CHEMISTRY LETTERS, pp. 429 - 432, 1984.

STEREOSELECTIVE HYDROLYSIS OF AMINO ACID ESTERS BY MODIFIED POLY(ETHYLENIMINE)S WITH COVALENTLY-LINKED DIPEPTIDE CONTAINING A HISTIDYL RESIDUE

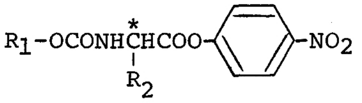
Yoshiharu KIMURA, Mamoru NANGO,* Yasuji IHARA,†
and Nobuhiko KUROKI
Department of Applied Chemistry, College of Engineering,
University of Osaka Prefecture, Sakai, Osaka 591
†Yamaguchi Women's University, 3-2-1 Sakurabatake, Yamaguchi 753

Stereoselective hydrolyses of chiral substrates were examined in poly(ethylenimine) derivatives with optically active groups. A high stereoselective effect, $k_L/k_D=3.6$, is observed. The effect of the substrate structure influenced both the rate constant and the stereoselective ratio in the hydrolyses by poly(ethylenimine) derivatives.

In the previous articles,$^{1,2)}$ we first observed stereoselective hydrolysis of amino acid p-nitrophenyl esters(**1**) by poly(ethylenimine)s with optically active L-histidine moieties. Similar workers have been reported with imidazole-containing polymer.$^{3-5)}$ Following this direction, this paper described a high stereoselective effect in the hydrolysis of the chiral substrates by modified poly(ethylenimine)s derivatives with covalently-linked dipeptide derivative.

Polymer(**2**) was prepared by the following sequence of steps.$^{1,2)}$ Lauryl ($C_{12}H_{25}$) groups were attached to poly(ethylenimine) by alkylation of the polymer with lauryl bromide in absolute ethanol. The dipeptide-containing polymer was produced from lauryl poly(ethylenimine) or quaternized lauryl poly(ethylenimine) in water containing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide by coupling of CBZ-L-Leu-L-His to attach (L-$C_{20}H_{25}N_4O_4$) groups by amide linkages to the macromolecule. Quaternized poly(ethylenimine) derivative was prepared as described in the previous paper.$^{2)}$ Integration of the peak in the ^{1}H NMR spectra of these

[Substrate]



ester	R_1	R_2
(**1a**) CBZ-Ala	$CH_2C_6H_5$	CH_3
(**1b**) MOC-Phe	CH_3	$CH_2C_6H_5$
(**1c**) CBZ-Phe	$CH_2C_6H_5$	$CH_2C_6H_5$

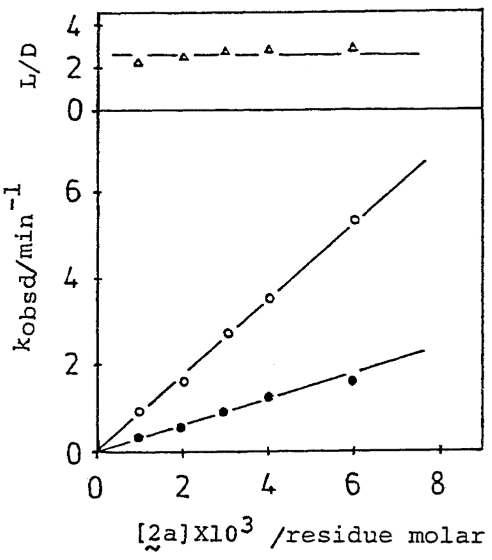


Fig. 1. Variation of pseudo-first-order rate constant(k_{obsd}) and stereoselective ratio(L/D) for hydrolysis of MOC-Phe p-nitrophenyl ester as a function of polymer(**2a**) concentration. [Substrate]=2X10^{-5} M; pH 7.30, 0.01 M Bis-tris buffer, 25 °C. (●), D-form; (○), L-form of substrate.

[Polymer]

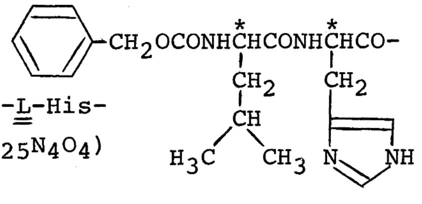
(**2a**) CBZ-L-Leu-L-His-L-PEI m≈1400

$(C_2H_4N)_m$ $(C_{12}H_{25})_{0.10m}$ $(L\text{-}C_{20}H_{25}N_4O_4)_{0.07m}$

(**2b**) CBZ-L-Leu-L-His-Q-L-PEI m≈1400

$(C_2H_4N)_m$ $(C_{12}H_{25})_{0.25m}$ $(L\text{-}C_{20}H_{25}N_4O_4)_{0.07m}$ $(C_2H_4N)_{0.07m}$ $(CH_3)_{1.60m}$ Cl_m

