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STEREOSELECTIVE HYDROLYSIS OF AMINO ACID ESTERS IN MODIFIED LINEAR POLY(ETHYLENIMINE) DOMAINS

Yoshiharu KIMURA, Shinichi KANDA, Mamoru NANGO,*
Yasuji IHARA,† Joichi KOGA, and Nobuhiko KUROKI
Department of Applied Chemistry, College of Engineering,
University of Osaka Prefecture, Sakai, Osaka 591
†Yamaguchi Women's University, 3-2-1 Sakurabatake, Yamaguchi 753

A large rate enhancement and a stereoselective preference are exhibited in the hydrolysis catalysed by N-decanoyl-L-histidine(2a) and a dipeptide containing L-histidyl residue(2b) in the domain of linear poly(ethylenimine) derivatives.

Poly(ethylenimine) is highly branched polymer and has several kinds of amino groups which occasionally make the behavior of catalysis puzzling. In contrast, linear poly(ethylenimine) has only secondary amino groups on the polymer chain and thus, provides simpler local macromolecular environment in aqueous solution than the branched one. This paper describes the results of catalytic activities of N-decanoyl-L-histidine and of a dipeptide containing a L-histidyl residue in the presence of linear poly(ethylenimine) derivatives to elucidate the effect of the polymer structure. A comparison of catalytic effect on both rate constants and stereoselectivity of the catalysts in the polymer domains provides some insights into the influence of local macromolecular environment on the behavior of the imidazole nucleophile in the polymer domains. There are several studies of stereoselective hydrolyses with high stereoselectivity in micellar and vesicular systems,[1-3] but no reports of high stereoselectivity in hydrolysis with macromolecular system.

Three types of poly(ethylenimine)(PEI), linear PEI(M.W.=50000, represented as PEI)(1a), branched PEI_{18}(M.W.=1800)(1b) and branched PEI_{600}(M.W.=50000)(1c) are used. Polymers(1d-1n) were prepared by the following sequence of steps. Lauryl ($C_{12}H_{25}$) groups were attached to PEI by alkylation of the polymer with lauryl bromide in absolute ethanol.[4,5] Quaternized PEI derivatives were prepared as described in the previous paper.[4,5] Any residual primary and secondary amino groups on the polymer were blocked with acetyl moieties(C_2H_3O) by treatment with acetic anhydride. Catalysts(2a - 2b) and substrates(3a - 3b) were obtained elsewhere.[2]

Stereoselective hydrolysis of MOC-Phe p-nitrophenyl ester in modified poly-(ethylenimine) domains with the presence of catalyst(2) was examined. In all of these experments the concentrations were [polymer] > [catalyst] > [substrate]. The pseudo-first-order rate constant(k_{obsd}) are shown in Table 1. As is apparent

[Polymer] (1a) PEI (1b) PEI_{18} (1c) PEI_{600}

(1d) Laurylated(L)-PEI $(C_2H_4N)_m$ $(C_{12}H_{25})_{0.15m}$ $m \approx 1400$

(1e) L-PEI_{18} $(C_2H_4N)_m$ $(C_{12}H_{25})_{0.15m}$ $m \approx 40$

(1f) L-PEI_{600} $(C_2H_4N)_m$ $(C_{12}H_{25})_{0.15m}$ $m \approx 1400$

(1g) Acetylated(Acyl)-L-PEI_{18} $(C_2H_4N)_m$ $(C_{12}H_{25})_{0.15m}$ $(C_2H_3O)_{0.35m}$ $m \approx 40$

(1h) Acyl-L-PEI_{600} $(C_2H_4N)_m$ $(C_{12}H_{25})_{0.15m}$ $(C_2H_3O)_{0.30m}$ $m \approx 1400$

(1i) Quaternized(Q)-L-PEI_{600} $(C_2H_4N)_m$ $(C_{12}H_{25})_{0.25m}$ $(C_2H_5N)_{0.10m}$ $(CH_3)_{1.60m}$ Cl_m

(1j) Acyl-Q-L-PEI_{600} $m \approx 1400$

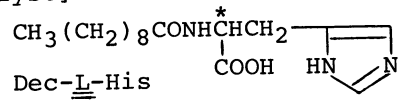
$(C_2H_4N)_m$ $(C_{12}H_{25})_{0.25m}$ $(C_2H_4N)_{0.10m}$ $(CH_3)_{1.60m}$ $(C_2H_3O)_{0.25m}$ Cl_m $m \approx 1400$

* Q-L(x)-PEI x : quaternized degrees of PEI (%).

