Catalyses by Polymer Complexes. V. The Heterotropic (Allosteric) Interaction of Histamine- and Hydroxamate-Containing Polymer Catalysts with Hydrophobic Ammonium Salts in the Hydrolysis of Phenyl Esters*

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ABSTRACT: Three nucleophilic copolymers, (*N*-methacryloylhistamine—methacrylic acid (MHistm—MAc), *N*-methacryloylhistamine—methacrylamide (MHistm—MAm), and methacrylohydroxamic acid—methacrylic acid (MHAc—MAc)) were synthesized, and their hydrolytic reactivities toward *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl hexanoate (PNPH) were studied in the absence and the presence of hydrophobic ammonium salts. The nucleophilic reactivity of MHistm—MAc toward PNPA was hardly affected by the addition of hydrophobic ammonium ions, while a marked increase of rates was found in the reaction with PNPH. Addition of these ammonium ions to the MHAc—MAc system enhanced the rate of the reaction with PNPH. This was produced by a pK_a lowering (0.3—0.4 pK unit) and by the increase in the second-order rate constant (*ca.* 3 fold), as inferred from the pH—rate profile. The rate-enhancing effect of the hydrophobic ammonium salts was analyzed by using the Hill equation which has been employed for analyzing the allosteric behavior in the enzyme system. The observed coefficient ($n=3\sim4$) suggests that polymer-bound ammonium ions facilitate the subsequent binding considerably.

KEY WORDS Hydrophobic Ion Pair / Polyion Complex / Hydrolysis / Polymeric Hydroxamic Acid / Polymeric Histamine / Heterotropic (Allosteric) Interaction /

We showed recently that anionic nucleophiles were activated in the presence of a variety of cationic hydrophobic aggregates in aqueous systems.[1-6] These rate enhancements can be attributed to the formation of "hydrophobic ion pair" between anionic nucleophiles and hydrophobic ammonium compounds.[1-3] It should be interesting to extend this concept to polyion systems, since polyanions and polycations are known to form very stable complexes owing to the coulombic and hydrophobic interactions.[7,8]

We thus prepared the following water-soluble copolymers and assessed the influence of positively charged, hydrophobic additives on the nucleophilic reactivity of the imidazole, and hydroxamate functions toward *p*-nitrophenyl esters.
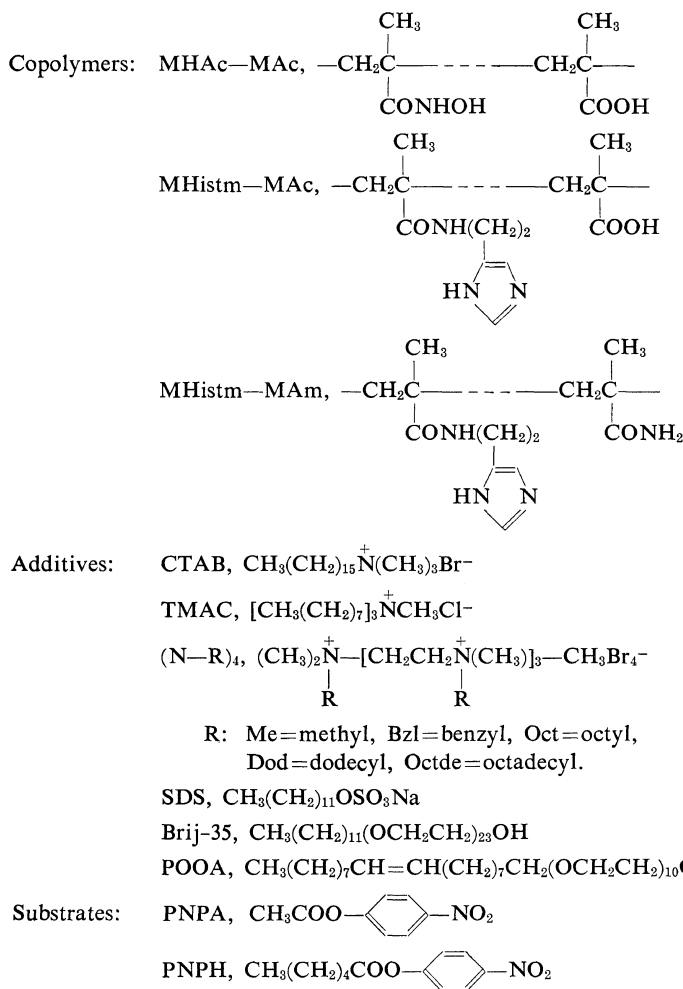
EXPERIMENTAL

