Significant Enantioselectivity in Alanine Ester Hydrolysis Catalyzed by Imidazole Attached β-Cyclodextrins

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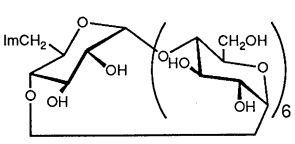
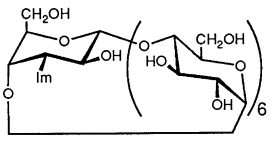
Imidazole-appended β-cyclodextrins, 3-deoxy-3-imidazolyl-β-cyclodextrin (3Im-βCyD) and 6-deoxy-6-imidazolyl-β-cyclodextrin (6Im-βCyD) were synthesized as substrate selective enzyme mimics. 6Im-βCyD shows marked enantioselectivity in the hydrolysis of Boc-alanine *p*-nitrophenyl ester rather than 3Im-βCyD. This selectivity could be interpreted by transition stability theory.

Since Bender reported enzyme modeling with cyclodextrins (CyD),[1] various kinds of enzyme mimics using modified CyDs have been constructed. Among them, imidazole group attached CyDs have been shown to act as catalysts for ester hydrolysis in neutral aqueous solution.[2] Especially, imidazole attached dimethyl-βCyD (Im-DMβCyD) showed an excellent rate enhancement in the hydrolysis of *p*-nitrophenyl acetate with a larger k_{cat} than that of chymotrypsin,[3] but it did not show significant enantioselectivity in the hydrolysis in spite of remarkable substrate specificity in binding.[4] This behavior of this enzyme mimic contrasts to that of many enzymes, for example, as shown by a specific esterase in bovine rod outer segment membranes, which shows stereoselective ester hydrolysis rather than stereospecific one.[5] The authors wish to report here, it is possible that an appropriately designed enzyme mimic shows remarkable enantioselective catalytic reaction (stereoselectivity is high)[5] with negligible enantioselectivity on its binding (stereospecificity is low)[5].

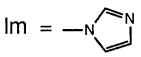
In order to realize high degree of stereoselectivity in ester hydrolysis, the authors have synthesized imidazole appended βCyD, 6-deoxy-6-imidazolyl-β-cyclodextrin (6Im-βCyD) and 3-deoxy-3-imidazolyl-β-cyclodextrin (3Im-βCyD), and examined their enantioselectivity in the hydrolysis of Boc-**D**- and Boc-**L**-alanine esters.



6Im-βCyD



3Im-βCyD



6Im-βCyD was prepared by the reaction of imidazole and 6-(*p*-toluenesulfonyl)-β-cyclodextrin.[6] While 3Im-βCyD was prepared by the reaction of imidazole and 2-(*p*-toluenesulfonyl)-β-cyclodextrin.[7,8] The concentration of substrate was varied from 5 x 10^{-5} to 5 x 10^{-4} M. The initial rates V_0 of the ester hydrolysis reaction of 3Im-βCyD or 6Im-βCyD were measured in pH 7.25 phosphate buffer solution (5 x 10^{-2} M) at 25 °C. In the absence of the catalyst, the rate constants of the hydrolysis for Boc-**D**-alanine *p*-nitrophenyl ester (Boc-**D**-Ala-*p*Np) and Boc-**L**-alanine *p*-nitrophenyl ester (Boc-**L**-Ala-*p*Np) were 1.58 x 10^{-4} and 1.62 x 10^{-4} s^{-1}. For the sake of keeping an excess condition of substrate, in any case, the concentrations of 3Im-βCyD and 6Im-βCyD were fixed at 2.5 x 10^{-5} M. In both cases, the plots of V_0 *vs.* [substrate] fit with theoretical curves of Michaelis-Menten equation finely (only the data of 6Im-βCyD is shown in Figure 1). These results suggest that the hydrolysis reaction catalyzed by 6Im-βCyD and 3Im-βCyD proceeds based on Michaelis-Menten mechanism. Apparently, the effects of 6Im-βCyD and 3Im-βCyD on the hydrolysis are more remarkable for Boc-**D**-Ala-*p*Np than for Boc-**L**-Ala-*p*Np. Kinetic parameters, k_{cat} and K_m, of the hydrolysis are summarized in Table 1.

