Functionalized Polymer Latices. 2. Catalytic Effects of Imidazole-Containing Latices on Hydrolyses of Phenyl Esters†

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ABSTRACT: Imidazole derivatives such as L-histidine and histamine were covalently bound to polymer latices by the carbodiimide method, and catalytic effects of these latices on the hydrolyses of phenyl esters were examined. In the hydrolyses of *p*-nitrophenyl acetate, 3-nitro-4-acetoxybenzoic acid, and *p*-nitrophenyl valerate, reaction rates increased linearly with the addition of imidazole-containing polymer latex, whereas in the hydrolysis of *N*-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester, saturation phenomena due to hydrophobic interaction between substrate and catalyst latex were observed with the addition of latices containing L-histidine and the *n*-hexyl group. When acrylic acid residues were present in the vicinity of imidazolyl groups, the pH profile of catalytic behavior of the polymer latices shifted to the alkaline region compared to imidazole and other soluble imidazole-containing polymers. In addition, the second-order rate constant, k_{cat}, obtained with imidazole-containing latices was larger than those of soluble imidazole derivatives, because of the enhanced nucleophilicity of the imidazolyl groups by surrounding carboxyl groups. The $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of the polymer latex system were larger than those of the corresponding linear polymer system and similar to those of the low molecular weight catalyst system, probably because of the restricted conformation and the high density of acrylic acid residues on the polymer latex surface. The $\Delta V^{\ddagger}$ was also measured.

Various kinds of functional polymers that contain one or more catalytically active groups have often been synthesized and studied as enzyme models.[1a-e] Among them, imidazole-containing polymers have been extensively examined,[2a-d] because imidazolyl groups are known to exist at the active site of various enzymes such as α-chymotrypsin, carboxypeptidase, ribonuclease, and so on.[3a,b] Almost of all the imidazole-containing catalysts examined have been linear polymers. Paying attention to the similarity between polymer latex particles and globular protein (enzyme) molecules, it is interesting to examine the catalytic effects of polymer latex particles as enzyme models. Polymer latices are known to disperse in solution, and estimating the reaction rate in the polymer latex system seems easier than estimating those in other heterogeneous systems.[4-6] In this paper we introduced histamine or L-histidine onto a polymer latex surface by the carbodiimide method and examined the catalytic effect of these imidazolyl group containing latices on the hydrolyses of phenyl esters. Thermodynamic parameters of these reaction systems are determined to clarify the enzyme-like behavior of heterogeneous polymer latices.

Experimental Section

Materials. *p*-Nitrophenyl acetate (PNPA) from Tokyo Kasei, Tokyo, was recrystallized twice from chloroform. *p*-Nitrophenyl valerate (PNPV) and *N*-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester (Z-Phe-ONP) were purchased from Sigma and used without further purification. 3-Nitro-4-acetoxybenzoic acid (NABA), an anionic phenyl ester, was synthesized following the method by Overberger et al.[7a,b] 1-Ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (EDC) was obtained from Nakarai Chemicals (Kyoto, Japan). 2,2′-Azobis(isobutyronitrile) (AIBN) and acrylamide (AAm) from Nakarai Chemicals were recrystallized from methanol and benzene, respectively. Potassium peroxydisulfate (KPS) was an analytical grade reagent from Merck. Monomers such as styrene (St), divinylbenzene (DVB), and acrylic acid (AA) were distilled by conventional methods before use. Other chemical reagents were commercially available. Deionized water was distilled before use.