Materials

p-Nitrophenyl methacrylate was prepared from an equimolar mixture of methacrylyl chloride and *p*-nitrophenol in an ice bath in the presence of excess pyridine. A small amount of hydroquinone was added to suppress polymerization. The reaction mixture was poured into water, and the phenyl ester produced was extracted with ether. The ether layer was washed with aqueous 0.5-*N* HCl solution and water, and dried over calcium chloride. The solvent was evaporated *in vacuo*, and recrystallization of the yellow residue gave colorless needles; yield 50%, mp 94.6—95.8°C (lit.[9] 94.5—95.5°C).

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* Contribution No. 414 from this department.

Copolymers: MHAc—MAc,

$$\begin{array}{cc} CH_3 & CH_3 \\ | & | \\ -CH_2C\!-\!-\!-\!-\!-\!-CH_2C\!-\!- \\ | & | \\ CONHOH & COOH \end{array}$$

MHistm—MAc,

$$\begin{array}{cc} CH_3 & CH_3 \\ | & | \\ -CH_2C\!-\!-\!-\!-\!-\!-CH_2C\!-\!- \\ | & | \\ CONH(CH_2)_2 & COOH \end{array}$$

HN　N

MHistm—MAm,

$$\begin{array}{cc} CH_3 & CH_3 \\ | & | \\ -CH_2C\!-\!-\!-\!-\!-\!-CH_2C\!-\!- \\ | & | \\ CONH(CH_2)_2 & CONH_2 \end{array}$$

HN　N

Additives: CTAB, $CH_3(CH_2)_{15}\overset{+}{N}(CH_3)_3Br^-$

TMAC, $[CH_3(CH_2)_7]_3\overset{+}{N}CH_3Cl^-$

(N—R)$_4$, $(CH_3)_2\overset{+}{N}\!-\![CH_2CH_2\overset{+}{N}(CH_3)]_3\!-\!CH_3Br_4^-$

　　　　　　|　　　　　　|
　　　　　　R　　　　　　R

R: Me=methyl, Bzl=benzyl, Oct=octyl,
Dod=dodecyl, Octde=octadecyl.

SDS, $CH_3(CH_2)_{11}OSO_3Na$

Brij-35, $CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH$

POOA, $CH_3(CH_2)_7CH\!=\!CH(CH_2)_7CH_2(OCH_2CH_2)_{10}OH$

Substrates: PNPA, $CH_3COO\!-\!\langle\bigcirc\rangle\!-\!NO_2$

PNPH, $CH_3(CH_2)_4COO\!-\!\langle\bigcirc\rangle\!-\!NO_2$

m-N,N-Dimethylaminophenyl methacrylate was similarly prepared by reaction of an equimolar mixture of methacryloyl chloride and *m-N,N*-dimethylaminophenol in an ice bath in the presence of aqueous 5-% NaOH; yield 81%, bp 83—85°C/0.07 mmHg. *Anal.* Found: C, 69.91; H, 7.31; N, 6.68. Calcd for $C_{12}H_{15}NO_2$: C, 70.21, H, 7.37; N, 6.82.

