A New Feature of Bifunctional Catalysis. Cyclodextrins Bearing Two Imidazole Moieties as Hydrolysis Enzyme Model

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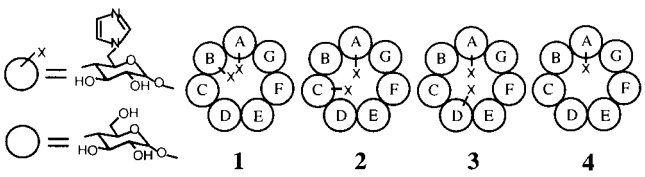
β-Cyclodextrin derivatives bearing two imidazole rings at AC, AD glucose residues (AC and AD isomers) exhibit much higher catalytic activity in hydrolysis of *p*-nitrophenyl acetate than AB isomer, showing a bell-shaped pH profile with a peak at pH 7.2. They accelerate the hydrolysis of Boc-alanine-*p*-nitrophenyl ester with remarkable D-substrate preference.

Cyclodextrins (CDs) form inclusion complexes with various organic guest species in aqueous solution and have been extensively studied as enzyme models.[1] In many cases, the catalytic activity of CDs themselves are low and one or two catalytic functional groups are attached to CDs to enhance their catalytic abilities.[2] The important feature of this chemistry is that the unique approach to examine catalysis mechanism is possible by using geometrical isomers with functional groups attached at different positions of CD framework such as AB, AC, and AD glucose units in β-CD. Breslow observed that AB isomer of two imidazole-appended β-CD is more active for hydrolysis of a cyclic phosphate as ribonuclease model[3] than AC and AD isomers while the AD isomer is more efficient than others as the catalysis for enolization.[4] On the other hand, we have attempted to construct effective catalysis for hydrolysis of ester substrates by using β-CD bearing one imidazole ring.[5] We wish to report here that simple ester hydrolysis can also be affected differently by the isomeric β-CD derivatives bearing two imidazole rings.



We have prepared β-CD derivatives bearing one imidazole(**4**) and two imidazoles at AB (**1**), AC (**2**), and AD (**3**) glucose residues.[6] Figure 1 shows pH profiles of k_{cat} of these CD derivatives in hydrolysis of *p*-nitrophenyl acetate (*p*NPA) in 0.02 M phosphate buffer. The isomers **2** and **3** exhibit bell-shaped profiles with a maximum of k_{cat} at pH 7.2. The presence of the bell-shape suggests that **2** and **3** work as bifunctional acid-base catalysis. On the other hand, the profile of isomer **1** is not simple and its k_{cat} values are much smaller than those of **2** and **3**. This result is quite different from that of the ribonuclease model reported by Breslow et al.,[3] and suggests that catalysis mechanism in ester hydrolysis is different from that of the

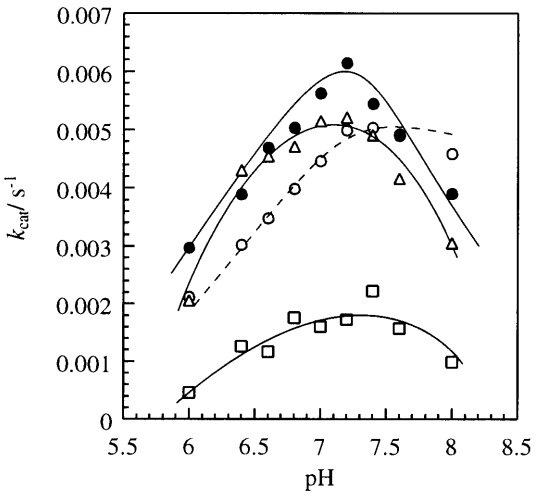
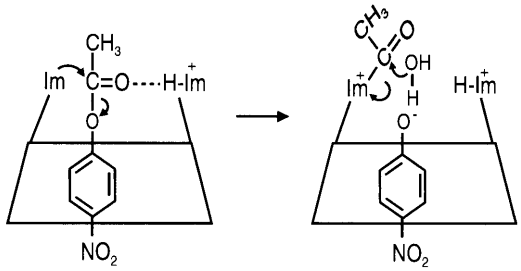


Figure 1. The pH profile of imidazole-appended β-CD derivatives (25μM), **1** (□), **2** (△), **3** (●), **4** (○) in 0.02 M phosphate buffer at 25 °C.

ribonuclease model. Figure 1 exhibits also the profile of **4**, which is not bell-shaped but the k_{cat} value is comparable to those of **2** and **3**. The data indicate that the acceleration achieved by cooperation of two imidazoles of **2** and **3** is not so remarkable. We calculated K_{TS},[7] which is dissociation constant of substrate-catalysis transition-state complex (Table 1). The values of K_{TS} show that **2** and **3** form more stabilized transition-state complex than **1**. The fact that **1** is not effective as catalyst suggests that two imidazole rings located at proximal position is difficult to exert the cooperative catalytic function. Orientation of the two imidazole rings may not be adequate when they interact with the carbonyl of *p*NPA included in the β-CD cavity. All these results suggest that two imidazoles and substrate carbonyl are required to be nearly in a line for the bifunctional acid-base catalysis (Scheme 1).



