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(20) This is based on estimating that the density of the modified layer of **4** is 1 g/mL.
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Stereoselective Hydrolysis of Amino Acid Esters in Branched or Linear Poly(ethylenimine) Derivatives

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ABSTRACT: The viscosity behavior of modified poly(ethylenimine) derivatives and fluorescence behavior of sulfonated fluorophore and binding behavior of sulfonated dye in branched or linear poly(ethylenimine) derivatives showed characteristics of the structural nature of the polymers. Stereoselective hydrolysis of chiral substrates was examined in branched or linear poly(ethylenimine) derivatives with covalently linked dipeptide-containing histidine. A high stereoselective effect, $k_L/k_D = 3.6$, is observed. Added copper ions influenced both the rate and stereoselective ratio as catalyzed by the polymers with covalently linked active groups, depending on the nature of the polymers. A large rate enhancement and a stereoselective preference also are exhibited in the hydrolysis as catalyzed by *N*-decanoyl-L-histidine (I) or a dipeptide containing an L-histidyl residue (II) in the environment of branched or linear poly(ethylenimine) domain. The dipeptide catalyst revealed the highest stereoselectivity, $k_L/k_D = 8.6$, in a linear poly(ethylenimine) derivative. The effect of the substrate structure influenced both the rate constant and stereoselective ratio in the hydrolyses by the modified poly(ethylenimine) derivatives.

Introduction

Developments in the area of synthetic macromolecular catalysts with enzymelike behavior are impressive.[1-4] However, there has been little study of stereochemical effect with synthetic macromolecular catalysts although the stereoselective preference is one of the most important properties in many enzymatic actions.[5-13] Our laboratory has been involved in the stereochemical effect on the catalysis in macromolecular systems as models for enzymatic reactions.[14]

In the previous article,[14a] we first observed stereoselective preference of amino acid *p*-nitrophenyl esters hydrolysis by branched poly(ethylenimine)s with optically active histidine moieties. Similar results have been reported by others with imidazole-containing polymers.[6,7] Following in this direction, this paper describes a high stereoselective effect in the hydrolysis of chiral esters by modified poly(ethylenimine) derivatives with a covalently linked dipeptide substituent containing a histidyl residue.[15] Furthermore, this paper describes the results of the catalytic activities of chiral ester hydrolysis in linear or branched poly(ethylenimine) domains.[16] The results of quarternization of branched poly(ethylenimine) and of the addition of surfactants on the kinetics of optically active ester hydrolysis in the branched poly(ethylenimine)s were reported previously,[14b] indicating that the rate and stereoselectivity are remarkably enhanced in the presence of quaternized branched poly(ethylenimine) derivatives. However, the branched poly(ethylenimine) has several kinds of amino groups which occasionally made the behavior of catalysis puzzling. In contrast, linear poly(ethylenimine) has only secondary amino groups on the polymer chain and this provides a simpler local macromolecular environment in aqueous solution than does the branched polymer.

A comparison of rate constants and stereoselectivity of the catalyst in the linear or branched polymer domains provides some insight into the influence of local macromolecular environment on the behavior of the imidazole nucleophile.

Experimental Section

A Jasco ORD/UV-5 spectropolarimeter was used to measure specific rotation, $[\alpha]_D$, at room temperature.

Branched poly(ethylenimine) (PEI) and linear poly(ethylenimine) (PEI) with an average molecular weight of about 50 000 were gifts from Professor I. M. Klotz (obtained from Dow Chemical Co.). These materials were purified by ultrafiltration in an Amicon ultrafiltration apparatus, using a Toyo Roshi UK-50 ultrafilter. *N*-Decanoyl-L-histidine (I) was prepared and purified by standard methods. CBZ-L-Leu-L-His (II) was prepared by the reaction of the *N*-hydroxysucciniimide ester of *N*-[(benzoyloxy)carbonyl]-L-leusine with L-histidine (Anal. Found: C, 59,30; H, 6.60; N, 13.20. Calcd: C, 59.68; H, 6.52; N, 13.92; mp 121-122 °C; $[\alpha]_D = +7.3°$; 80% EtOH-H_2O).[17] Lauryl iodide was distilled before use [bp 117 °C (1.1 mmHg)]. 1,8-Bis(dimethylamino)-naphthalene, or "proton sponge" (from Aldrich Co.), was recrystallized from ethanol-water. 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide (Sigma Chemical Co.), L-histidine methyl ester, $CuCl_2$ (Nakarai Chemical Co.), lauryl bromide, and *N*-(2-bromoethyl)phthalimide (Tokyo Kasei Co.) were used without further purification. The substrate *p*-nitrophenyl esters of L-*N*-carbobenzoxyalanine (CBZ-Ala) and L-*N*-CBZ-phenylalanine (CBZ-Phe) (from Sigma Chemical Co.) were used without further purification. The preparation of D-CBZ-Ala nitrophenyl ester has been described previously.[14b] The other substrates, D- and L-*N*-(methoxycarbonyl) (MOC)-Phe and D-CBZ-Phe were