Hexadecyltrimethylammonium bromide (CTAB), trioctylmethylammonium chloride (TMAC), and sodium dodecyl sulfate (SDS) are products of Wako Pure Chem. Ind. Polyoxyethylene (n=10) oleyl alcohol and Brij–35 were purchased from Tokyo Kasei. CTAB was recrystallized from ethanol before use, while other surfactants were used without further purification. Tetra-alkylated triethylenetetamines were prepared and characterized by Hirotsu[10] and Okahata, *et al.*[11] Preparations of *p*-nitrophenyl acetate (PNPA, mp 83.5—84.5°C) and *p*-nitrophenyl hexanoate (PNPH, bp 142—147°C/0.2 mmHg) have been described previously.[12]

Polymerization and Polymer Reaction

Polymerizations were carried out in benzene using azobisisobutyronitrile (1—3% of monomer) as the initiator at 70°C. Poly(*p*-nitrophenyl methacrylate) was recovered by pouring into ether (yield 85—90%), and poly(*m-N,N*-dimethyl-aminophenyl methacrylate) was recovered by pouring into methanol (yield 97%). The polymer catalysts were prepared from these polymers according to the following schemes.
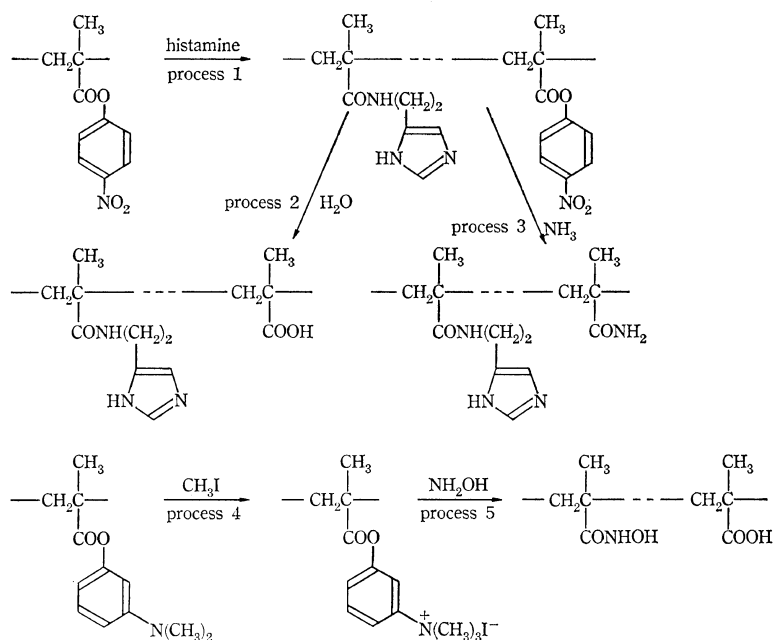
Process 1: 6.67 g (30 mmol) of poly(p-nitrophenyl methacrylate), 2.76 g (15 mmol) of histamine dihydrochloride, and 4.5 ml of triethylamine were dissolved in 70 ml of dimethylformamide, and heated at 90—100°C for 24 hr. Precipitates (Et$_3$N·HCl) were filtered and the solusion was poured into ether; recovered polymer, 8.4 g.

Process 2: 2 g of the histamine-containing polymer were dissolved in aqueous 1-N NaOH solution, and heated at 40°C for 24 hr. The polymer solution was subjected to extensive dialysis until the yellow color of p-nitrophenolate disappeared. The IR spectrum of the polymer recovered by pouring into tetrahydrofuran showed an amide absorption (1650 cm^{-1}) instead of an ester absorption (1750 cm^{-1}). The composition of the polymer (MHistm—MAc) was determined by titrating the MHistm unit (Table I).

Process 3: 2 g of the histamine-containing polymer were dissolved in 20 ml of dimethylformamide, and dry ammonia gas was introduced. A small amount of imidazole was added, in order to promote the aminolysis. The reaction was continued for 24 hr at room temperature. The polymer was recovered by pouring into acetone, and was further purified by reprecipitation from water and acetone. The IR

Table I. Composition of polymer calalysts

Polymer	MHistm, mol%	MHAc, mol%	pK_a	n'
MHistm—MAc[a]	18	—	7.25	—
MHistm—MAm[a]	20	—	6.20	3.17
MHAc—MAc	—	19[b]	10.4[c]	0.94

[a] Determined by titration at 30°C, $\mu=1.0$ with KCl. pK_a denotes the half-neutralized ($\alpha=0.5$) pH value. pK_{int} for MHistm—MAm=6.79. $n'=$ (pK_a−pH)/log[(1−α)/α]. n' value for MHistm—MAc could not be determined because of insufficient separation of the titration curve.