Polymer Latices. Two kinds of latices, AA-2 and AA-3, were used as carriers. Preparation of the AA-2 latex was described in a previous paper.[5] The AA-3 latex was prepared as follows: Water (400 mL) was poured into a three-necked 500-mL flask equipped with a reflux condenser and kept at 70 °C. Nitrogen gas was continuously passed through the flask. Then 70 g (77.8 mL) of styrene, 1 g (0.94 mL) of acrylic acid, and 0.5 g (0.52 mL) of divinylbenzene were added; finally, 115 mg of KPS was added as initiator. The mixture was stirred vigorously at 230–250 rpm by a Teflon stirring paddle. After 3 h, the temperature of the solution was raised to 80 °C, and the reflux condenser was taken away to evaporate unreacted monomers. Eight hours after the beginning of polymerization, heating and stirring of the solution were stopped. The polymer latex solution was cooled to room temperature, passed through a 150-mesh Nylon filtering cloth, and poured into a glass bottle that contained 100 mL of Amberlite MB-3 mixed-bed ion-exchange resin. The MB-3 resin was washed several times with an excess of distilled water before use. The bottle was sealed and slowly rolled for 30 min. The latex solution was then dialyzed for 1 week using a Visking cellulose tube. The recipes for the polymer latex solutions are shown in Table I. The diameter of the latices was determined from electron micrographs using a JEM-100U electron microscope. (Nihon Denshi Co., Tokyo, Japan) (Figure 1).

Modification of Polymer Latices. The following procedures for coupling histamine or L-histidine to the polymer latices were used: First 10 mL of AA-2 (containing 0.86 mmol of carboxyl group) was mixed with 30 mL of water, and the pH of the solution was adjusted to 4.5.[8] Then 1 g (9 mmol) of histamine (free base) and 1.7 g (8.9 mmol) of EDC were added, and pH of the solution was kept at 4.5 for 1 h by the addition of 0.1 N HCl or NaOH solution at room temperature. The reaction mixture was stored at 4 °C for 2 days under continuous stirring. After 2 days, the reaction mixture was centrifuged at 35000*g* for 10 min, and the supernatant solution was drained. Then 20 mL of 0.05 N NaOH solution was added, and the solution was vigorously stirred with a glass rod and kept at 2 °C for 1 h to hydrolyze the acylated imidazole ring of histamine.[9] The solution was then centrifuged and washed with water several times until the supernatant lost catalytic activity. Finally, the latex was suspended in 100 mL of water and used as a catalyst (His-AA-2 latex). To promote the substrate binding ability of the latex, *n*-hexyl groups were introduced into the AA-2 latex by reaction of AA-2 with *n*-hexylamine in the presence of EDC. Then L-histidine was introduced onto the latex using EDC (C_6-L-His-AA-2 latex). The imidazolyl and carboxyl contents of the modified latices were determined from both elemental analyses and conductometric titrations, and the results are given in Table II.

Homogeneous Imidazole-Containing Polymers. *N*-Methacryloyl-L-histidine and *N*-methacryloylhistamine were prepared by coupling L-histidine or histamine with an equal amount of methacryloyl chloride in the presence of an equal amount of triethylamine in dioxane–water by the modified methods of Okamoto et al.[10] and Imanishi et al.[11] The *N*-methacryloylhistamine or *N*-methacryloyl-L-histidine was copolymerized with

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Table I
Preparation of Carrier Latices

	H_2O, mL	styrene, mL	acrylate, mL	DVB, mL	KPS, mL	charge number	charge density, charge/Å^2	diameter, Å
AA-2[a]	500	50	5	0.5	230	1.6×10^6	0.20	1600 ± 40
AA-3[b]	400	78	0.94	0.5	115	6.8×10^5	0.019	3420 ± 100

[a] 60 °C, 4 h. [b] 70 °C, 3 h.

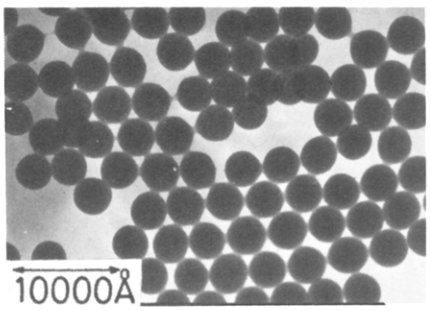

Figure 1. Electron micrograph of polymer latex AA-3.