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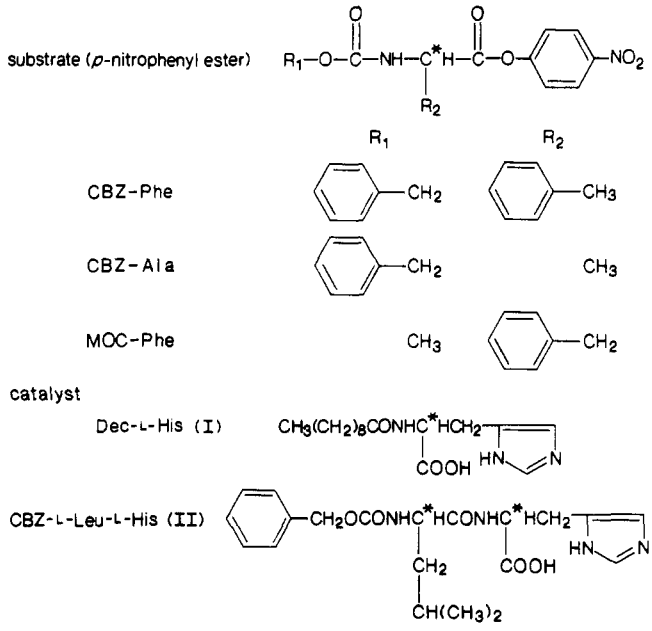
Chart I



prepared and purified by standard method.[14b] These esters were characterized by their melting points, C, H, and N analyses, and specific rotation, which were in good agreement with those from the literature. The purity of these esters was also confirmed from the kinetic analyses. 2-*p*-Toluidinylnaphthalene-6-sulfonate (TNS) (Sigma Co.) was recrystallized from a 2% NaOH solution, and the sodium salt was dried under vacuum at 25 °C for 24 h. Formulas of the substrates and catalysts (I–II) are presented in Chart I. Methyl orange (Tokyo Kasei Co.) was recrystallized from MeOH–H_2O.

Modification of Polymers. Lauryl branched poly(ethylenimine) (L(15)-PEI) (III) and lauryl linear poly(ethylenimine) (L(15)-$\overline{\text{PEI}}$, L(24)-$\overline{\text{PEI}}$, L(37)-$\overline{\text{PEI}}$, and L(50)-$\overline{\text{PEI}}$) (IV–VII). Corresponding lauryl iodide was added to branched or linear poly(ethylenimine) in absolute ethanol containing a "proton sponge". The resulting solution was stirred at 50 °C for 24 h. The polymer-containing solution was dialyzed against 40% ethanol–water and then against water. The aqueous solution was lyophilized. Integration of the proton magnetic resonance (^{1}H NMR) spectrum of the products in D_2O indicated a corresponding mole of lauryl groups per residue mole of polymer, respectively. Thus, the modified polymer may be represented by the following stoichiometric formulas: L(15)-PEI (III), $(C_2H_4N)_m(C_{12}H_{25})_{0.15m}$, m = 1400; L(15)-$\overline{\text{PEI}}$ (IV), $(C_2H_4N)_m(C_{12}H_{25})_{0.15m}$, m = 1400; L(24)-$\overline{\text{PEI}}$ (V), $(C_2H_4N)_m(C_{12}H_{25})_{0.24m}$, m = 1400; L(37)-$\overline{\text{PEI}}$ (VI), $(C_2H_4N)_m(C_{12}H_{25})_{0.37m}$, m = 1400; L(50)-$\overline{\text{PEI}}$ (VII), $(C_2H_4N)_m(C_{12}H_{25})_{0.50m}$, m = 1400.

Quaternized Lauryl Branched Poly(ethylenimine) (Q(160)-L(25)-PEI) (VIII) and Quaternized Lauryl Linear Poly(ethylenimine) (Q(155)-L(15)-$\overline{\text{PEI}}$, Q(146)-L(24)-$\overline{\text{PEI}}$, Q(133)-L(37)-$\overline{\text{PEI}}$, and Q(120)-L(50)-$\overline{\text{PEI}}$) (IX–XII). The quaternized polymer with free primary amines (VIII) was prepared by the following four-step procedures as described in a previous paper:[14b,f] (1) introduction of protected primary amines by reaction of *N*-(2-bromoethyl)phthalimide with polymer in absolute ethanol; (2) alkylation of polymer with $C_{12}H_{25}$ groups by reaction with lauryl bromide in absolute ethanol; (3) quaternization of polymer with dimethyl sulfate; (4) removal of the phthalimide protecting groups by treatment with hydrazine in absolute ethanol and release of primary aminoethyl group (C_2H_6N) in the polymer. Quaternized lauryl linear poly(ethylenimine)s IX–XII were prepared by quaternization of lauryl-linear poly(ethylenimine)s IV–VII with dimethyl sulfate. Integration of the ^{1}H NMR spectra of the polymer dissolved in D_2O indicated the following stoichiometric composition: Q(160)-L(25)-PEI (VIII), $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_6N)_{0.10m}(CH_3)_{1.60m}Cl_m$, m = 1400; Q(155)-L(15)-$\overline{\text{PEI}}$ (IX), $(C_2H_4N)_m(C_{12}H_{25})_{0.15m}(CH_3)_{1.55m}Cl_m$, m = 1400; Q(146)-L(24)-$\overline{\text{PEI}}$ (X), $(C_2H_4N)_m(C_{12}H_{25})_{0.24m}(CH_3)_{1.46m}Cl_m$, m = 1400; Q(133)-L(37)-$\overline{\text{PEI}}$ (XI), $(C_2H_4N)_m(C_{12}H_{25})_{0.37m}(CH_3)_{1.33m}Cl_m$, m = 1400; Q(120)-L(50)-$\overline{\text{PEI}}$ (XII), $(C_2H_4N)_m(C_{12}H_{25})_{0.50m}(CH_3)_{120m}Cl_m$, m = 1400.