[b] Determined by elemental analysis.

[c] Determined kinetically (see Results section).

spectrum of the resultant polymer showed no ester absorption around 1750 cm^{-1}. The polymer composition was determined by titrating the MHistm unit (Table I).

Process 4: 9.4 g (49 mmol) of poly(m-N,N-dimethylaminophenyl methacrylate) were dissolved in 60 ml of dimethylformamide, and 25 g (176 mmol) of methyl iodide were added. The reaction mixture was stirred at room temperature for 2.5 hr, and 10 g (52 mmol) of methyl iodide were again added. The polymer war recovered by pouring into acetone, and

repreicipitated from dimethylformamide and ether—acetone (1 : 1). *Anal.* Found: C, 43.06; H, 5.44; N, 3.98. Calcd for $C_{13}H_{18}NO_2I$: C, 44.97; H, 5.22; N, 4.03.

Process 5: 3.47 g (1 mmol) of poly(*m*-methacryloyloxy *N,N,N*-trimethylanilinium iodide and 1 g (3 mmol) of hydroxylamine which had been freed by excess triethylamine were dissolved in 30 m*l* of dimethylformamide, and heated at 80°C for 24 hr. The solvent was evaporated *in vacuo*, and the residue was taken in methanol. The polymer was recovered by pouring into aqueous 0.01-*N* HCl solution. The IR spectrum showed two carbonyl absorptions at 1710 cm^{-1} (—COOH) and 1670 cm^{-1} (—CONH—). The polymer composition was determined by elemental analysis (Table I).

Rate Measurements

The hydrolytic rate of *p*-nitrophenyl esters was determined at 30°C by following the increase in the absorption of *p*-nitrophenolate anion at 401 nm. In all cases, excess polymer catalysts (*i.e.*, excess MHistm and MHAc units) were present, so that pseudo first-order kinetics were observed. From the pseudo first-order rate constant ($=k_{total}-k_{H_2O} : k_{H_2O}$, the rate of spontaneous decay) thus obtained, the apparent second-order rate constant (k_{obsd}) was calculated. The reactions of MHistm—MAc and MHistm—MAm with PNPA were carried out mainly at pH 9.1 (0.02-*M* borate buffer), where the imidazole group is mostly dissociated (pK_a of MHistm 6—7; Table I). Therefore, k_{obsd} is equal to the true second-order rate constant (k_a). The reaction of MHAc—MAc was carried out mainly at pH 10.0 (0.02-*M* carbonate buffer), and the true second-order constant (k_a) was evaluated from the pH—rate profile (Figure 5).

RESULTS

Catalytic Hydrolysis with the Imidazole-Containing Copolymer

First, the rates of the spontaneous decay of PNPA and PNPH substrates (k_{H_2O}) were eva-

luated under various reaction conditions. The results are summarized in Table II. Additives did not affect very much the spontaneous decay although the hydrolysis was somewhat faster in the presence of $(N\text{-Octde})_4$.

The effect of added ammonium salts on the catalytic hydrolysis of PNPA with MHistm—MAc (eq 1) is illustrated in Figure 1.