Table II
Composition of Acrylate Derivatives on the Surface of Latices[a]

	molar distribution of acrylic functions		
	free COOH, %	Im, %	n-C_6H_{13}, %
His-AA-2 latex	71	29	0
L-His-AA-2 latex	73	27	0
C_6-L-His-AA-2 latex	50	11	39
His-AA-3 latex	35	65	0
L-His-AA-3 latex	45	55	0

[a] Mole percent of total acrylate derivatives in the latices: AA-2, 12.3; AA-3, 0.57.

acrylamide in methanol at 65 °C using AIBN as initiator (His-AAm and L-His-AAm). To enhance the ability of substrate binding a small amount of vinyl lauryl ether was introduced into His-AAm and L-His-AAm (C_{12}-His-AAm and C_{12}-L-His-AAm). Poly(*N*-vinylimidazole) (PVIm) was prepared by free-radical polymerization of *N*-vinylimidazole in benzene using AIBN as initiator.[7a] Poly(acrylic acid) was directly coupled with histamine (His-AA) using EDC in a similar way to imidazole-containing latices and purified by repeated ultrafiltration and dilution with H_2O using a Visking dialyzing tube (8/32). The His-AA was further coupled with *n*-hexylamine using EDC and purified by repeated ultrafiltration and dilution (C_6-His-AA). Details of the preparation of the homogeneous polymer catalysts were described elsewhere.[12] The composition of each of these homogeneous polymer catalysts was determined from both elemental analyses and conductometric titrations (Table III).

Kinetic Measurements. The initial rate for the esterolytic reaction was followed by the increase in the absorbance at 400 nm (PNPA, Z-Phe-ONP, and PNPV) or 418 nm (NABA) using a high-sensitivity spectrophotometer (SM-401, Union Engineering, Hirakata, Japan). The extinction coefficients of the product phenolate for each reaction system were obtained by reference experiments for buffer solutions containing *p*-nitrophenol or 3-nitro-4-hydroxybenzoic acid in the presence of various amounts of latex particles. Reaction at high pressure was studied with a Union high-pressure spectrophotometer.[13] Tris buffer was used because of its insensitivity to pressure.[14]

Results and Discussion

We first examined the esterolyses of PNPA, NABA, and PNPV catalyzed by His-AA-2 latex. The polymer latex solution showed acceleration effects on the esterolyses of phenyl esters (Figure 2). Since the supernatant of the centrifuged latex solution did not show any catalytic activity, the catalytic activity of the latex solution could be attributed to the latex particles themselves. From the figure it is apparent that the reaction rates increase linearly with the addition of latex solution, suggesting that the reactions rates are expressed as $V_0 = V_{sp} + k_{cat}[C]_0[S]_0$, where V_0 and V_{sp} are the observed reaction rate and spontaneous reaction rate, respectively. The second-order rate constant of the latex-catalyzed reaction is k_{cat}, and $[S]_0$ and $[C]_0$ are the initial concentrations of substrate and imidazole residue of the added polymer latex solution. These linear relationships of catalytic activity are attributed to weak interactions between substrates and polymer latices because of hydrophilic acrylic acid residues on the surface of polymer latices.

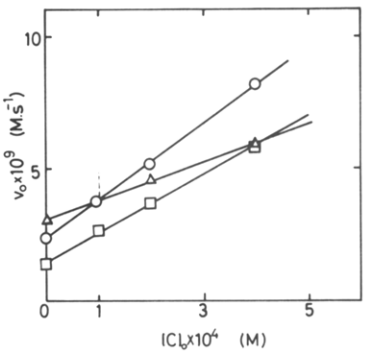

Figure 2. Catalytic effects of His-AA-2 latex on the hydrolyses of phenyl esters at 25 °C: (○) PNPA; (□) PNPV; (△) NABA.

