Stereospecific Acceleration of Hydrolysis of *p*-Nitrophenyl D-Glycoside by α-Cyclodextrin

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The effects of cyclodextrin (CD) on alkaline hydrolysis of *p*-nitrophenyl α- and β-D-glycoside were investigated; α-CD selectively accelerates hydrolysis of *p*-nitrophenyl α-D-mannoside, β-D-glucoside, and β-D-galactoside in comparison to that of the corresponding β-D-mannoside, α-D-glucoside, and α-D-galactoside, respectively.

In the field of biomimetic chemistry, the development of models for understanding the catalytic specificity of enzymes at the molecular level has attracted much attention over the last two decades. Especially, the models of hydrolase such as lipase, protease, and ribonuclease have been most actively developed.[1] On the other hand, there is only one report on a model of glycosidase which catalyzes the stereospecific hydrolytic cleavage of the glycosidic bond,[2] although glycosidase has found wide application in synthetic organic chemistry, and the elucidation of its catalytic mechanism, which is still a controversial subject,[3] is thus very important. These facts prompted us to develop a model capable of mimicking the catalytic action of glycosidase. In designing a glycosidase model, it is necessary to incorporate some functional groups, which interact with the sugar hydroxyl groups or the aglycone moiety of glycoside in an aqueous solution, into the model compound. Recently, we found that alkyl- and arylboronic acids, which interact with the sugar hydroxyl groups of glycosides in an aqueous solution, selectively accelerate the alkaline hydrolysis of *p*-nitrophenyl α-D-glucoside in comparison with that of the corresponding β-D-glucoside. On the other hand, in the case of *p*-nitrophenyl D-mannoside, the hydrolysis of the β-anomer instead of the α-anomer was selectively accelerated by the addition of these boronic acids.[4]

Cyclodextrins (CDs) are reported to incorporate the aryl moiety of *p*-nitrophenyl ester into their hydrophobic cavities in an aqueous solution and then to accelerate the hydrolysis of the ester, in some cases with enantioselectivity,[5] *via* a nucleophilic attack by one of the 2-hydroxyl groups of the CDs.[6] Therefore, a combination of *p*-nitrophenyl glycoside and CD is expected to afford a new glycosidase model system in which CD can stereoselectively accelerate the hydrolysis of the glycosidic bond through the encapsulation of the aglycone moiety of the glycoside into the cyclodextrin cavity.

Here, we report that α-CD selectively accelerates the hydrolysis of *p*-nitrophenyl β-D-glucoside, *p*-nitrophenyl β-D-galactoside, and *p*-nitrophenyl α-D-mannoside compared to that of the corresponding α-D-glucoside, α-D-galactoside, and β-D-mannoside, respectively. In particular, the hydrolysis of *p*-nitrophenyl α-D-mannoside was remarkably accelerated in comparison with that of the β-anomer to result in a reversal of the selectivity in the alkaline hydrolysis. Furthermore, the function of α-CD was found to be contrary to that of boronic acid.

The hydrolysis of *p*-nitrophenyl α- and β-D-glycoside was carried out separately in 100 mM (= mmol dm^{-3}) phosphate buffer (pH 12.0) at 25 °C. In order to determine the reaction rate, the hydrolysis was spectrophotometrically monitored by measuring the absorbance of the released *p*-nitrophenoxide ion at 400 nm. The rate constants (k_α and k_β) were calculated using the absorbance data obtained in the restricted region where the rates obeyed the first-order kinetics. Each experiment was repeated at least three times to ensure reproducibility (± 10%).

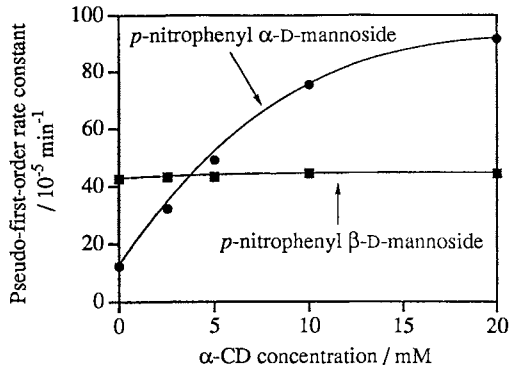

Figure 1. Plots of the observed pseudo-first-order rate constants *vs.* α-CD concentration in 100 mM phosphate buffer (pH 12.0) at 25 °C. [*p*-Nitrophenyl α (or β)-D-mannoside] = 5.0 mM.