Table II. Spontaneous hydrolysis of PNPA and PNPH[a]

Substrate	Reaction medium		pH	$k_{H_2O} \times 10^4$, sec^{-1}
PNPA	None		9.10	3.55
	CTAB	0.1 mM	9.10	3.53
	{CTAB	0.1 mM	9.10	3.48
	poly(MAc)	2.5 mM		
	{$(N$-Octde)$_4$	0.1 mM	9.10	4.80
	poly(MAc)	2.5 mM		
	{$(N$-Bzl)$_4$	0.5 mM	9.24	5.40
	poly(MAc)	2.5 mM		
PNPH	None		9.15	2.29
	poly(MAc)	2.5 mM	9.15	2.25
	{CTAB	0.1 mM	9.10	2.05
	poly(MAc)	2.5 mM		
	{$(N$-Octde)$_4$	0.1 mM	9.13	3.01
	poly(MAc)	2.5 mM		

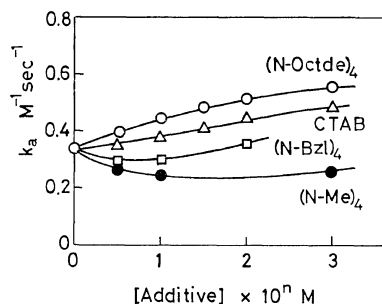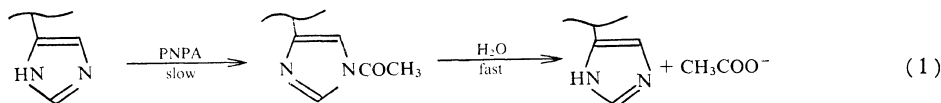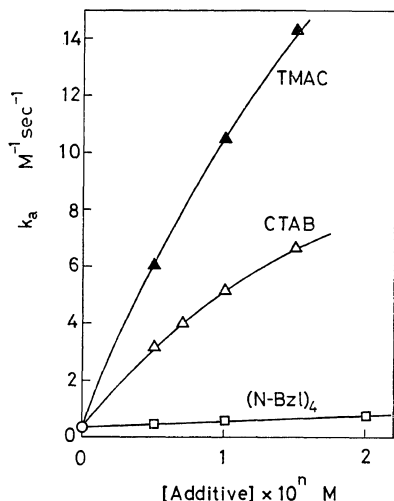[a] 30°C, $\mu=0.02$ with KCl, 3 vol-% ethanol.

Figure 1. Second-order rate constants for the reaction of MHistm—MAc and PNPA plotted against the concentration of added ammonium salts: pH 9.10±0.03, 3 vol-% ethanol; $\mu=0.01$ with KCl; [MHistm unit]$=4.23 \times 10^{-4}$ *M*; [PNPA]$=4.36 \times 10^{-5}$ *M*; $n=3$ for $(N$-Bzl)$_4$ and $(N$-Me)$_4$; $n=4$ for $(N$-Octde)$_4$ and CTAB.
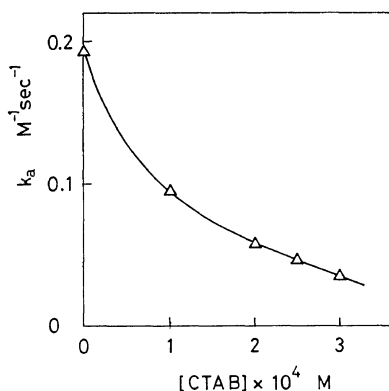

$$\text{(1)}$$

The addition of $(N\text{-Me})_4$ slightly retarded the hydrolytic rate, while $(N\text{-Bzl})_4$ had a very small effect. On the other hand, CTAB and $(N\text{-Octde})_4$ produced rate enhancements. The concentration of added CTAB is below its critical micelle concentration (cmc) under kinetic conditions.* The cmc of $(N\text{-Octade})_4$, if present, is said to be below 5×10^{-5} M.[11] These rate augmentations were only 1.4—1.6 fold.

More distinct rate enhancements were observed by employing PNPH, which is a more hydrophobic substrate. As shown in Figure 2, $(N\text{-Bzl})_4$ was only slightly effective. In contrast, CTAB and TMAC (typical phase-transfer catalysts) readily produced rate augmentations of 20—40 fold, and the rate increased with an increase in the concentration of these ammonium salts. Apparently, the hydrophobicity around the polymer chain was strengthened by the binding of hydrophobic ammonium ions (CTAB

Figure 3. Second-order rate constants for the reaction of MHistm—MAm and PNPH plotted against the concentration of CTAB: pH 9.00 ± 0.03, 3 vol-% ethanol; $\mu=0.01$ with KCl; [MHistm]$=4.50\times10^{-4}M$; [PNPH]$=4.54\times10^{-5}$ M.