Acetyl lauryl branched poly(ethylenimine) (Acyl(30)-L(15)-PEI) (XIII) and acetyl quaternized lauryl branched poly(ethylenimine) (Acyl(25)-Q(160)-L(25)-PEI) (XIV). The general procedure followed was that described in a previous papers.[14f] This acetylation was carried out with polymers III and IV. These modified polymers were purified by the same ultrafiltration procedure as for poly(ethylenimine) (PEI or $\overline{\text{PEI}}$). The extents of acetylation (C_2H_3O) were determined from peak area in the respective ^{1}H NMR spectra. Stoichiometric compositions for the acetylated derivatives are as follows: Acyl(30)-L(15)-PEI (XIII), $(C_2H_4N)_m(C_{12}H_{25})_{0.15m}(C_2H_3O)_{0.30m}$, m = 1400; Acyl(25)-Q(160)-L(25)-PEI (XIV), $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_4N)_{0.10m}(CH_3)_{1.60m}(C_2H_3O)_{0.25m}Cl_m$, m = 1400.

Dipeptide-Containing Poly(ethylenimine) Derivatives XV, XVI, and XVII. Equivalent molar CBZ-L-Leu-L-His (II) and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide were added to an aqueous solution of poly(ethylenimine) derivatives L(15)-PEI (III), L(15)-$\overline{\text{PEI}}$ (IV), and Q(160)-L(25)-PEI (VIII) at pH 6.0. The resulting solution was stirred at room temperature for 10 days. The aqueous solution was lyophilized. Integration of the ^{1}H NMR spectrum in D_2O indicated 0.07 mol of dipeptide groups ($C_{20}H_{25}N_4O_4$) per residue mole of polymer. Thus, modified polymers may be represented by the stoichiometric formula: dipeptidyl lauryl branched poly(ethylenimine) [CBZ-L-Leu-L-His(7)-L-(15)-PEI (XV)], $(C_2H_4N)_m(C_{12}H_{25})_{0.15m}(C_{20}H_{25}N_4O_4)_{0.07m}$, m = 1400; dipeptidyl lauryl linear poly(ethylenimine) [CBZ-L-Leu-L-His-(7)-L(15)-$\overline{\text{PEI}}$ (XVI)], $(C_2H_4N)_m(C_{12}H_{25})_{0.15m}(C_{20}H_{25}N_4O_4)_{0.07m}$, m = 1400; dipeptidyl quaternized lauryl branched poly(ethylenimine) [CBZ-L-Leu-L-His(7)-Q(160)-L(25)-PEI (XVII)], $(C_2H_4N)_m(C_{12}H_{25})_{0.25m}(C_2H_6N)_{0.10m}(C_{20}H_{25}N_4O_4)_{0.07m}(CH_3)_{1.60m}Cl_m$, m = 1400.

Kinetic Measurements. Reaction rates were followed, at pH 7.3 in 0.01 M Bis-Tris buffer and at pH 8.0 in 0.01 M Tris buffer, at 25 °C by the appearance of *p*-nitrophenolate ion, measured by increased absorbance at 400 nm as recorded with an Hitachi Model 124 spectrophotometer. A stock solution of 0.03 M substrate was prepared in dioxane and a stock solution of 0.02 M catalyst was prepared in 80% ethanol–water or dimethyl sulfoxide. The reference cell contained appropriate controls to measure the cleavage of nitrophenyl ester in the presence of all other constituents except the polymer. Pseudo-first-order rate constants (k_{obsd}) were calculated from the variation of log ($A_\infty - A_t$) versus time (t) by use of the least-squares methods. Correlation coefficients were above 0.999.

Nuclear Magnetic Resonance Spectra. Resonance was scanned in an Hitachi–Perkin-Elmer R20 instrument, operating at 60 MHz, with an associated data collecting unit (Hitachi A-1600). Samples in D_2O were prepared at a concentration of approximately 9% by weight. Each final spectrum was the result of 100–350 scans.

Viscosity Measurement. Measurements were performed in 0.01 M Bis-Tris buffer at pH 7.3 at 25 ± 0.01 °C. An Ubbelohde dilution-type viscometer was used.

Fluorescence of TNS. Fluorescence spectra were measured by an Hitachi MPF-2A instrument at 25 °C. Solutions were prepared in 0.01 M Bis-Tris buffer at pH 7.3. Excitation was 320 nm and emission was 435–445 nm. All the emission spectra was corrected with guinidine sulfate.

Extent of Binding of Sulfonated Dye to Modified Poly(ethylenimine)s. Binding of methyl orange by these polymers was measured by an equilibrium dialysis method[18] at 25 °C. All the solutions were prepared in 0.01 M Bis-Tris buffer, pH 7.0.