Scheme 1. Plausible reaction mechanism.

Table 1. Kinetic parameters of imidazole substituted β-CD (25 μM) in 0.02 M phosphate buffer at 25 °C

Catalysts [a]	Substrate [b]	k_{cat} /10^{-3} s^{-1}	K_m /10^{-3} M	k_{cat}/K_m /$s^{-1}M^{-1}$	k_{cat}/k_{un} [c]	K_{TS} [d] /10^{-6} M
	*p*NPA	2.2	3.9	0.6	468	7.8
1	Boc-L-Ala-ONP	6.1	1.1	5.5	222	5.0
	Boc-D-Ala-ONP	63	0.8	79	2410	0.3
	*p*NPA	5.2	3.0	1.7	1110	2.8
2	Boc-L-Ala-ONP	12	0.9	13	438	2.1
	Boc-D-Ala-ONP	82	0.7	117	3140	0.2
	*p*NPA	6.1	2.7	2.3	1300	2.0
3	Boc-L-Ala-ONP	11	0.8	14	401	2.0
	Boc-D-Ala-ONP	77	0.6	128	2950	0.2
	*p*NPA	4.9	2.9	1.7	1040	2.8
4	Boc-L-Ala-ONP	12	1.0	12	438	2.3
	Boc-D-Ala-ONP	77	0.7	110	2950	0.2

[a] [Catalysis]: 2.5 x 10^{-5} M for *p*NPA, 1.25 x 10^{-5} M for Boc-L or D-Ala-ONP. [b] [*p*NPA]: 5.0 x 10^{-4} ~ 2.5 x 10^{-3} M, [Boc-L or D-Ala-ONP]: 1.0 x 10^{-4} ~ 5.0 x 10^{-4} M. [c] The uncatalytic rate constant (k_{un}): 4.70 x 10^{-6} s^{-1} for *p*NPA, 2.74 x 10^{-5} s^{-1} for Boc-L-Ala-ONP and 2.61 x 10^{-5} s^{-1} for Boc-D-Ala-ONP in 0.02 M phosphate buffer at 25 °C. [d] $K_{TS} = k_{un}/(k_{cat}/K_m)$.[7]

We also examined the catalytic abilities and stereo-selectivity of **1**-**4** with Boc-D-alanine-*p*-nitrophenyl ester (Boc-D-Ala-ONP) and Boc-L-alanine-*p*-nitrophenyl ester (Boc-L-Ala-ONP) as substrates at pH 7.2. The results of kinetic parameters are shown in Table 1. CDs usually exhibit low degrees of chiral recognition for enantiometric guests probably due to their symmetrical round-shaped cavity. However we observed remarkable chiral recognition abilities of **1**-**4** for the enantiometric substrates used here. The order of k_{cat} of **1**, **2**, **3** and **4** is **1**<**3, 4**≤**2** for both substrates with 7~10-fold higher rate constant for D-isomer of Boc-Ala-ONP in each case. The order of k_{cat} values for *p*NPA substrate is **1**<**4**, **2**<**3**, so the trend of catalytic activity is similar for both substrates in the point that **1** is worse in its catalytic activity than **2** and **3**. The K_m values of **1**-**4** are similar for each of the D and L-isomers. This result indicates that K_m is determined mostly by the cavity of CD, and not influenced by the attached functional groups. Since the k_{cat} values exhibit remarkable degrees of the chiral recognition in the reaction with D-isomer preference, the k_{cat} values are reflected in the values of k_{cat}/K_m, which may be regarded as the parameters of over-all reaction rates. Compounds **1**-**4** show smaller K_{TS} values for the D-isomer than those for the L-isomer. The result is consistent with the fact that **2** and **3** exhibit larger k_{cat} values for the D-isomer than for the L-isomer, suggesting that the binding stability in the transition state is the key factor for the stereo-selective hydrolysis.

References and Notes

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6 All compounds (**1**-**4**) were characterized *via* their TOFMS and ^{1}H NMR spectra.

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