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Spacer-Assisted Catalytic Action of Imidazole-Appended γ-Cyclodextrin

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Catalytic activity of γ-cyclodextrin bearing an imidazole moiety (**1**) was compared with that of the corresponding β-cyclodextrin derivative (**2**) in the hydrolysis of *p*-nitrophenyl acetate. The catalysts **1** and **2** hydrolyzed the ester 80 and 172 times, respectively, as fast in the complexes as the buffer without any catalyst. The binding of **1** for the ester was 40% weaker than that of **2**. When sodium 1-naphthaleneacetate was present in the catalytic systems, the hydrolysis rate was enhanced for **1**, but depressed for **2**. This rate enhancement of **1** suggests that sodium 1-naphthaleneacetate acts as a spacer which facilitates **1** to bind the substrate by narrowing the large γ-cyclodextrin cavity. The time dependency of *p*-nitrophenol formation under the conditions of large excess of *p*-nitrophenyl acetate to **1** or **2** exhibited that the inhibitory effect of the product was remarkable for **2**, but negligible for **1**.

Cyclodextrins (CDs) are a series of cyclic oligomers consisting of six or more α-1,4-linked D-glucopyranose units. The number of glucopyranose unit is designated by a Greek letter: α for six, β for seven, γ for eight, so on. They have a hydrophobic cavity in which various hydrophobic guests can be included.[1] Because of their remarkable ability to form inclusion complexes, CDs, particularly α- and β-CDs, have been used as enzyme models.[1,2] Ester hydrolysis accelerated by CDs has been extensively studied as a serine protease model in which an alkoxide anion of secondary hydroxyls of CDs attacks the carbonyl of bound substrates.[3-5] Since pK_a of the hydroxyls is 12.1,[4] the acceleration by CDs occurs only at high pHs, in contrast to chymotrypsin which exhibits maximal rate at pH 8. Another fault of native CDs as enzyme models is that they are not real catalyst because acylated CDs thus produced are stable. There have been many attempts to convert CDs to better enzyme models by attaching appropriate functional groups.[1] Imidazole is one of those functional groups, which itself works as a catalyst at around pH 8, and actually plays an important role in catalytic action of chymotrypsin. From this viewpoint, imidazole-modified α- and β-CDs have been studied as effective catalysts which have both binding site (CD unit) and functional group (imidazole).[6-8]

The use of γ-CD as a binding site of such enzyme models has not yet been attempted so far mainly due to its large cavity size. However, in recent years, γ-CD has excited much attention because it was shown that γ-CD can include two guest molecules in its large cavity.[9] It was also shown that good-sized molecules or attached moieties can act as spacers which enable γ-CD to include a variety of guests by narrowing the large cavity.[10-12] In connection with this unique property of γ-CD, we have prepared imidazole-appended γ-CD (**1**) in the present work to examine its catalytic activity, turnover ability and effects of additives by using the corresponding β-CD derivative (**2**) as a reference catalyst.

Table 1. Pseudo-First-Order Rate Constants for Ester Hydrolysis[a)]

Catalyst	$10^5 k_{obsd}/s^{-1}$
None	4.83±0.27
β-CD	10.6±0.04
γ-CD	8.49±0.20
1	63.4±1.4
2	182±7
3	29.7±0.05

a) 25 °C in pH 8.16 (I=0.200 (KCl)) 0.05 mol dm^{-3} Tris-HCl buffer with 0.40% (v/v) acetonitrile added; [Catalyst]=2.5×10^{-3} mol dm^{-3}; [*p*-Nitrophenyl acetate]=2.5×10^{-5} mol dm^{-3}.

Results and Discussion

Table 1 shows the pseudo-first-order rate constants obtained at 25 °C in pH 8.16 buffer solution by using *p*-nitrophenyl acetate as a substrate. Even under these mild conditions, the ester decomposes spontaneously. β- and γ-CDs enhance the hydrolysis rate slightly, showing 2.2 and 1.8-fold larger values, respectively, than the simple buffer solution. The data suggest stronger binding ability of β-CD than that of γ-CD.[12] The imidazole-appended β-CD **2** revealed 38-fold increase in the rate constant, while the rate increase of the corresponding γ-CD derivative **1** was 13-fold. The inferior activity of **1** is probably due to the large cavity size of its γ-CD unit. It is obvious that the CD parts of **1** and **2** contribute remarkably as binding sites to the acceleration of the reaction, as shown by two and six times larger rate constants for **1** and **2**, respectively, than that of the reference imidazole compound **3**. The reaction, therefore, proceeds according to the Scheme 1,

$$\mathrm{ImCD} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ImCD \cdot S} \xrightarrow{k_2} \mathrm{ImCD \cdot S'} \xrightarrow{k_3} \mathrm{ImCD} + \mathrm{P_2}$$
$$\mathrm{ImCD \cdot S'} + \mathrm{P_1}$$

Scheme 1.