Results and Discussion

Viscous Behavior of Modified Poly(ethylenimine)s. The viscous behavior of branched and linear poly(ethylenimine) derivatives is illustrated in Figure 1. The reduced viscosity is fairly independent of the concentration in all cases except the quaternized polymers. Comparing polymers, one finds that the reduced viscosity at high concentration of these polymers increases in the order $\overline{\text{PEI}}$

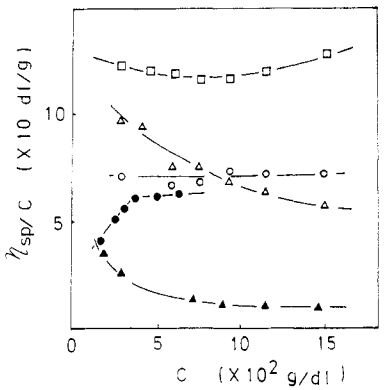


Figure 1. Reduced viscosity as a function of concentration for poly(ethylenimine) derivatives in 0.01 M Bis-Tris buffer, pH 7.3 at 25 °C: (□) $\overline{\text{PEI}}$; (●) L(15)-PEI (III); (○) L(15)-$\overline{\text{PEI}}$ (IV); (▲) Q(160)-L(25)-PEI (VIII); (△) Q(155)-L(15)-$\overline{\text{PEI}}$ (IX).

Table I
Fluorescence Intensity[a] of TNS in the Presence of Modified Poly(ethylenimine) Derivatives

polymer	I	I_{rel}[b]
$\overline{\text{PEI}}$	0.0	0.0
PEI	0.0	0.0
Acyl(30)-L(15)-PEI (XIII)	42.5	1.00
L(15)-$\overline{\text{PEI}}$ (IV)	42.9	1.01
Acyl(25)-Q(160)-L(25)-PEI (XIV)	62.3	1.48
Q(155)-L(15)-$\overline{\text{PEI}}$ (IX)	18.3	0.43
Q(146)-L(24)-$\overline{\text{PEI}}$ (X)	79.8	1.89
Q(133)-L(37)-$\overline{\text{PEI}}$ (XI)	77.3	1.74
Q(120)-L(50)-$\overline{\text{PEI}}$ (XII)	68.0	1.61

[a] Measurement conditions: pH 7.3, 0.01 M Bis-Tris buffer, 25 °C; [polymer] = 1.2×10^{-4} residue molar, [TNS] = 1×10^{-6} M; excitation wavelength, 320 nm, emission wavelength, 435–445 nm. [b] Relative intensity of fluorescence for XIII (I_0): $I_{rel} = I/I_0$.

> L(15)-$\overline{\text{PEI}}$ (IV) ≳ Q(155)-L(15)-$\overline{\text{PEI}}$ (IX) > L(15)-PEI (III) > Q(160)-L(25)-PEI (VIII), indicating that linear poly(ethylenimine) derivatives have less compact structure in aqueous solution than the corresponding branched poly(ethylenimine) derivatives and also that apolar groups on the polymers cause more compact structure. The shape of the curve for Q(155)-L(15)-$\overline{\text{PEI}}$ (IX) shows a similar trend to that for Q(160)-L(24)-PEI (VIII) which is reminiscent of those of a linear, flexible polyelectrolyte in salt-free or extremely dilute solution as described in a previous paper.[14b]

Fluorescence Behavior of TNS in Modified Poly(ethylenimine)s. The relative fluorescence of TNS in aqueous solution of various poly(ethylenimine) derivatives is listed in Table I. As is apparent in Table I, TNS, which has been shown to be apolar fluorescent probe, is practically nonfluorescent in aqueous solution of PEI or $\overline{\text{PEI}}$, but fluorescent enhancement is observed in the polymers with apolar groups. Thus, the observed TNS fluorescence may be due to the binding of the fluorophore to the apolar areas of polymer surface. Comparing polymers, the intensity of fluorescence increases in the order Q(146)-L(24)-$\overline{\text{PEI}}$ (X) > Q(133)-L(37)-$\overline{\text{PEI}}$ (XI) > Q(120)-L(50)-$\overline{\text{PEI}}$ (XII) > Acyl(25)-Q(160)-L(25)-PEI (XIV) > L(15)-$\overline{\text{PEI}}$ (IV) ≳ Acyl(30)-L(15)-PEI (XIII) > Q(155)-L(15)-$\overline{\text{PEI}}$ (IX) > $\overline{\text{PEI}}$ ≈ PEI. In spite of the structural difference between these polymers, the fluorescence intensity of TNS for 15% laurylated linear poly(ethylenimine) (IV) is similar to that for the 15% laurylated branched one (III). The intensity for polymer IV largely decreases by quaternization as indeed is observed for polymer IX. Interestingly, comparing the degree of alkylation for quaternized linear poly(ethylenimine) derivatives, the fluorescence intensity of TNS exhibits no significant relation with the degree of alkylation on the polymer, indicating that the binding of TNS to these polymers largely depends on the macromolecular environment due to the degree of alkylation and quaternization on the polymers.

