. Soc.*, **96**, 4500–4507 (1974).
(9) A. J. Kirby and G. J. Lloyd, *J. Chem. Soc., Perkin Trans. 2*, 637–642 (1974).
(10) T. Kunitake, F. Shimada, and C. Aso, *J. Am. Chem. Soc.*, **91**, 2716–2723 (1969).
(11) T. Kunitake, S. Shinkai, and S. Hirotsu, *J. Polym. Sci., Polym. Lett. Ed.*, **13**, 377–381 (1975).
(12) C. G. Overberger and I. Cho, *J. Polym. Sci., Polym. Chem. Ed.*, **6**, 2741–2754 (1968).
(13) J. H. Cooley, W. D. Bills, and J. R. Throckmorton, *J. Org. Chem.*, **25**, 1734–1736 (1960).
(14) A. Katchalsky, N. Shavit, and H. Eisenberg, *J. Polym. Sci.*, **13**, 69–84 (1954).
(15) R. Lumry, E. L. Smith, and R. R. Glantz, *J. Am. Chem. Soc.*, **73**, 4330–4340 (1951).
(16) M. L. Bender and T. H. Marshall, *J. Am. Chem. Soc.*, **90**, 201–207 (1968).
(17) T. Kunitake, Y. Okahata, and S. Hirotsu, *Bull. Chem. Soc. Jpn.*, in press.
(18) W. B. Gruhn and M. L. Bender, *Bioorg. Chem.*, **4**, 219–236 (1975).

Multifunctional Hydrolytic Catalyses. 8. Remarkable Acceleration of the Hydrolysis of *p*-Nitrophenyl Acetate by Micellar Bifunctional Catalysts[1]

Toyoki Kunitake,* Yoshio Okahata, and Tetsuo Sakamoto

Contribution No. 397 from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan. *Received March 16, 1976*

Abstract: Surfactant molecules which contain the hydroxamate and imidazole functions were synthesized, and their catalytic actions in aqueous cetyltrimethylammonium bromide micelles were examined for the hydrolysis of *p*-nitrophenyl acetate at 30 °C. The catalysis proceeded mainly via acylation and imidazole-catalyzed deacylation at the hydroxamate group. Both of these processes were remarkably accelerated in the case of bifunctional micelles, due to activation of anionic nucleophiles in cationic micellar environments. The overall catalytic efficiency exceeded even that of α-chymotrypsin at pH 8 and was more than 5000 times higher than that of imidazole. Micellar monofunctional catalysts and a nonmicellar bifunctional catalyst were much less effective. Therefore, the combination of bifunctionality and micellar microenvironments was essential for the highly efficient catalysis.

The high proteolytic activity of serine proteases is derived from fast acylation and deacylation at the seryl hydroxyl group. The remarkable efficiency of these processes is currently explained by the multifunctionality and the microenvironment of the catalytic site.[2]

In the previous papers of this series,[3,4] we have established that combinations of complementary functional groups such as hydroxamate and imidazole give rise to enhanced catalytic efficiency. However, the esterolytic efficiency of these bifunctional catalysts was still much lower than that of α-chymotrypsin.

The reactivity of anionic nucleophiles is remarkably enhanced in cationic micelles.[5] Therefore, it is expected that the efficiency of the bifunctional catalysts is further enhanced in