Figure 2. Second-order rate constants for the reaction of MHistm—MAc and PNPH plotted against the concentration of added ammonium salts. [PNPH]$=4.54\times10^{-5}$ M. Other reaction conditions are recorded under Figure 1: $n=3$ for $(N\text{-Bzl})_4$; $n=4$ for TMAC and CTAB.
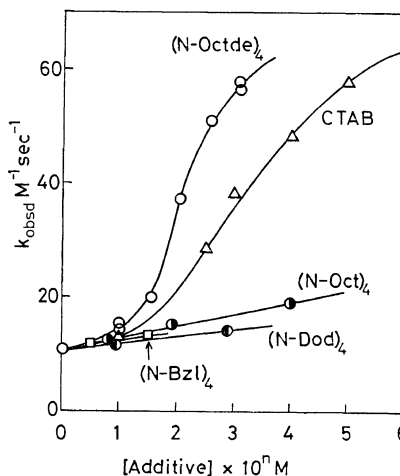
Figure 4. Apparent second-order rate constants at pH 10.00 ± 0.03 for the reaction of MHAc—MAc and PNPA plotted against the concentration of added ammonium salts: 3 vol-% ethanol, $\mu=0.06$ with KCl; [MHAc]$=2.01\times10^{-4}$ M; [PNPA]$=2.05\times10^{-5}$ M; $n=3$ for $(N\text{-Bzl})_4$; $n=4$ for $(N\text{-Octde})_4$, $(N\text{-Dod})_4$, $(N\text{-Oct})_4$, and CTAB.

and TMAC) and the rate increase was due to concentration of hydrophobic PNPH.

The addition of anionic (SDS $0.3\,\text{m}M$) and nonionic (Brij-35, $0.3\,\text{m}M$) surfactants were found to be ineffective.

In the catalytic hydrolysis of PNPH with MHistm—MAm, the addition of CTAB lowered

* The cmc of CTAB determined with dichlorophenolindophenol at 30°C, 3 vol-% ethanol was: 8.0×10^{-4} M at $\mu=0.01$ and 3.8×10^{-4} M at $\mu=0.06$. Therefore, the micelle would not be formed under kinetic conditions, except at the relatively high concentrations in Figure 4.

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the reaction rate as shown in Figure 3. This result contrasts well with those observed for MHistm—MAc catalyst.

Catalytic Hydrolysis with the MHAc—MAc Copolymer

It is known that the acylation of the hydro-xamate anion takes place fast, while the de-acylation is relatively slow.[14-16] Therefore, the k_{obsd} value with MHAc—MAc copolymer under the pseudo first-order condition is equivalent to the acyl-transfer rate from PNPA to the MHAc unit (eq 2).
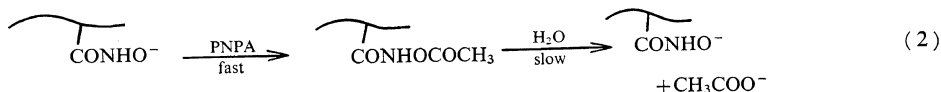
$$\text{CONHO}^- \xrightarrow[\text{fast}]{\text{PNPA}} \text{CONHOCOCH}_3 \xrightarrow[\text{slow}]{\text{H}_2\text{O}} \text{CONHO}^- \qquad (2)$$
$$+ \text{CH}_3\text{COO}^-$$

Figure 4 shows the apparent second-order rate constant (k_{obsd}) for the reaction of MHAc—MAc and PNPA and pH 10.0 plotted against the concentration of added ammonium salts. As expected, the addition of $(N\text{-Bzl})_4$ and $(N\text{-Me})_4$ was not very effective. The more hydrophobic CTAB and $(N\text{-Octde})_4$ produced rate enhancements by 6—7 times. The addition of anionic (SDS) and nonionic (POOA) surfactants decelerated the reaction (see Table III).

pH Dependence of the Reaction of MHAc—MAc and PNPA

The pH dependence of the rate for the reaction of MHAc—MAc and PNPA was studied in the presence and the absence of ammonium salts (Figure 5). The apparent second-order rate constant, k_{obsd} is easily afforded by eq 3, where α is the dissociated fraction of MHAc units. In the polymeric system, however, α should be related to pH through the modified Henderson—Hasselbach equation (eq 4),[17] so that α is expressed by eq 5.