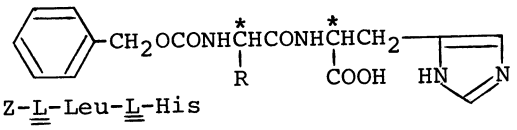
$(C_2H_4N)_m$ $(C_{12}H_{25})_{(x/100)m}$ $(CH_3)_{(1.7-x/100)m}Cl_m$ $m \approx 1400$

(1k) Q-L(15)-PEI (1l) Q-L(24)-PEI (1m) Q-L(37)-PEI (1n) Q-L(50)-PEI

[Catalyst]



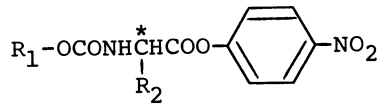
(2a) Dec-L-His



(2b) CBZ-L-Leu-L-His

R = $CH_2CH(CH_3)_2$

[Substrate]



ester	R_1	R_2
(3a) CBZ-Ala	$CH_2C_6H_5$	CH_3
(3b) MOC-Phe	CH_3	$CH_2C_6H_5$

in Table 1, in the absence of catalyst, linear PEI has smaller k_{obsd} by a factor of about 15-40 than PEI_{18} or branched PEI, which has reactive primary amino groups on the polymer. Interestingly, after the removal of primary amino groups by acylation of PEI derivative and the attachment of non-polar groups by alkylation to PEI derivatives, linear PEI with non-polar groups on the polymer provides suitable domains for the hydrolysis of the ester with all of the catalysts. For example, the hydrolysis rate for both catalysts 2a and 2b is in the order L-PEI > Acyl-L-PEI $\geqslant$ Acyl-L-PEI_{18}. However, stereoselectivity(L/D) does not show any differences between linear PEI and branched one. It becomes worthwhile , therefore, to extend further study of linear PEI with non-polar groups.

More extensive kinetic measurements were also made with the linear PEI derivatives. The hydrolysis rate of MOC-Phe p-nitrophenyl ester catalyzed by CBZ-L-Leu-L-His in polymer(1k-1n) was measured under conditions where initial concentration of catalyst(C_o) $\gg$ initial concentration of substrate(S_o). A substantial difference in rate between the L-form and D-form is observed upon addition of catalyst(2b). Both the rate curves show saturation behavior. The kinetics of hydrolysis were analysed by an extention of equation used in enzymatic catalysis.[4,5] If C represents catalyst and Pd represents polymer domain, then, one may write the following scheme.

Table 1. Pseudo-First-Order Rate Constant(k_{obsd}) and Stereoselective Ratio(L/D) for Hydrolysis[a] of p-Nitrophenyl Ester of MOC-Phe by Catalyst Containing a L-Histidyl Residue in the Presence of Polymer

Polymer	No Catalyst k_{obsd} X10 min^{-1}	Dec-L-His (2a) k_{obsd} X10 min^{-1}			CBZ-L-Leu-L-His (2b) k_{obsd} X10 min^{-1}		
	L	L	D	L/D	L	D	L/D
PEI(1a)	0.02	0.15	0.13	1.2			
PEI_{18}(1b)	0.30	0.68	0.61	1.1			
PEI_{600}(1c)	0.43	0.60	0.55	1.1			
Acyl-L-PEI_{18}(1g)	0.06	0.64	0.39	1.6	0.28	0.09	3.1
Acyl-L-PEI_{600} (1h)	0.06	0.79	0.52	1.5	0.33	0.08	4.1
L-PEI(1d)	0.41	32.5	14.1	2.3	24.6	3.1	7.9
Acyl-Q-L(25)-PEI_{600}(1j)	0.40	29.5	14.0	2.1	29.1	4.1	7.1
Q-L(15)-PEI(1k)	0.46	15.5	7.5	2.1	7.5	1.0	7.5