CBZ-L-Leu-L-His-
($L\text{-}C_{20}H_{25}N_4O_4$)



polymers dissolved in D_2O indicated the stoichiometric compositions presented in formular **2a** and **2b**. Reaction rates were followed at pH 7.3 and 25 °C by spectrphotometric assey(at 400 nm) of p-nitrophenolate ion released on hydrolysis of the enantiomeric ester(**1**). Compounds(**1**) were used as described elsewhere.$^{1)}$

Figure 1 illustrates the variation of pseudo-first-order rate constant(k_{obsd}) and stereoselective ratio(L/D) for MOC-Phe p-nitrophenyl ester as a function of concentration of **2**. In all of these experiments the concentrations were [polymer]≫[substrate]. The rate varied with increases 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 the k_{obsd} against the polymer concentration. The k_{cat} values for various experiments are shown in Table 1. For the polymer **2b**, k_{obsd} at first increased with concentration of the polymer and then saturation behavior appeared at high concentration of the polymer. The kinetics of hydrolysis were then analyzed in a format similar to that used in enzymatic catalysis as described in Eqs.1 and 2.$^{1,2)}$ If S represents substrate and C represents one catalytic site on the polymer, then one may write the following scheme:

Table 1. Second-Order Rate Constant(k_{cat}) and Stereoselective Ratio(L/D) for Hydrolysis[a)] of p-Nitrophenyl Ester of CBZ-Ala(1a), MOC-Phe(1b), and CBZ-Phe(1c) by Dipeptide-Containing Polymer

Polymer	CBZ-Ala (1a) k_{cat} $\times10^{-2}$ M^{-1} min^{-1}			MOC-Phe (1b) k_{cat} $\times10^{-2}$ M^{-1} min^{-1}			CBZ-Phe (1c) k_{cat} $\times10^{-2}$ M^{-1} min^{-1}		
	L	D	L/D	L	D	L/D	L	D	L/D
CBZ-L-Leu-L-His-L-PEI (2a)	3.93	2.08	1.9	8.76	3.04	2.9	9.78	2.75	3.6
CBZ-L-Leu-L-His-Q-L-PEI (2b)	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]= 0-5X10^{-3} residue molar, [Substrate]=2X10^{-5} M. (1 M = 1 mol dm^{-3})

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S\,C \xrightarrow{k_2} \text{Product} + C \tag{1}$$

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

The kinetic constants k_2 and K_M/n, where n is the number of catalytic site on molecule of polymer, can be evaluated when saturation kinetics are obtained, as indeed has been observed for the polymer 2b(Fig.1). Values of these parameters are listed in Table 2.

As is apparent in Table 1, the highest stereoselectivity(L/D=3.6) is observed for hydrolysis of CBZ-Phe p-nitrophenyl ester by the polymer 2a. The variation in both hydrolysis rate and stereoselectivity among 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 isomer of CBZ-Ala p-nitrophenyl ester, were greater than those for CBZ-Ala p-nitrophenyl ester in all examined. Nevertheless, it is apparent that the polymer containing a L-histidine residue stereoselectively hydrolyzes 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 2, 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 mainly determined by acyl transfer to the imidazole function at the active site of the optically

Table 2. Kinetic Parameters for Hydrolysis$^{a)}$ of p-Nitrophenyl Ester of CBZ-Ala(**1a**) and MOC-Phe(**1b**) by Dipeptide-Containing Polymer(**2b**)

Substrate	k_2 min^{-1}			K_M/n $\times 10^3$ M			nk_2/K_M $\times 10^{-2}$ M^{-1} min^{-1}		
	L	D	L/D	L	D	D/L	L	D	L/D
CBZ-Ala(**1a**)	2.67	2.13	1.3	6.43	6.48	1.0	4.15	3.29	1.3
MOC-Phe(**1b**)	5.71	2.86	2.0	7.61	8.34	1.1	7.50	3.43	2.2

a) Reaction conditions : pH 7.30, 0.01 M Bis-tris buffer, 25 °C; [Polymer]=0-10$\times 10^{-3}$ residue molar, [Substrate]=2$\times 10^{-5}$ M.

active polymer. Thus, 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.

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

References

1) M. Nango, H.Kozuka, Y. Kimura, N. Kuroki, Y. Ihara, and I. M. Klotz, J. Polym. Sci., Polym. Lett. Ed., **18**, 647 (1980).

2) Y. Kimura, M. Nango, N. Kuroki, Y. Ihara, and I. M. Klotz, J. Polym. Sci., Polym. Chem. Ed., in press.

3) J. M. van der Eijk, R. J. M. Nolte, V. E. M. Richters, and W. Drenth, Recl. Trav. Chem. Pays-Bas, **100**, 222 (1981).

4) I. Cho, J-S Shin, Makromol. Chem., **183**, 2041 (1982); **184**, 147 (1983).

5) N. Sassky, M.Reix, M-O Sepulchre, and J-P Guetté, Makromol. Chem., **184**, 17 (1983).

(Received December 6, 1983)