The Dependence of Catalytic Activities of Secondary Functional β-Cyclodextrins on Cavity Structures

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Secondary imidazole-appended β-cyclodextrin 5 with a nondistorted cavity synthesized from a novel intermediate 3-amino-3-deoxy-β-cyclodextrin exhibits much greater catalytic activity in the ester hydrolysis than its isomer 6 with a distorted cavity, indicating that the catalytic activities of secondary functional cyclodextrins are dependent on cavity structures.

Key words nondistorted cyclodextrin; imidazole; ester hydrolysis

Cyclodextrins (CDs) and some of their functional derivatives[1] have been studied extensively as artificial enzymes in biomimetic chemistry.[2] These studies, however, have been largely confined to the primary hydroxyl side, while the reported secondary functional CDs[3] obtained from the ring opening of manno-2,3-epoxido-CDs are of the distorted CD type. The distortion of CD cavities causes a decrease in binding abilities.[4] Thus such secondary functional CDs may not exhibit great overall catalytic activity (k_2/K_m) or even lose a significant amount of catalytic activity. In this case, to attain optimum catalysis by secondary functional CDs, study of the effect of cavity structures on their catalytic activities is required. Herein, we report the synthesis of secondary functional CDs with nondistorted cavities and the first investigation of the dependence of catalytic activities of secondary imidazole-appended CDs on their cavity structures.

Secondary imidazole-appended β-CD **5** (65.9%) with a nondistorted cavity and its isomer **6** (65.7%) with a distorted cavity were synthesized from the reaction of a novel intermediate 3-amino-3-deoxy-β-CD **3** and 3-amino-3-deoxy-(2*S*,3*R*)-β-CD **4**,[5] respectively, with imidazol-1-ylacetic acid in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) (Chart 1). Compound **3** was prepared as follows: the reaction of allo-2,3-epoxido-β-CD[6] with NaN_3 in H_2O, followed by purification by reverse-phase chromatography with a gradient elution from H_2O to 40% aqueous MeOH, afforded **1** (65%) and **2** (25%). Staudinger reduction of **1** gave **3** (82.6%). All the compounds were characterized by FAB MS, NMR (^1H, ^{13}C, and 2D), and elemental analysis. The $^4C_1$ glucosidic structures of the modified sugar residues in **3** and **5** and the $^1C_4$ altrosidic structure in **6** were deduced from the ^1H–^1H coupling constants.[7] Thus, the $^4C_1$ glucosidic conformations in native CDs are maintained in **3** and **5**, while the distortion of the macrocycle in **6** is required because of the equatorial C1 (A′)–O4 (G′) and axial C4 (A′)–O4 (A′) bonds (Residue A′, Chart 1). That is, compounds **3** and **5** possess nondistorted cavities, while **6** has a distorted cavity.

The catalytic activities of **5** and **6** were investigated by measuring the hydrolysis rate of ester **7** spectroscopically at 420 nm. The pseudo-first-order rate constants for the hydrolysis of **7** as well as the pK_a values of imidazoles in **5** and **6** are shown in Table 1. Secondary imidazole-appended β-CD **6** with a distorted cavity is not more efficient than imidazole in the hydrolysis of **7**, while **5** with a nondistorted cavity can hydrolyze **7** *ca*. 22 and 24 times faster than imidazole and **6**, respectively, indicating that the nondistorted cavity contributes remarkably to the acceleration of the reaction. From the maximum catalytic rate constants (k_2) and Michaelis constants (K_m) (Table 2), estimated by the Lineweaver–Burk plots,[8] it is revealed that the overall catalytic activity (k_2/K_m) of **5** is *ca*. 108 and 61 times as large as those of imidazole and **6**, respectively. These results suggest that efficient catalysis can be expected from secondary functional CDs with nondistorted cavities.

The differences in catalytic activities between **5** and **6** could be explained by the differences in their cavity struc-

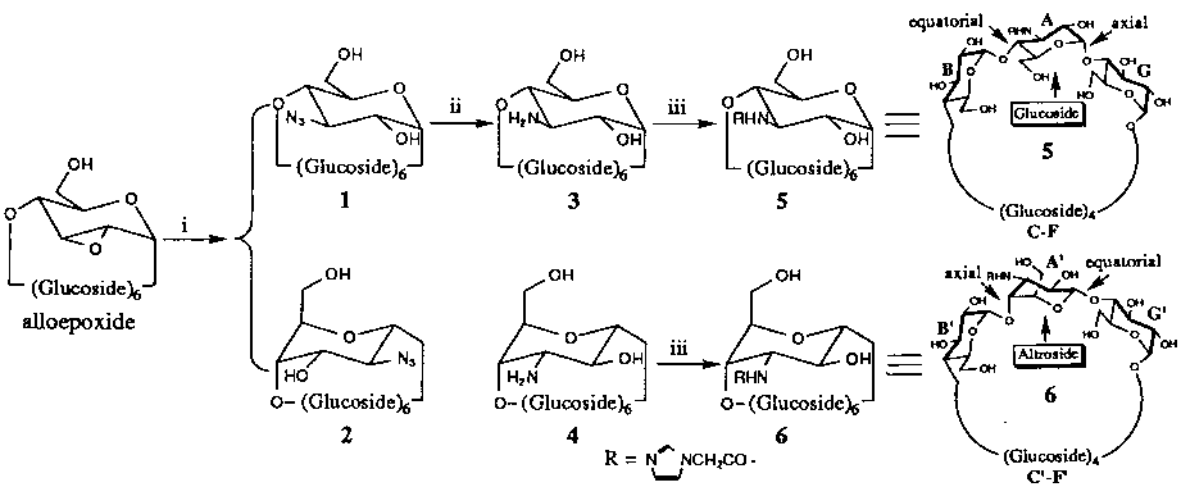

Chart 1. Synthesis of 3-Amino-3-deoxy-β-cyclodextrin **3** and Imidazole-Appended β-Cyclodextrins **5** and **6**