a) Reaction condition : pH 7.30, 0.01 M Bis-tris buffer, 25 °C; [Polymer]=$2X10^{-3}$ residue molar, [Substrate]=$2X10^{-5}$ M, [Catalyst]= $2X10^{-4}$ M. (1 M = 1 mol dm^{-3})

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

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

For $C_o \gg S_o$ $\quad k_{obsd} = k_2\cdot C_o / (K_M + C_o)$ (3)

where C·Pd indicates the polymer-catalyst complex. 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 observed for the complex(C·Pd). Values of these parameters are listed in Table 2. As Table 2 indicates, the highset stereoselectivity(L/D=8.6) is observed for hydrolysis of MOC-Phe p-nitrophenyl ester by CBZ-L-Leu-L-His in the presence of Q-L(25)-PEI(I_1). The values of the dissociation constant(K_M) decrease with increasing extent of non-polar groups, indicating that this trend is primarily a reflection of increased binding ability of substrate. The stereoselectivity for K_M with increasing extent of non-polar groups on the polymer, however, is not much observed. Interestingly, the rate constant(k_2), a second-order rate constant(k_2/K_M) and stereoselective ratio(L/D) in terms of k_2 and k_2/K_M show a maximum value for Q-L(25)-PEI(I_1), representing that kinetic activation step, represented by k_2, are crucial for an effect on the hydrolysis rate and the stereoselective ratio in the terms of these polymer domains.

Table 2. Kinetic Parameters for Hydrolysis[a] of p-Nitrophenyl Ester of MOC-Phe(3b) by Dipeptide Catalyst in the Presence of Quaternized Linear Polymer(PEI)

Polymer	CBZ-L-Leu-L-His (2b) + MOC-Phe (3b) ester								
	k_2 / min^{-1}			K_M / $X10^4$ M			k_2/K_M / $X10^{-3} min^{-1} M^{-1}$		
	L	D	L/D	L	D	(D/L)	L	D	L/D
Q-L(15)-PEI(l_k)	1.43	0.27	5.3	2.38	3.38	1.4	6.01	0.80	7.5
Q-L(24)-PEI(l_l)	11.1	1.59	7.0	1.40	1.72	1.2	79.3	9.24	8.6
	2.94[b]	1.74[b]	1.7[b]	0.87[b]	1.30[b]	1.5[b]	33.8[b]	13.3[b]	2.5[b]
Q-L(37)-PEI(l_m)	7.14	1.23	5.8	1.34	1.69	1.3	53.3	7.28	7.3
Q-L(50)-PEI(l_n)	4.55	0.81	5.6	1.00	1.30	1.3	45.5	6.23	7.3

a) Reaction condition : pH 7.30, 0.01 M Bis-tris buffer, 25 °C, [Polymer]=$2X10^{-3}$ residue molar, [Substrate]=$2X10^{-5}$ M, [Catalyst]=0-$2.7X10^{-4}$ M. b) CBZ-Ala(3a).

In addition, stereoselectivity depends on the structure of the substrate (Table 2). The stereoselectivity ratio for MOC-Phe p-nitrophenyl ester were greater than that for CBZ-Ala p-nitrophenyl ester, which is isomer of MOC-Phe p-nitrophenyl ester, in the same polymer domain(l_l). As is apparent in Table 2, the larger part of the difference in the stereoselectivity for the substrate is attributed to k_2. It is possible, therefore, that specific interaction of the imidazole group in the catalyst with substrate at the transition state in these polymer domains are crucial for an effect on the stereoselectivity in these hydrolytic reaction.

References

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