With C_6-L-His-AA-2 latex we could not observe the saturation phenomenon in the hydrolysis of PNPV. In the hydrolysis of the more hydrophobic substrate Z-Phe-ONP, however, a clear saturation phenomenon is observed (Figure 3). Both the dissociation constant, K, and the reaction rate constant of the C_6-L-His-AA-2 latex–substrate complex, k_2 (0.16 mM and 8.7×10^{-4} s^{-1}, respectively), are estimated by using double-reciprocal plots of $(V_0 - V_{sp})^{-1}$ vs. $[C]_0^{-1}$ and eq 1 and 2, where $V_{sp} = k_{sp}[S]_0$. Similar

$$\begin{array}{ccccccc} C + S & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & CS & \xrightarrow{k_2} & CS' & \xrightarrow{k_3} & C + P_2 \\ {\scriptstyle k_{sp}}\downarrow & & & & + & & \\ P & & & & P_1 & & \end{array} \quad (1)$$

$$\frac{1}{(V_0 - V_{sp})} = \frac{1}{[S]_0(k_2 - k_{sp})} + \frac{1}{[S]_0(k_2 - k_{sp})} \frac{K}{[C]_0} \quad (2)$$

saturation phenomena were also observed in the hydrolyses of PNPV and Z-Phe-ONP catalyzed by C_{12}-His-AAm and C_{12}-L-His-AAm. The kinetic constants obtained are listed in Table IV.

The pH effects on the catalytic activity of imidazole-containing catalysts are shown in Figure 4. The pH profile of His-AAm is similar to that of imidazole except the position of the maximal value of k_{cat}, in contrast to that of His-AA-2 latex and His-AA, which is shifted to the alkaline region. The ratios of imidazole to carboxyl residues to His-AA-2 latex and His-AA are about 1:2.5 and 1:5.7. This means that imidazole groups are surrounded by carboxyl groups. The environment of the imidazole

Table III
Composition of Homogeneous Polymer Catalysts[a]

catalyst	acrylamide	N-methacryloylhistamine (or -L-histidine)	vinyl lauryl ether
His-AAm	0.90	0.10	0
L-His-AAm	0.82	0.18	0
C_{12}-His-AAm	0.74	0.21	0.05
C_{12}-L-His-AAm	0.76	0.22	0.02
catalyst	acrylic acid	N-acryloylhistamine	N-acryloylhexylamine
His-AA	0.85	0.15	0
C_6-His-AA	0.58	0.20	0.22

[a] Mole fraction.

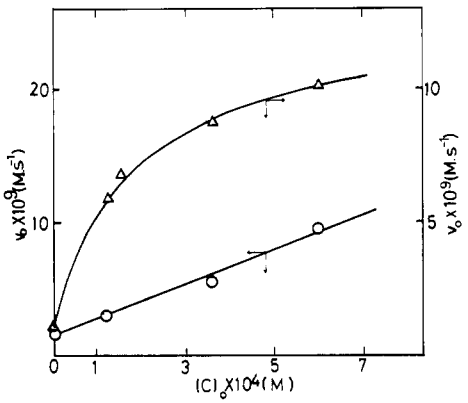

Figure 3. Catalytic effect of C_6-L-His-AA-2 latex on the hydrolyses of phenyl esters at 25 °C: (○) PNPV; (△) Z-Phe-ONP, pH 8.2 Tris-HCl buffer.

Table IV
Second-Order Rate Constants for the Catalytic Hydrolyses of Phenyl Esters at 25 °C[a]

catalyst	substrate		
	PNPA	PNPV	Z-Phe-ONP
imidazole	0.47	0.22	0.44
His-AA-2 latex	0.30	0.26	
L-His-AA-2 latex	0.27	0.17	0.40
C_6-His-AA-2 latex		0.40	5.4[b] (0.16)[c]
PVIm	0.035	0.028	0.039
His-AAm	0.19	0.18	0.59
L-His-AAm	0.082	0.062	0.37
C_{12}-His-AAm	0.099	0.16[b] (4.7)[c]	0.96[b] (3.4)[c]
C_{12}-L-His-AAm	0.085	0.39[b] (5.6)[c]	0.73[b] (2.9)[c]
His-AA	0.022		0.17
C_6-His-AA	0.11		2.8[b] (1.8)[c]