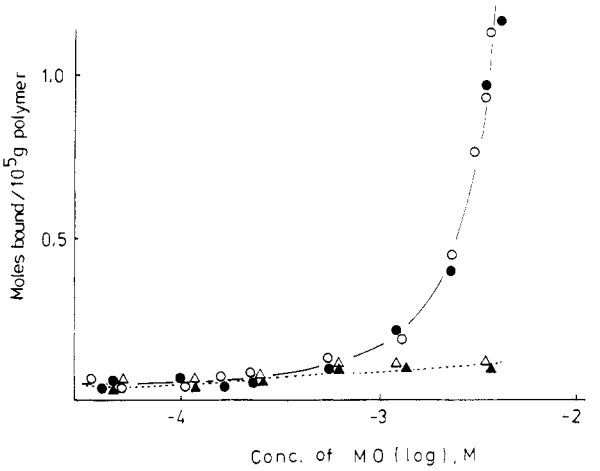


Figure 2. Extent of binding of methyl orange (MO) to poly(ethylenimine) derivatives in 0.01 M Bis-Tris buffer, pH 7.0 at 25 °C: (△) PEI; (▲) $\overline{\text{PEI}}$; (○) L(15)-PEI (III); (●) L(15)-$\overline{\text{PEI}}$ (IV).

Binding Behavior of a Sulfonated Dye by Poly(ethylenimine) Derivatives. The extent of the binding of methyl orange (MO) by poly(ethylenimine) derivatives as a function of the dye concentration is shown in Figure 2. As is apparent in Figure 2, the extent of binding of MO by branched PEI or linear PEI is insignificant in the concentration ranged used. In contrast, binding of MO by laurylated branched PEI (III) or laurylated linear $\overline{\text{PEI}}$ (IV) progressively increased with increasing dye concentration, where the difference in the binding behavior of the dye between linear PEI and branched PEI was not observed. This binding behavior of the dye by these laurylated polymers corresponds with the fluorescence behavior of TNS in these polymers (Table I). Thus, hydrophobic portion in these polymers provides a crucial effect on the binding behavior in both branched and linear PEI.

Stereoselective Hydrolysis of Chiral Esters by Modified Poly(ethylenimine)s with a Covalently-Linked Dipeptide Containing a Histidyl Residue. Figure 3 illustrates the variation of pseudo-first-order rate constants (k_{obsd}) and stereoselective ratio (L/D) for MOC-Phe *p*-nitrophenyl ester catalyzed by polymers XV, XVI, and XVII as a function of concentration of the polymer. In all of these experiments the concentrations were [polymer] ≫ [substrate]. The rate varied with increase of polymer concentration, but the stereoselective ratio was constant. The second-order rate constants (k_{cat}) were obtained from the linear slope in a graph of k_{obsd} against the polymer concentration. The k_{cat} values for various experiments are shown in Table II. For polymer XVII, k_{obsd} at first increased with concentration of polymer and then saturation appeared at a high concentration of polymer. The kinetics of hydrolysis were, therefore, analyzed in a format similar to that used in enzymatic catalysis as described in eq 1 and 2, where S represents substrate

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S{\cdot}C \xrightarrow{k_2} \text{product} + C \quad (1)$$

$$K_M = (k_{-1} + k_2)/k_1 \quad (2)$$

Table II
Second-Order Rate Constant (k_{cat}) and Stereoselective Ratio (L/D) for Hydrolysis[a] of an Amino Acid *p*-Nitrophenyl Ester by a Dipeptide-Containing Polymer

polymer	CBZ-Ala *p*-nitrophenyl ester $10^{-2}k_{cat}$, M^{-1} min^{-1}			MOC-Phe *p*-nitrophenyl ester $10^{-2}k_{cat}$, M^{-1} min^{-1}			CBZ-Phe *p*-nitrophenyl ester $10^{-2}k_{cat}$, M^{-1} min^{-1}		
	L	D	L/D	L	D	L/D	L	D	L/D
CBZ-L-Lue-L-His(7)-L(15)-PEI (XV)	3.93	2.08	1.9	8.76	3.04	2.9	9.78	2.75	3.6
CBZ-L-Leu-L-His(7)-L(15)-$\overline{\text{PEI}}$ (XVI)	0.59	0.49	1.2	1.63	0.71	2.3			
CBZ-L-Leu-L-His(7)-Q(160)-L(25)-PEI (XVII)	2.11	1.63	1.3	4.31	1.93	2.2			

[a] Reaction conditions: pH 7.30, 0.01 M Bis-Tris buffer, 25 °C; [polymer] = 0–5 × 10^{-3} residue molar, [substrate] = 2 × 10^{-5} M.

Table III
Kinetic Parameters for Hydrolysis[a] of the *p*-Nitrophenyl Ester of CBZ-Ala and MOC-Phe by Dipeptide-Containing Polymer XVII[b]

substr *p*-nitrophenyl ester	k_2, min^{-1}			$10^3K_M/n$, M			$10^{-2}nk_2/K_M$, M^{-1} min^{-1}		
	L	D	L/D	L	D	L/D	L	D	L/D
CBZ-Ala	2.67	2.13	1.3	6.43	6.48	1.0	4.15	3.29	1.3
MOC-Phe	5.71	2.86	2.0	7.61	8.34	0.91	7.50	3.43	2.2

[a] Reaction conditions: pH 7.30, 0.01 M Bis-Tris buffer, 25 °C; [polymer] = 0–10 × 10^{-3} residue molar, [substrate] = 2 × 10^{-5} M. [b] XVII: CBZ-L-Leu-L-His(7)-Q(160)-L(25)-PEI.

and C represents one catalytic site on the polymer.[19] The kinetic constants k_2 and K_M/n, where n is the number of catalytic sites on one molecule of polymer, can be evaluated when saturation kinetics are obtained, as has been observed for polymer XVII (Figure 3C). Values of these parameters are listed in Table III.