Reagents and conditions: i, NaN_3, $(CH_3)_3N \cdot HCl$, H_2O, 80 °C, 3 d; ii, Ph_3P, DMF, then $NH_3 \cdot H_2O$; iii, imidazol-1-ylacetic acid, DCC, HOBt, DMF.

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Table 1. pK_a Values of **5** and **6** and Pseudo-first-order Rate Constants for the Hydrolysis of **7**

Catalyst	pK_a [a]	k_{obs}/s^{-1} [b]	Catalyst	pK_a [a]	k_{obs}/s^{-1} [b]
None	___	$(1.45\pm0.04)\times10^{-4}$	**5**	6.62	$(3.61\pm0.01)\times10^{-2}$
β-CD	___	$(2.40\pm0.08)\times10^{-5}$	**6**	5.90	$(1.52\pm0.02)\times10^{-3}$
Imidazole	6.95	$(1.65\pm0.01)\times10^{-3}$			

a) Measured spectroscopically (220 nm) in aqueous solution at 25 °C, except for imidazole by titration. *b*) In 20% DMSO phosphate buffer (0.02 M, pH 7.40, I=0.200 [KCl]) at 25 °C with 0.74% (v/v) MeCN added, [catalyst]=1.99×10^{-3} M, [ester **7**]=2.98×10^{-5} M.

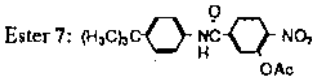
Ester 7: 

Table 2. Kinetic Parameters for the Hydrolysis of **7** Catalyzed by **5** and **6** [a]

Catalyst	k_2/s^{-1}	K_m/M	k_2/k_{un}	$k_2/K_m/M^{-1}s^{-1}$ [b]
5	4.74×10^{-2}	5.79×10^{-4}	327	81.9
6	4.26×10^{-3}	3.15×10^{-3}	29.4	1.35

a) In 20% DMSO phosphate buffer (0.02 M, pH 7.40, I=0.200 [KCl]) at 25 °C with 0.74% (v/v) MeCN added, [ester **7**]=2.98×10^{-5} M. *b*) The second-order rate constant of imidazole in the same condition is $0.76\,M^{-1}s^{-1}$.

tures, rather than by the differences in the availability of unprotonated imidazolyl groups based on their pK_a values. That is, the distortion of the cavity in **6** decreases the binding ability ($1/K_m$), and causes an unfavorable mutual orientation between the imidazolyl group and the ester carbonyl in the inclusion complex of **6** and **7**, leading to a greatly decreased acceleration in the intracomplex reaction (k_2/k_{un}). Thus the inferior catalytic activity (k_{obs}/k_{un}, k_2/K_m) of **6** was observed.

In conclusion, secondary functional β-CDs with nondistorted cavities can be obtained from 3-amino-3-deoxy-β-CD, a novel intermediate allowing the selective functionalization of the secondary hydroxyl side of CDs. The present results reveal the cavity-dependent catalytic activities of secondary functional CDs and supply a clear guideline for the creation of versatile artificial enzymes using secondary functional CDs, *i.e.*, appropriate incorporation of functional groups with no distortion of the cavities of the native CDs.

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References and Notes

1) Wenz G., *Angew Chem. Int. Ed. Engl.*, **33**, 803—822 (1994).
2) Breslow R., Dong S. D., *Chem. Rev.*, **98**, 1997—2011 (1998) and references cited therein.
3) Breslow R., Czarnik A. W., *J. Am. Chem. Soc.*, **105**, 1390—1391 (1983); Akkaya E. U., Czarnik A. W., *J. Am. Chem. Soc.*, **110**, 8553—8554 (1988); Ikeda H., Nagano Y., Du Y.-Q., Ikeda T., Toda F., *Tetrahedron Lett.*, **35**, 5045—5048 (1991).
4) Fujita K., Okabe Y., Ohta K., Yamamura H., Tahara T., Nogami Y., Koga T., *Tetrahedron Lett.*, **37**, 1825—1828 (1996).
5) Fujita K., Egashira Y., Imoto T., Fujioka T., Mihashi K., Tahara T., Koga T., *Chem. Lett.*, **1989**, 429—432.
6) Fujita K., Tahara T., Imoto T., Koga T., *J. Am. Chem. Soc.*, **108**, 2030—2034 (1986).
7) The ^1H–^1H coupling constants of the modified sugar units in **3**: $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.5 Hz, and $J_{3,4}$=10.0 Hz ($^4C_1$); **5**: $J_{1,2}$=3.5 Hz, $J_{2,3}$=10.2 Hz, and $J_{3,4}$=10.2 Hz ($^4C_1$); **6**: $J_{1,2}$=6.8 Hz, $J_{2,3}$=9.9 Hz and $J_{3,4}$=3.7 Hz ($^1C_4$).
8) Lineweaver H., Burk D., *J. Am. Chem. Soc.*, **56**, 658—666 (1934).