[a] pH 8.2 Tris-HCl buffer, k_{cat} value in $M^{-1} s^{-1}$. [b] k_2/K value in $M^{-1} s^{-1}$. [c] K value in mM.

residue is more acidic than in the bulk phase, resulting in the shift of the pH profile to alkaline region.[6]

Shimizu et al. and Overberger et al. examined the catalytic activity of poly(4(5)-vinylimidazole-*co*-acrylic acid) in the hydrolysis of a cationic ester and observed that the pK of imidazole shifted to alkaline pH with increasing acrylic acid content.[15,16] Shinkai et al. synthesized poly(*N*-methacryloylhistamine-*co*-acrylic acid) and poly(*N*-methacryloylhistamine-*co*-acrylamide) and also observed the pK shift of imidazole to alkaline in the presence of carboxylic group.[17] Similar pH shifts were observed in the catalytic activities of enzymes and microbial cells entrapped in polyelectrolyte gels[18,19] or enzymes immobilized onto the polymer latices.[5,20]

The maximal value of k_{cat} for His-AA-2 latex is about two times higher than that for imidazole and seven times than that for His-AAm. This is probably because of the enhancement of the nucleophilicity of imidazolyl groups by neighboring carboxyl groups. When the percent of

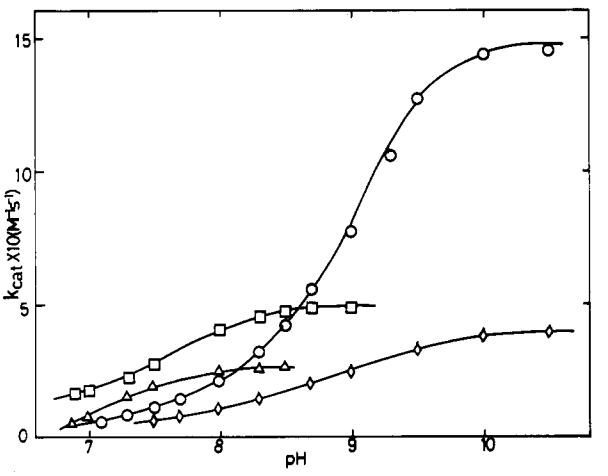

Figure 4. pH effects on the catalytic activity of imidazole-containing compounds at 25 °C: (□) imidazole; (△) His-AAm; (◇) His-AA; (○) His-AA-2 latex. [PNPA] = 50 μM.

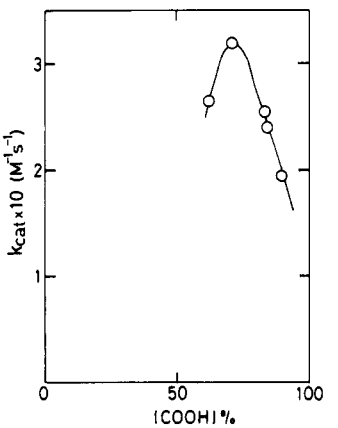

Figure 5. Effects of copolymer composition of the latex surface on k_{cat} value for His-AA-2 latex catalyzed hydrolysis of PNPA at 25 °C. [PNPA] = 50 μM, pH 8.2 Tris-HCl buffer.

acrylate groups to imidazolyl groups (100 × [COOH]/[COOH] + [Im])) on the latex surface is changed, k_{cat} shows a maximum as shown in Figure 5, though not much data are available. This change also suggests the cooperative effect of acrylic acid on the nucleophilicity of imidazole group. Overberger et al. found that the most catalytically active species was the carboxylate–imidazole–carboxylate triad.[16] Shimizu et al. also reported a cooperative effect for the hydrolytic reaction of cationic ester catalyzed by poly(4(5)-vinylimidazole-*co*-acrylic acid) of less than 40 mol % in the 4(5)-vinylimidazole content.[15] In the case of hydrolysis of neutral ester, PNPA, catalyzed by random copolymers that contain both imidazolyl and carboxyl groups, Overberger et al. could not so clearly observe such an enhancing effect and maximum, probably because the random copolymer is extended by the elec-