As is apparent in Table II, the highest stereoselectivity (L/D = 3.6) is observed for hydrolysis of CBZ-phe *p*-nitrophenyl ester by polymer XV. The variation in both hydrolysis rate and stereoselectivity between the polymers is fairly large, indicating that the rates and stereoselectivity are affected by the specific interaction of polymer and substrate. The stereoselectivity depends on the structure of the substrate. The stereoselective ratios for MOC-phe *p*-nitrophenyl ester, which is the isomer of CBZ-Ala *p*-nitrophenyl ester, were greater than those for CBZ-Ala *p*-nitrophenyl ester in all cases examined. Nevertheless, it is apparent that the polymer containing a L-histidine residue stereoselectively hyrolyzes the L enantiomer of the substrates, *p*-nitrophenyl esters of CBZ-Ala, CBZ-Phe, and MOC-phe in all cases. As is apparent in Table III, the larger part of the stereoselectivity in the second-order rate parameter nk_2/K_M is contributed by k_2 for both substrates, indicating that the stereoselective control is mainly determined by acyl transfer to the imidazole function at the active site of the optically active polymer. Thus, the amino acid residue next to the imidazole contributes to an increase in the stereoselectivity by increasing the rate of the hydrolysis of one enantiomer, perhaps by apolar interaction or hydrogen bonding.

The Effect of Metal Ion on Hydrolysis Rate and Stereoselectivity by Dipeptide-Containing Poly(ethylenimine)s. It had occurred to several investigators that metal complexes might accelerate the hydrolysis rate and the stereoselective preference in the hydrolysis of amino acid *p*-nitrophenyl esters.[8,12,13] Thus, we also examined the potential of this approach for dipeptide-containing polymers.

Table IV shows pseudo-first-order rate constants (k_{obsd}) and stereoselective ratio (D/L) for hydrolysis of MOC-Phe as catalyzed by these polymers in the presence of copper ions. As is apparent in Table IV, added copper ions showed increases in both the rate and the stereoselective

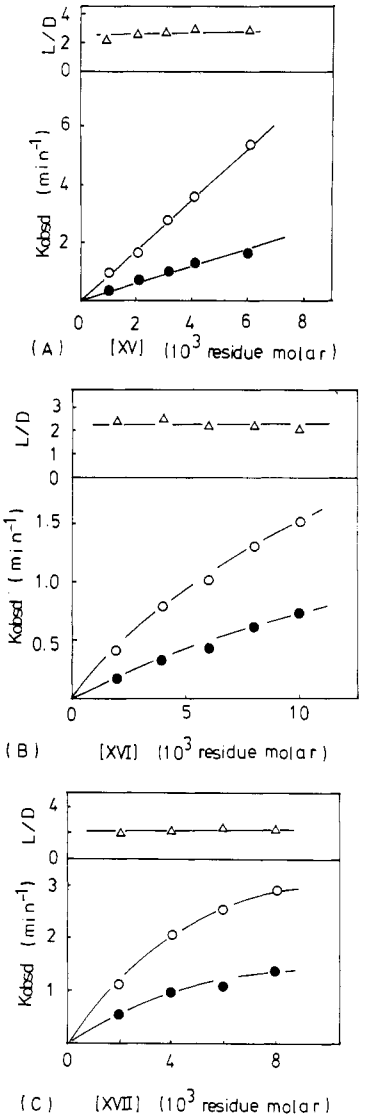


Figure 3. Variation of pseudo-first-order rate constant (k_{obsd}) and stereoselective ratio (L/D) for hydrolysis of D- and L-MOC-Phe *p*-nitrophenyl ester as a function of poly(ethylenimine) derivative concentration. [substrate] = 2 × 10^{-5} M; pH 7.3, 0.01 M Bis-Tris buffer, 25 °C; (●) D-form, (○) L-form of substrate; (A) CBZ-L-Leu-L-His(7)-L(15)-PEI (XV), (B) CBZ-L-Leu-L-His(7)-L(15)-$\overline{\text{PEI}}$ (XVI), (C) CBZ-L-Leu-L-His(7)-L(15)-PEI (XVII).

preference for the linear polymer XVI as described in a previous paper.[14c,d] In contrast, added ions showed decreases in both the rate and stereoselectivity for the branched polymer XV. It is clear that histidine and primary amino groups in the polymer play a role in creating sites with a high affinity for Cu^{2+}. For the linear poly(ethylenimine) XVI, which has no primary amino groups on the polymer, the histidine groups on the polymer may be responsible to the copper ion effect. In contrast, for

Table IV
Pseudo-First-Order Rate Constant (k_{obsd}) and Stereoselective Ratio (L/D) of Hydrolysis[a] of the p-Nitrophenyl Ester of MOC-Phe by PEI Derivatives

polymer[b]	substr p-nitrophenyl ester	no metal k_{obsd}, min^{-1}	Cu^{2+} [c] k_{obsd}, min^{-1}
CBZ-L-Leu-L-His(7)-L(15)-PEI (XV)	L	2.65	0.78
	D	1.05	0.56
	L/D	2.5	1.4
CBZ-L-Leu-L-His(7)-L(15)-$\overline{PEI}$ (XVI)	L	0.33	0.89
	D	0.22	0.51
	L/D	1.5	1.7

[a] Reaction conditions: pH 8.0, 0.01 M Tris buffer, 25 °C. [b] [polymer] = 2×10^{-3} residue molar; [substrate] = 2×10^{-5} M. [c] [$CuCl_2$] = 2×10^{-4} M; L-histidine content in the polymer:Cu^{2+} = 1:1.

the branched poly(ethylenimine) XV, which has primary amino and histidine groups on the polymer, both groups on the polymer may be responsible to the copper ion effect. Thus, the decreasing effect of copper ions on both the rate and the stereoselectivity for ester hydrolysis in the branched polymer may be due to the inhibition of the active primary amino groups on the branched polymer by added copper ions.