Table 2. Kinetic Parameters for Ester Hydrolysis by **1** and **2**[a)]

Catalyst	$10^3k_2/s^{-1}$[b)]	K_m/mmol dm^{-3}[c)]	$10(k_2/K_m)$/dm^3 mol^{-1} s^{-1}
1	3.86±0.42	14.9±2.0	2.60±0.07
2	8.33±0.28	8.90±0.08	9.35±0.23

a) Hydrolysis of *p*-nitrophenyl acetate at 25 °C in pH 8.16 (I=0.200 (KCl)) 0.05 mol dm^{-3} Tris-HCl buffer with 0.40% (v/v) acetonitrile added. b) Rate constant of intracomplex hydrolysis. c) Dissociation constant of the substrate-catalyst complexes.

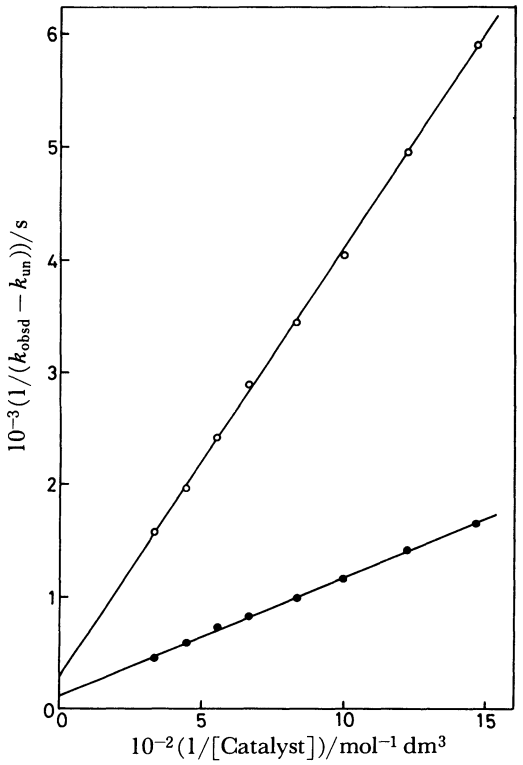


Fig. 1. Lineweaver-Burk plots for hydrolysis of *p*-nitrophenyl acetate with **1** (○) and **2** (●) (25 °C in pH 8.16 Tris-HCl buffer).

where ImCD is **1** or **2**, S is *p*-nitrophenyl acetate, P_1 is *p*-nitrophenol and P_2 is acetic acid.

The rate constants for the intracomplex reaction k_2 and the dissociation constants (Michaelis constants) K_m were evaluated by the Lineweaver-Burk plots[13)] of Eq. 1 (Fig. 1) as shown in Table 2,

$$\frac{1}{k_{obsd}-k_{un}} = \frac{K_m}{(k_2-k_{un})[Catalyst]} + \frac{1}{(k_2-k_{un})}, \quad (1)$$

where k_{obsd} and k_{un} denote the rate constants in the presence and absence of catalyst, respectively. The catalysts **1** and **2** hydrolyze the ester 80 and 172 times, respectively, as fast in the complexes as the buffer without any catalyst. The catalyst **2** has a dissociation constant that is 40% smaller than that of **1**. These data demonstrate that size-fitting between CD cavity and guest and positions of imidazole and ester carbonyl in the complex are better for **2**, resulting in 3.6-fold larger overall reaction rate k_2/K_m than that of **1**.