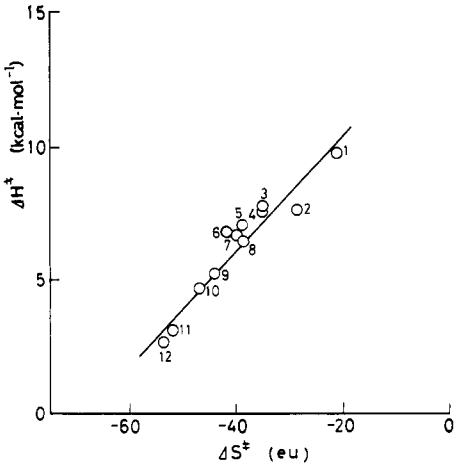

Figure 6. Isokinetic relationships in catalytic processes of hydrolysis of PNPA: (1) histamine; (2) L-histidine; (3) His-AA-2 latex; (4) L-His-AA-2 latex; (5) His-AA; (6) PVIm; (7) C_6-His-AA; (8) imidazole; (9) His-AAm; (10) L-His-AAm; (11) C_{12}-His-AAm; (12) C_{12}-L-His-AAm.

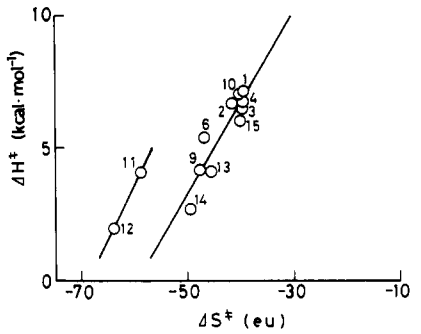

Figure 7. Isokinetic relationships in catalytic process of hydrolysis of PNPV. Catalyst numbers 1–12 are the same as in Figure 6; (13) L-His-AA-3 latex; (14) His-AA-3 latex; (15) C_6-L-His-AA-2 latex.

trostatic repulsion and partly because of weak interaction between a substrate and catalyst.

The thermodynamic parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) for the hydrolyses of PNPA and PNPV catalyzed by histamine, His-AAm, and other imidazole-containing catalysts are shown in Figures 6 and 7. Bruice et al.[21] reported that $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of imidazole-catalyzed hydrolysis of PNPA were 7.8 kcal·mol^{-1} and −27.2 eu, respectively. Bender et al. reported 6.5 kcal·mol^{-1} and −38.2 eu for these quantities.[22] Our data (6.5 kcal·mol^{-1} and −39 eu, respectively) agreed well with those of Bender et al. The large negative entropy of imidazole-catalyzed reaction of the neutral ester was attributed to an increase in polarity and subsequent electrostriction of solvent water.[2d] The $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the hydrolysis of PNPA catalyzed by the various homogeneous polymer catalysts are similar to each other but smaller than those of imidazole catalysis. Overberger et al. reported that $\Delta S^{\ddagger}$ of poly(4(5)-vinylimidazole)-catalyzed hydrolysis of PNPA was −9.7 eu than that of imidazole and attributed this to the higher degree of order in the polymeric activated complex.[23] Our data also showed similar trends. The differences in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for soluble polymers examined here might be attributed to the differences in the environment of imidazole residues (the influences of polar or apolar environment on the catalytic properties of soluble polymers will be discussed in a following paper[12]). On the other hand, the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the hydrolysis catalyzed by the latices are larger than those for the homogeneous polymer catalysts and are comparable to those for small-molecule catalysts.