Stereoselective Hydrolysis of Chiral Esters by Catalysts in Modified Linear Poly(ethylenimine) Derivatives. Stereoselective hydrolysis of Moc-phe p-nitrophenyl ester was examined in the presence of modified poly(ethylenimine) with catalyst I and II. In all of the experiments the relative concentrations were [polymer] > [catalyst] > [substrate]. The pseudo-first-order rate constant (k_{obsd}) are shown in Table V. As is apparent, in the absence of catalyst, linear PEI shows a smaller k_{obsd} by a factor of about 15–40 than branched PEI, which has reactive primary amino groups on the polymer. Interestingly, after the removal of primary amino groups by acylation of the branched poly(ethylenimine) and the attachment of nonpolar groups by alkylation, linear PEI with nonpolar groups becomes more effective than the branched polymer in the hydrolysis of the ester with both of the catalysts. For example, the hydrolysis rate with both catalysts I and II is in the order L(15)-$\overline{PEI}$ (IV) > Acyl-(30)-L(15)-PEI (XIII). However, stereoselectivity (L/D) does not show any large differences between linear PEI and the branched polymer.

More extensive kinetic measurements were also made, therefore, with the linear PEI derivatives. The hydrolysis rates of MOC-Phe p-nitrophenyl ester catalyzed by CBZ-L-Leu-L-His (II) or Dec-L-histidine (I) in polymers IX–XII were measured under conditions where initial concentration of catalyst (C_0) ≫ initial concentration of substrate (S_0). A substantial difference in rate between the L-form and D-form of substrate was observed upon addition of catalyst II. The rate curves for each substrate showed saturation behavior. The kinetics of hydrolysis were analyzed by an extention of the kinetics equation used in enzymatic catalysis. If C represents catalyst and P_d represents polymer domain, then one may write[14b]

$$S + C{\cdot}P_d \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} S{\cdot}C{\cdot}P_d \xrightarrow{k'_2} \text{product} + C{\cdot}P_d \quad (3)$$

$$K'_M = (k'_{-1} + k'_2)/k'_1 \quad (4)$$

where $C{\cdot}P_d$ indicates the polymer–catalyst complex. For $C_0 \gg S_0$

$$k_{obsd} = k'_2 C_0/(K'_M + C_0) \quad (5)$$

The kinetic constants k'_2 and K'_M can be evaluated when saturation kinetics are observed for both substrate and complex, as indeed has been seen for $S{\cdot}C{\cdot}P_d$. Values of these parameters are listed in Table VI. As Table VI indicates, the highest stereoselectivity (L/D = 8.6) is observed for hydrolysis of MOC-Phe p-nitrophenyl ester by CBZ-L-Leu-L-His (II) in the presence of Q(146)-L(24)-$\overline{PEI}$ (X). The values of the dissociation constant (K'_M) decrease with increasing extent of nonpolar groups, reflecting increased binding affinity for the substrate. However, there is little change in the stereoselectivity for K'_M with in-

Table V
Pseudo-First-Order Rate Constant (k_{obsd}) and Stereoselective Ratio (L/D) for Hydrolysis[a] of the p-Nitrophenyl Ester of MOC-Phe by a Catalyst Containing a Histidyl Residue in the Presence of Polymer

polymer	no cat. $10k_{obsd}$, min^{-1}	cat. I $10k_{obsd}$, min^{-1}			cat. II $10k_{obsd}$, min^{-1}		
	L	L	D	L/D	L	D	L/D
$\overline{PEI}$	0.02	0.15	0.13	1.2			
PEI	0.43	0.60	0.55	1.1			
Acyl(30)-L(15)-PEI (XIII)	0.06	0.79	0.52	1.5	0.33	0.08	4.1
L(15)-$\overline{PEI}$ (IV)	0.41	32.5	14.1	2.3	24.6	3.10	7.9
Acyl(25)-Q(160)-L(25)-PEI (XIV)	0.40	29.5	14.0	2.1	29.1	4.10	7.1
Q(155)-L(15)-$\overline{PEI}$ (IX)	0.46	15.5	7.50	2.1	7.54	1.04	7.5

[a] Reaction conditions: pH 7.3, 0.01 M Bis-Tris buffer, 25 °C; [polymer] = 2×10^{-3} residue molar, [substrate] = 2×10^{-5} M, [catalyst] = 2×10^{-4} M.