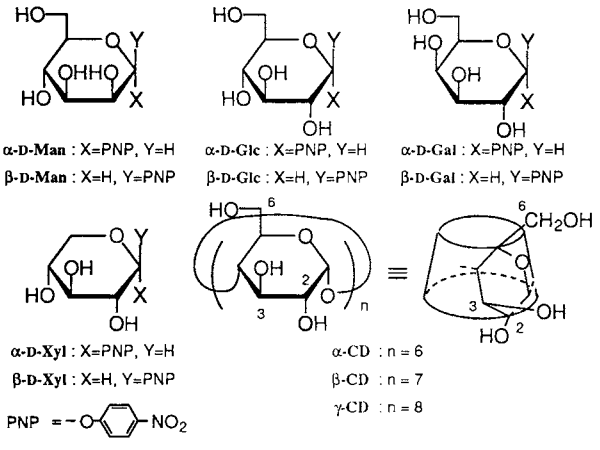

Table 1. Kinetic constants for the hydrolysis of *p*-nitrophenyl α- and β-D-mannoside in the presence of α-, β-, and γ-CD

Additive	Concn./mM	k_α[a]	k_β[a]
none		12	42
α-CD	10	75 (6.3)[b]	44 (1.0)[b]
	20	91 (7.6)[b]	44 (1.0)[b]
β-CD	10	11 (0.92)[b]	34 (0.81)[b]
γ-CD	10	10 (0.83)[b]	31 (0.74)[b]

[a]Pseudo-first-order rate constant (10^{-5} min^{-1}), observed in 100 mM phosphate buffer (pH 12.0) at 25 °C, [*p*-nitrophenyl α- or β-D-mannoside] = 5.0 mM. [b]Relative ratio to the value observed in the absence of CD is designated in parentheses.

Figure 1 shows the plots of the observed pseudo-first-order rate constants for hydrolysis of *p*-nitrophenyl α- and β-D-mannoside *vs.* α-CD concentration in phosphate buffer (pH 12.0). In the absence of α-CD, the β-mannoside was hydrolyzed faster than the α-mannoside (k_α/k_β = 0.29). The rate constant for the α-mannoside remarkably increased along with the increase in α-CD concentration, whereas the rate constant for the β-mannoside was only slightly affected by the increase in α-CD concentration. This phenomenon resulted in a reversed α/β selectivity from β-selective to α-selective hydrolysis by the addition of α-CD of higher concentration than 3.8 mM. On the other hand, the rate constants for both the α- and β-mannoside were decreased by the addition of β- or γ-CD whose apolar cavities are larger than that of α-CD (Table 1). Comparison of ^{1}H-NMR spectra of α-, β-, and γ-CD in D_2O in the absence and presence of *p*-nitrophenyl mannoside showed that the *p*-nitrophenyl group of both the α- and β-mannoside is located near the H-3 atom in the α-CD cavity,[7] while it is placed near the H-5 atom in the case of β- and γ-CD, similarly to the case of phenyl β-D-glucoside reported previously.[8] This indicates that the *p*-nitrophenyl group of the mannoside is incorporated more deeply into the cavity of β- or γ-CD than that of α-CD. The results of both the hydrolysis and the NMR experiments may indicate that the stereoselective hydrolysis by CD largely depends on the inclusion position of the *p*-nitrophenyl group of the mannoside inside the CD cavity.

Table 2. Kinetic constants for the hydrolysis of *p*-nitrophenyl α- and β-D-glycoside in the presence of α-CD

Substrate	[α-CD] /mM	k_α[a]	k_β[a]	K_α[c]	K_β[c]
D-Man	0	12	42	170	110
	20	91 (7.6)[b]	44 (1.0)[b]		
D-Glu	0	45	3.0	250	240
	20	49 (1.1)[b]	11 (3.7)[b]		
D-Gal	0	39	2.5	190	260
	20	70 (1.8)[b]	17 (6.8)[b]		
D-Xyl	0	120	6.4	230	200
	20	130 (1.1)[b]	54 (8.4)[b]		

[a]Pseudo-first-order rate constant (10^{-5} min^{-1}), observed in 100 mM phosphate buffer (pH 12.0) at 25 °C, [*p*-nitrophenyl α- or β-D-glycoside] = 5.0 mM. [b]Relative ratio to the value observed in the absence of α-CD is designated in parentheses. [c]Association constant (M^{-1}) between α-CD and *p*-nitrophenyl α- or β-D-glycoside, determined by ^{1}H-NMR titration method in D_2O at 25 °C. [α-CD]/[*p*-nitrophenyl α- or β-D-glycoside] = 0 - 4.0