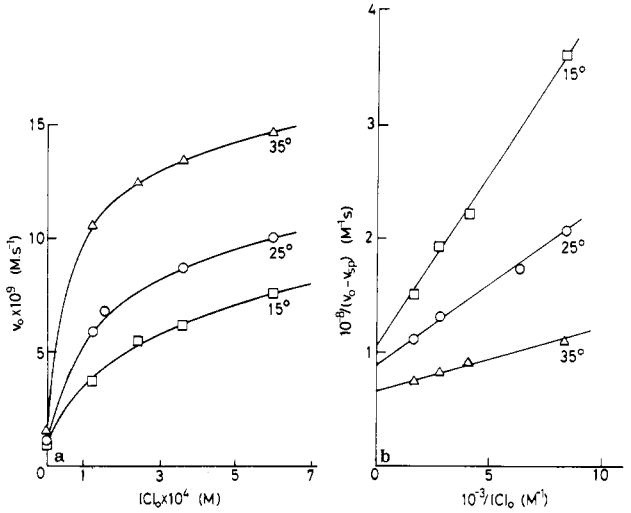

Figure 8. (a) Catalytic effects of C_6-L-His-AA-2 latex on the hydrolysis of Z-Phe-ONP at various temperatures. (b) Double-reciprocal plots of (a).

This trend might be attributed partly to the loss of freedom of the active group in the initial state ($\Delta S^{\ddagger}$ increase) and partly to the high density of acrylic acid. Since catalysis of polymer latex was carried out by both imidazolyl and carboxyl groups cooperatively, decrease of $\Delta S^{\ddagger}$ by the electrostriction in the transition state might be relatively smaller than that for linear polymer because of a large amount of hydrated water to the acrylic acid in the vicinity of imidazole group in the ground state. Overberger et al. suggested that the dimension of macromolecules was temperature dependent.[23] In the case of the functional latices, however, this dimensional factors seems to be smaller than for soluble macromolecules because of restricted conformation. Thus, the obtained thermodynamic parameters of latices would be closer to those of low molecular weight catalysts than those of linear polymer catalysts.

$\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the PNPA hydrolysis and biomolecular hydrolysis of PNPV showed satisfactory linear relationships, whereas $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the hydrolysis of PNPV through intracomplex showed another relationship similarly to those by Kunitake et al.[25] (Figure 7).

By the double reciprocal plots of $(V_0 - V_{sp})^{-1}$ vs. $[C]_0^{-1}$ at various temperatures for the hydrolysis of Z-Phe-ONP catalyzed by C_6-L-His-AA-2 latex (Figure 8a,b), we estimate the thermodynamic parameters of both the substrate binding and the reaction of the catalyst–substrate complex. These results are shown in Table V together with those for linear polymer catalysts, C_{12}-L-His-AAm and C_6-His-AA. The positive enthalpy change and large positive entropy change for the linear polymer catalysts C_6-His-AA and C_{12}-L-His-AAm are similar to those evaluated by Kunitake et al.[24] (ΔH, ΔS, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ values for the hydrolysis of *p*-acetoxybenzoic acid catalyzed by a copolymer of *N*-(*p*-4(5)-imidazolybenzyl)acrylamide and acrylamide were 5.40 kcal·mol^{-1} and 24.9 eu and 4.17 kcal·mol^{-1} and −60 eu, respectively). On the other hand, ΔH and ΔS values of C_6-L-His-AA-2 latex catalyzed reaction are quite different. Extremely large ΔH value might be attributed to the stability of hydrophobic domain on the latex (though the hydrophobic interaction increases to 58 °C,[25] the molecular motion by the thermal energy might destabilize the hydrophobic domain in the linear polymer). The difference in ΔS values can be accounted for as follows: in the case of the homogeneous catalyst, the so-called "looping effect" decreases the ΔS value: that is, the polymer catalyst forms catalytic loops upon sub-