Table VI
Kinetic Parameters for Hydrolysis[a] of the p-Nitrophenyl Ester of MOC-Phe by Dipeptide Catalyst CBZ-L-Leu-L-His (II) in the Presence of Quaternized Linear Polymers

polymer	k'_2, min^{-1}			$10^4 K'_M$, M			$10^{-3} k'_2/K'_M$, min^{-1} M^{-1}		
	L	D	L/D	L	D	L/D	L	D	L/D
Q(155)-L(15)-$\overline{PEI}$ (IX)	1.43	0.27	5.3	2.38	3.38	0.71	6.01	0.80	7.5
Q(146)-L(24)-$\overline{PEI}$ (X)	11.1	1.59	7.0	1.40	1.72	0.83	79.3	9.24	8.6
	2.94[b]	1.74[b]	1.7[b]	0.87[b]	1.30[b]	0.67[b]	33.8[b]	13.3[b]	2.5[b]
	10.3[c]	5.05[c]	2.1[c]	1.29[c]	1.26[c]	1.0[c]	79.8[c]	40.1[c]	2.0[c]
Q(133)-L(37)-$\overline{PEI}$ (XI)	7.14	1.23	5.8	1.34	1.69	0.77	53.3	7.28	7.3
Q(120)-L(50)-$\overline{PEI}$ (XII)	4.55	0.81	5.6	1.00	1.30	0.77	45.5	6.23	7.3
Acyl(25)-Q(160)-L(25)-PEI (XIV)	4.76	0.77	6.2	1.36	1.73	0.77	35.0	4.45	7.9

[a] Reaction condition: pH 7.30, 0.01 M Bis-Tris buffer, 25 °C, [polymer] = 2×10^{-3} residue molar, [substrate] = 2×10^{-5} M, [catalyst] = 0–2.7×10^{-4} M. [b] Substrate was p-nitrophenyl ester of CBZ-Ala. [c] Catalyst was Dec-L-His (I).

creasing extent of nonpolar groups on the polymer. Interestingly, the stereoselective ratio (L/D) for k'_2 and for k'_2/K'_M shows a maximum value for Q(146)-L(24)-$\overline{\text{PEI}}$ (X). The kinetic activation step, represented by k'_2, is crucial for an effect on the hydrolysis rate and the stereoselective ratio in these polymer domains.

Hydrolysis rate and stereoselectivity in quaternized branched PEI Acyl(25)-Q(160)-L(25)-PEI (XIV) is fairly similar to those in the Q(120)-L(50)-$\overline{\text{PEI}}$ (XII), which has the most alkylated linear $\overline{\text{PEI}}$, revealing that both polymers have a similar contribution from both k'_2 and K'_M in the macromolecular environment. Alternatively, comparing catalysts I and II in the same polymer domain (X), the stereoselectivity is more enhanced by catalyst II than by catalyst I in all cases. This trend is primarily a reflection of decreased rate, represented by k'_2, for the hydrolysis of the D-MOC-Phe with Dec-L-His (I) in comparison to the rate for the hydrolysis of the same substrate with CBZ-L-Leu-L-His (II).

In addition, stereoselectivity depends on the structure of the substrate (Table VI). In the same polymer domain (X) the stereoselective ratio for the MOC-phe *p*-nitrophenyl ester is greater than that for the CBZ-Ala *p*-nitrophenyl ester, which is an isomer of the former. As is apparent in Table VI, the larger part of the difference in the stereoselectivity for the substrate can be attributed to k'_2. It is possible, therefore, that a specific interaction of the imidazole group in the catalyst with substrate at the transition state in these polymer domains is crucial for an effect on the stereoselectivity in these hydrolytic reactions, as well as for the stereoselective hydrolysis by modified poly(ethylenimine)s with covalently-linked dipeptide (XV–XVII, Table II).

Conclusions

Stereoselective hydrolysis of chiral substrates as catalyzed by an optically active imidazole moiety in the hydrophobic environment of poly(ethylenimine) derivatives with both linear and branched backbones was examined, where the hydrophobic environment on the polymer provided a crucial role for the stereoselective hydrolysis reaction. A large rate enhancement and a stereoselective preference were exhibited in the hydrolysis catalyzed by L-histidine residues in the hydrophobic domain of macromolecules in which a significant difference of structural nature between linear and branched polymer was not observed.

Entrapped L-histidine moieties in the macromolecular domains had a larger catalytic ability for stereoselective preference than the covalently linked L-histidine moieties on the macromolecules, indicating that the entrapped histidine catalyst in the hydrophobic macromolecular domains can be found in a better location for stereoselective hydrolysis than the covalently linked catalyst on the macromolecules. Furthermore, the dipeptide catalyst containing L-histidine in the macromolecular domains had more pronounced stereoselectivity than the corresponding monopeptide catalyst,[14] where the effect of the substrate structure greatly influenced much both the rate constant and stereoselective preference in the hydrolysis.

It will be of interest to see if this stereoselectivity is manifested with other substrates and whether it can be further enhanced by alternative active catalysts on the modified macromolecules.

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Registry No. I, 55258-10-1; II, 79778-48-6; TNS-Na, 53313-85-2; CBZ-Phe-OC_6H_4-*p*-NO_2, 2578-84-9; CBZ-Ala-OC_6H_4-*p*-NO_2, 1168-87-2; MOC-Phe-OC_6H_4-*p*-NO_2, 1456-03-7; $CuCl_2$, 7447-39-4; methyl orange, 547-58-0.

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