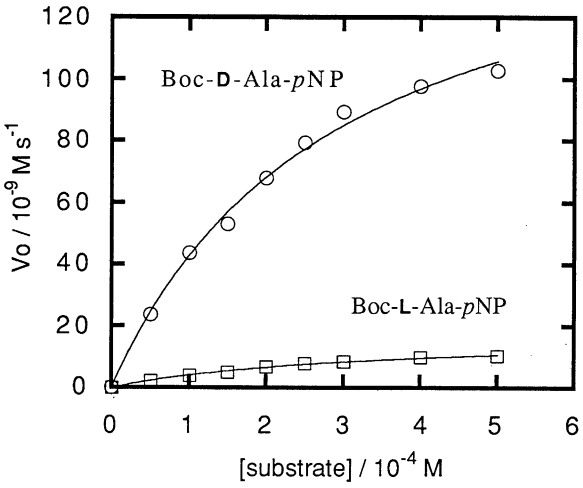


Figure 1. Hydrolysis of Boc-(**D** or **L**)-alanine *p*-nitrophenyl ester catalyzed by 6Im-βCyD in pH = 7.25 phosphate buffer solution (5 x 10^{-2} M). [6Im-βCyD] = 2.5 x 10^{-5} M.
The solid lines were obtained by least square curve fitting with Michaelis-Menten equation.

The stereoselectivity of 6Im-βCyD is more significant than that of 3Im-βCyD. The k_{cat} and K_m values of 6Im-βCyD for Boc-**D**-Ala-*p*Np were 66.8 x 10^{-4} s^{-1} and 2.89 x 10^{-4} M, while the values for Boc-**L**-Ala-*p*Np were 7.25 x 10^{-4} s^{-1} and 3.54 x 10^{-4} M, respectively. There is not significant difference in K_m for the substrates, but k_{cat} value for Boc-**D**-Ala-*p*Np is 9.2 times as large as that for Boc-**L**-Ala-*p*Np. These results strongly indicate that the catalytic effect of 6Im-βCyD is stereoselective rather than stereospecific. It implies that the reaction is facilitated particularly for the D isomer in the complex. Although we have no means to know about the exact structural feature of the complex. One approach to interpret the reactivity and selectivity of the catalyst mediated reaction is using the parameter of transition state stability (K_{TS}).[9] K_{TS} is the apparent dissociation constant of the complex of the substrate in the transition state and catalyst. K_{TS} can be obtained with measurable terms of k_{un} and k_{cat} / Km ($K_{TS} = k_{un} / (k_{cat} / Km)$). The K_{TS} values for Boc-**D**-

Table 1. Kinetic parameters for the hydrolysis of Boc-(D or L)-alanine *p*-nitrophenyl esters catalyzed by Im-βCyDs at 25 °C in pH = 7.25 phosphate buffer solution

catalyst	substrate	k_{cat} / 10^{-4} s^{-1}	K_m / 10^{-4} M	k_{cat} / K_{un}	k_{cat} / K_m 10^{-4} s^{-1}	K_{TS} / 10^{-5} M
6Im-βCyD	Boc-D-Ala-*p*Np	66.8	2.89	42.3	23.5	0.672
	Boc-L-Ala-*p*Np	7.25	3.54	4.48	2.05	7.90
3Im-βCyD	Boc-D-Ala-*p*Np	19.4	5.53	12.0	3.43	4.61
	Boc-L-Ala-*p*Np	3.43	2.30	2.12	1.50	10.8

Ala- and Boc-L-Ala-*p*Np were 0.672 x 10^{-5} M and 7.90 x 10^{-5} M, respectively. This result suggested that the transition state stability in the complex of Boc-D-Ala-*p*Np and 6Im-βCyD is superior to that of Boc-L-Ala-*p*Np and 6Im-βCyD. This may reflect the difference of the mutual orientation of ester and imidazole moiety in the complexes.