We also investigated the effect of α-CD on the rate of hydrolysis of other glycosides such as *p*-nitrophenyl D-glucoside, *p*-nitrophenyl D-galactoside, and *p*-nitrophenyl D-xyloside (Table 2). In these cases, the hydrolysis of their β-anomers instead of α-anomers was selectively accelerated by the addition of α-CD, that is contrary to the case of *p*-nitrophenyl mannoside. This result shows that the hydrolysis of the glycoside bearing the *trans* C-2 hydroxyl group to the *p*-nitrophenoxy group, such as in the cases of *p*-nitrophenyl β-D-glucoside, β-D-galactoside, β-D-xyloside, and α-D-mannoside, was more remarkably accelerated by the addition of α-CD. Meanwhile, the hydrolysis of the corresponding anomeric isomer, in which the configurations of the C-2 hydroxyl group and the *p*-nitrophenoxy group are *cis* to each other, was slightly accelerated by addition of α-CD. This effect of CD is contrary to that of boronic acids which we reported previously,[4] where boronic acids interact with the sugar moiety of glycoside. The association constants (K_α and K_β) between the *p*-nitrophenyl glycoside and α-CD in D_2O, which were determined by ^{1}H-NMR titration method, are shown in Table 2. Although a slight difference in the association constants between the α- and β-anomer was observed, these association constants did not directly correlate with the selective acceleration of the hydrolysis of the glycosides by α-CD.

In order to clarify which hydroxyl groups of α-CD participate in such stereospecific acceleration, as a preliminary study, hydrolysis of *p*-nitrophenyl α- and β-D-galactoside was carried out in the presence of partially methylated α-CD derivatives such as hexakis(2-*O*-methyl)α-CD and hexakis(6-*O*-methyl)α-CD.[9] The hydrolysis of both the α- and β-D-galactosyl ether was accelerated by the addition of hexakis(6-*O*-methyl)α-CD to a similar level to the addition of α-CD. However, the hydrolysis were hardly accelerated by the addition of hexakis(2-*O*-methyl)α-CD. These results imply that the C-2 hydroxyl groups of α-CD should participate in the acceleration of hydrolysis of *p*-nitrophenyl α- and β-D-galactoside, though the detailed mechanism of the selective acceleration is left uncertain.

In conclusion, α-CD selectively accelerated the hydrolysis of *p*-nitrophenyl α-D-mannoside compared to that of the corresponding β-D-mannoside, reversing the α/β selectivity in the hydrolysis of *p*-nitrophenyl D-mannoside in an alkaline solution. On the other hand, α-CD exhibited the β-selective acceleration effect on the hydrolysis of *p*-nitrophenyl D-glucoside, *p*-nitrophenyl D-galactoside, and *p*-nitrophenyl D-xyloside. The selectivity is related to the stereochemical relationship between the hydroxyl group at the C-2 position and the *p*-nitrophenyl group of glycoside. Further work on the mechanism of the selective hydrolysis of glycoside by α-CD is now in progress.

References and Notes

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7 [CDs] = 4.0 mM, [*p*-nitrophenyl D-mannoside] = 8.0 mM. NMR data (δ_H, 400 MHz, D_2O, ref. DSS) for α-CD: 3.93 (H3), 3.79 (H5); with α-mannoside additive: 3.87 (H3), 3.79 (H5); with β-mannoside additive: 3.87 (H3), 3.81 (H5). For β-CD: 3.82 (H3), 3.70 (H5); with α-mannoside additive: 3.75 (H3), 3.65 (H5); with β-mannoside additive: 3.80 (H3), 3.67 (H5). For γ-CD: 3.93 (H3), 3.84 (H5); with α-mannoside additive: 3.85 (H3), 3.76 (H5); with β-mannoside additive: 3.91 (H3), 3.82 (H5). The difference in the signals of ^{1}H-NMR of sugar moiety between mannoside and CDs was distinguished by ^{1}H-^{1}H and ^{1}H-^{13}C COSY.

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9 Hydrolysis rate constants (10^{-6} min^{-1}) of *p*-nitrophenyl β-D-galactoside in pH 11.0 phosphate buffer at 25 °C: 2.4 (none), 4.9 (α-CD), 2.5 ((2-*O*-methyl) α-CD)), 5.2 ((6-*O*-methyl)α-CD)). [*p*-nitrophenyl α-D-galactoside] = 0.50 mM, [additive] = 1.0 mM.