Table V
Thermodynamic Parameters for the Catalytic Hydrolysis of Z-Phe-ONP

	ΔG, kcal·mol^{-1}	ΔH, kcal·mol^{-1}	ΔS, eu	$\Delta G^{\ddagger}$, kcal·mol^{-1}	$\Delta H^{\ddagger}$, kcal·mol^{-1}	$\Delta S^{\ddagger}$, eu
C_{12}-L-His-AAm	−3.5 ± 0.2	3.1 ± 0.2	22 ± 1	21.0 ± 0.2	7.6 ± 0.2	−45 ± 1
C_6-His-AA	−3.7 ± 0.2	3.4 ± 0.2	24 ± 1	21.0 ± 0.2	5.8 ± 0.2	−49 ± 1
C_6-L-His-AA-2 latex	−5.2 ± 0.2	9.9 ± 0.2	51 ± 1	21.6 ± 0.2	4.0 ± 0.2	−59 ± 1

strate binding and the catalytically active site is relatively fixed.

On the contrary, the limited mobility of catalytic group on the latex does not permit looping to be substantial; in other words, a cooperative action of binding site and active site is not as likely in the latex catalysis.

Kunitake et al. attributed the negative activation entropy of intracomplex reaction ($\Delta S^{\ddagger}$) to the destruction of hydrophobic interaction.[24] Contrarily, Taniguchi et al. estimated a large contribution of increase in polarity (−16 mL·mol^{-1}) and suggested small contribution of hydrophobic interaction (−4 mL·mol^{-1}) to the negative volume of activation in the esterolysis of phenyl esters by poly(1-vinyl-2-methylimidazole-*co*-1-vinylpyrrolidone).[2d] Though the reason was not still clear, the tendencies of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of both C_{12}-L-His-AAm and C_6-L-His-AA-2 latex catalyzed reaction are nearly the same as Kunitake's data except the large $\Delta H^{\ddagger}$ values for C_{12}-L-His-AAm.

Pressure effects on the catalytic activity of imidazole derivatives on the hydrolyses of PNPA or Z-Phe-ONP are examined. The obtained $\Delta V^{\ddagger}$ values for PNPA hydrolysis are −20 ± 2 mL·mol^{-1} (imidazole), −20 ± 3 mL·mol^{-1} (His-AA-2 latex), and −19 ± 2 mL·mol^{-1} (His-AAm). The $\Delta V^{\ddagger}$ values for the hydrolyses of Z-Phe-ONP are −17 ± 2 mL·mol^{-1} (imidazole), −22 ± 3 mL·mol^{-1} (His-AA-2 latex), and −11 ± 2 mL·mol^{-1} (His-AAm). The negative $\Delta V^{\ddagger}$ values are partly due to the increase in polarity in the course of activation and partly because the transition state involves bonding between two previously discrete molecular species.[26] These results are consistent with the finding that $\Delta S^{\ddagger}$ of His-AA-2 latex catalyzed reaction showed a similar tendency with those of small-molecule catalysts. The unexpected large $\Delta V^{\ddagger}$ value of His-AAm in the hydrolysis of Z-Phe-ONP could be probably attributed to the factor that the dimensional factor did not strongly influence the $\Delta V^{\ddagger}$ value in comparison with $\Delta S^{\ddagger}$ values.

In conclusion, heterogeneous but homogeneously dispersed catalysts could be obtained easily by using polymer latices as carriers. By the presence of carboxylic residues in the vicinity of imidazole groups fixed on the latex surface, thermodynamic properties of the catalyst latex were different from those of corresponding homogeneous polymer catalysts.

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Registry No. PNPA, 830-03-5; PNPV, 1956-07-6; Z-Phe-ONP, 2578-84-9; NABA, 1210-97-5; His-AAm, 86120-14-1; L-His-AAm, 86120-16-3; C_{12}-His-AAm, 86120-17-4; C_{12}-L-His-AAm, 86128-97-4; PVIm, 25232-42-2.

References and Notes

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