On the other hand, 3Im-βCyD showed modest stereoselectivity with the k_{cat} value of the Boc-D-Ala-*p*Np is 5.7 times as large as that of Boc-L-Ala-*p*Np while the Km value of the former is 2.4 times as large as that of the latter one. The profitability shown by these parameters are opposite, i.e. this reaction proceeds stereoselectively. The K_{TS} values for Boc-D-Ala-*p*Np and Boc-L-Ala-*p*Np are 4.61 x 10^{-5} M and 10.8 x 10^{-5} M, respectively. Then, the stability of the complex in the transition state (K_{TS}) is more important than that of initial one (Km).

6Im-βCyD and 3Im-βCyD showed acceleration for the Boc-alanine ester hydrolysis with striking stereoselectivity rather than stereospecificity. Stereoselectivity of 3Im-βCyD was inferior to that of 6Im-βCyD in spite of the asymmetrical shape of cavity of 3Im-βCyD.[10] These results suggest that the shape of host molecules is not so important in determine the selectivity at least in the present system. Namely, the orientation or the flexibility of the imidazole moiety of 6Im-βCyD for Boc-D-Ala-*p*Np is better than that of 3Im-βCyD for the ester carbonyl to be attached. All these results demonstrate that in our enzyme mimics, catalytic reaction proceeds with chiral discrimination based on stereoselectivity rather than stereospecificity.

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References and Notes

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6 Anal. Found: C, 44.46; H, 6.17; N, 2.38%. Calcd for $C_{45}H_{71}O_{34}N_2 \cdot 3H_2O$: C, 44.62; H, 6.34; N, 2.33%. ^{1}H-NMR (D_2O, 500 MHz) : d = 3.14 (dd, 1H), 3.29 (d, 1H), 3.38 (t, 1H), 3.51 ~ 3.98 (m, majority), 4.04 (t, 1H), 4.26 (dd, 1H), 4.54 (d, 1H), 5.00 (d, 2H), 5.05 (broad, 1H), 5.07 (d, 1H), 7.02 (s, 1H, imidazole), 7.19 (s, 1H, imidazole), 7.71 (s, 1H, imidazole).

7 2-(*p*-Toluenesulfonyl)-β-cyclodextrin is converted into 3-deoxy-3-imidazolyl-β-cyclodextrin (3Im-βCyD) via 2,3-mannoepoxide-β-cyclodextrin. A. Ueno, and R. Breslow, *Tetrahedron Lett..*, **23**, 3451 (1982); R. Breslow, and A.W. Czernik, *J. Am. Chem. Soc.*, **105**, 1390 (1983); R. Breslow, A.W. Czernik, M. Lauer, R. Leppkes, and S. Zimmerman, *J. Am. Chem. Soc.*, **108**, 1969 (1986).

8 Anal. Found: C, 44.75; H, 6.25; N, 2.41%. Calcd for $C_{45}H_{71}O_{34}N_2 \cdot H_2O$: C, 44.93; H, 6.20; N, 2.33%. ^{1}H-NMR (D_2O, 500 MHz) : d = 3.44 (d,1H), 3.46 (d,1H), 3.52 ~ 4.01 (m, majority), 4.08 (broad, 1H), 4.30 (dd, 1H), 4.43 (m, 1H), 4.56 (d, 1H), 4.58 (d, 1H), 4.83 (d, 1H), 5.00 (d, 1H), 5.01 (d, 1H), 5.04 (m, 2H), 5.14 (d, 1H), 5.16 (d, 1H), 7.05 (s, 1H, imidazole), 7.35 (s, 1H, imidazole), 7.89 (s, 1H, imidazole).

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