Table III. Catalytic hydrolysis of PNPA with MHAc—MAc[a]

Additive		k_{obsd} at pH 10.0, $M^{-1}\text{sec}^{-1}$	k_a, $M^{-1}\text{sec}^{-1}$	pK	n'
	mM				
None		10.7	32	10.4	0.94
CTAB	0.30	38.3	85	10.1	1.03
$(N\text{-Octde})_4$	0.30	57.7	93	10.0	0.92
$(N\text{-Dod})_4$	0.29	13.9			
$(N\text{-Oct})_4$	0.20	15.5			
$(N\text{-Bzl})_4$	1.50	13.3			
SDS	0.44	7.4			
POOA	1.0	4.8			

[a] 30°C, $\mu = 0.06$ with KCl, 3 vol-% ethanol, pH 10.0 ± 0.03 with $0.02\,M$ carbonate.
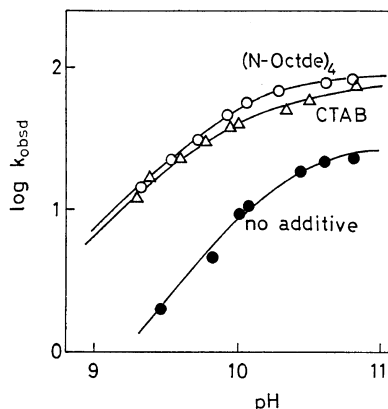
Figure 5. pH dependence for the reaction of MHAc—MAc and PNPA: $[(N\text{-Octde})_4 = 3.08 \times 10^{-4}\,M$; $[\text{CTAB}] = 3.00 \times 10^{-4}\,M$. Ammonium salt was not added where the circles are filled.

$$k_{obsd} = k_a \alpha \qquad (3)$$

$$\text{pH} = pK_a - n' \log[(1-\alpha)/\alpha] \qquad (4)$$

$$\alpha = \frac{1}{(a_H/K_a)^{1/n'} + 1} \qquad (5)$$

From eq 3 and 4, eq 6 follows.

$$\log\left(\frac{k_a}{k_{obsd}} - 1\right) = \frac{1}{n'}(pK_a - \text{pH}) \qquad (6)$$

Equation 6 shows that, if the k_a value is determined separately, then n' and pK_a are calculated from a linear relation of pH and $\log[(k_a/k_{obsd}) - 1]$. k_a values were evaluated by extrapolating the curves in Figure 5 to higher pH values. Thus, the k_a values listed in Table III were obtained. As illustrated in Figure 6, plots of pH vs. $\log[(k_a/k_{obsd}) - 1]$ provided straight lines ($r > 0.99$), from which n' and pK_a values were determined by the least squares method. As given
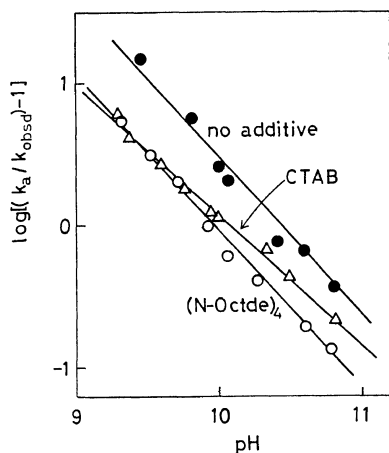
In an attempt to analyze the sigmoidal profile quantitatively, we assumed that Scheme I applies

$$MHAc + nA \underset{K}{\overset{K}{\rightleftharpoons}} MHAc \cdot A_n$$

$$\downarrow k'_{obsd} [PNPA] \qquad \downarrow k''_{obsd} [PNPA]$$

$$product \qquad\qquad product$$
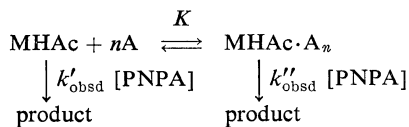
Scheme I

where k'_{obsd} and k''_{obsd} denote the apparent second-order rate constant for the uncomplexed polymer and that for the polymer complexed with additive A, respectively. K is the association constant defined as $K=[MHAc \cdot A_n]/[MHAc][A]^n$. It should be noted that K is also a function of the dissociation of the hydroxamic acid. Modification of the equation derived by Connors, *et al.*,[27] gives eq 7

$$\frac{k'_{obsd}}{k_{obsd}-k'_{obsd}} = \frac{1}{qK[A]^n} + \frac{1}{q} \qquad (7)$$

where $q=(k''_{obsd}/k'_{obsd})-1$. Equation 7 can be rewritten as eq 8 or 9. Thus, if k''_{obsd} can be determined independently, plots of $\log[k'_{obsd}/(k_{obsd}-k'_{obsd})-1/q]$ *vs.* $\log[A]$ would give a linear relation.

$$\log\left(\frac{k'_{obsd}}{k_{obsd}-k'_{obsd}} - \frac{1}{q}\right) = -n\log[A] - \log qK \qquad (8)$$

or

$$\log\left(\frac{k_{obsd}-k'_{obsd}}{k''_{obsd}-k_{obsd}}\right) = n\log[A] + \log K \qquad (9)$$

Thus, determination of k''_{obsd} values which would satisfy the relation of eq 8 was attempted by trial-and-error computation using the data of Figure 4. Figure 7 shows plots of eq 8 for $k''_{obsd} = 68\ M^{-1}\,sec^{-1}$ (CTAB) and $72\ M^{-1}\,sec^{-1}$ ((N-Octde)$_4$). The correlation coefficients were better than 0.99. From the slope $(-n)$ and the intercept $(-\log qK)$, n and K were obtained: for CTAB $n=3.0$, $K=3.6\times10^{10}\ M^{-n}$; for (N-Octde)$_4$ $n=3.6$, $K=1.6\times10^{13}\ M^{-n}$.

The n value in eq 8 may be equated to the coefficient in Hill's empirical equation.[28] A positive homotropic behavior is seen when the enzyme binds the substrate cooperatively: binding of the substrate stimulate (or inhibits) the interaction with additional substrate(s). When this is caused by a compound other than a substrate (*i.e.*, effector), the behavior is designated
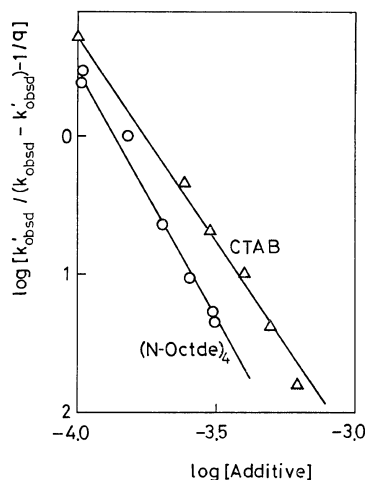

Figure 7. $\log[Additive]$ *vs.* $\log[k'_{obsd}/(k_{obsd}-k'_{obsd}) -1/q]$.

as a positive heterotropic response. This phenomenon is known to play an important role in the regulation of the metabolism.[29,30] Allosteric enzymes usually possess Hill's coefficients of 1.3—1.8.[30-33] Therefore, the positive heterotropic behavior observed in the present system is relatively large, indicating that polymer-bound ammonium ions facilitate subsequent binding considerably.*

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* Recently, an attempt to apply the Hill's equation to micelle-catalyzed system was reported by Piszkiewicz,[34] in which n values of 1 to 5 were observed.

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