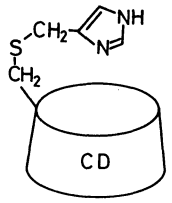
It seems interesting to examine the effect of additives

Table 3. Spacer Effect of Sodium 1-Naphthaleneacetate on Ester Hydrolysis Catalyzed by **1** and **2**[a)]

Catalyst	Spacer[b)]/mol dm^{-3}	$10^5k_{obsd}/s^{-1}$
1	0	63.4±1.4
1	0.005	74.9±0.3
1	0.01	78.7±0.04
2	0	182±7
2	0.005	162±0.3
2	0.01	150±4
3	0	29.7±0.05
3	0.005	29.7±0.06
3	0.01	29.9±0.04

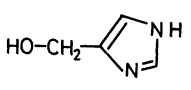
a) 25 °C in pH 8.16 (I=0.200 (KCl)) 0.05 mol dm^{-3} Tris-HCl buffer with 0.40% (v/v) acetonitrile added; [Catalyst]=2.5×10^{-3} mol dm^{-3}; [*p*-Nitrophenyl acetate]=2.5×10^{-5} mol dm^{-3}. b) Sodium 1-naphthaleneacetate.

in the catalytic system of **1** since the additives might operate as spacers which enable the substrate to be included more strongly by narrowing the large γ-CD cavity. Table 3 shows the variation of the pseudo-first-order rate constant observed when sodium 1-naphthaleneacetate (**4**) was added as a spacer. The rate value for **2** diminished with increasing concentration of **4**, while **1** showed 24% increase in the value at [**4**]=0.01 mol dm^{-3}. The results imply that **4** acts as an inhibitor for **2** but as an effector for **1**. This effector behavior of **4** should be due to the spacer effect of **4** as shown in Fig. 3. This spacer effect was also confirmed by the fact that the catalyst **3**, which has no cavity, afforded the same rate value independently of the concentration of **4**. Since similar rate enhancement was



1 CD= γ-CD

2 CD= β-CD



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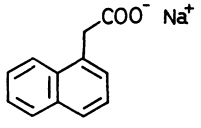

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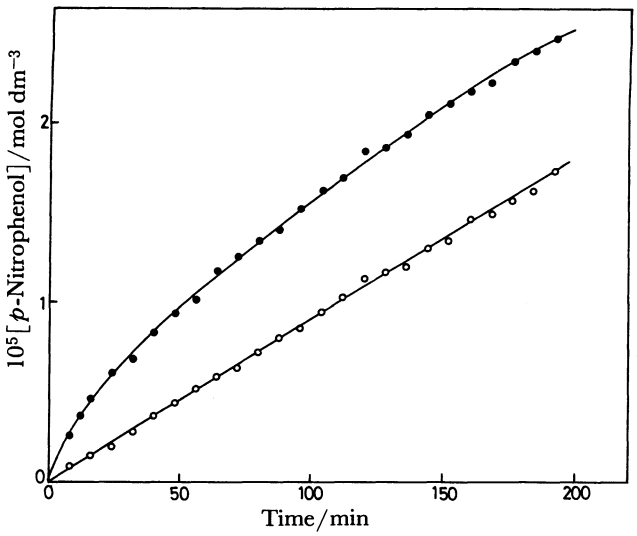

Fig. 2. Concentration of *p*-nitrophenol produced by hydrolysis of *p*-nitrophenyl acetate as a function of time under the conditions of large excess of substrate to **1** (○) or **2** (●) (25 °C in pH 7.02 (I=0.200 (KCl)) phosphate buffer). [*p*-Nitrophenyl acetate]=1.00×10^{-3} mol dm^{-3}. [**1**] or [**2**]=1.00×10^{-5} mol dm^{-3}.

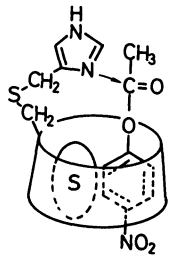

Fig. 3. Schematic representation of spacer(S)-assisted hydrolysis of *p*-nitrophenyl acetate in **1**.

observed when cyclohexanone and (−)-borneol were used in place of **4**,[14] this spacer effect is considered to occur widely in γ-CD systems.

The turnover abilities of **1** and **2** were examined under the conditions of large excess (100-fold) of *p*-nitrophenyl acetate. Figure 2 shows the time dependency of the amount of *p*-nitrophenol. The amounts of hydrolyzed *p*-nitrophenyl acetate are 1.7-fold and 2.5-fold larger for **1** and **2**, respectively, than the quantity of the catalysts at 200 min of the reaction time. This confirms that the deacylation step in Scheme 1 proceeds, regenerating the catalysts which can hydrolyze other substrate molecules again. The plots of *p*-nitrophenol vs. time exhibit different features for **1** and **2**, being curved for **2** and linear for **1**. The depressed rate observed for **2** in the period after the initial stage may be explained in terms of the inhibitory effect of produced *p*-nitrophenol. It means that the product competes with the substrate for complexing with **2**. In contrast to the remarkable binding ability of **2** to *p*-nitrophenol, the catalyst **1**, which has a large γ-CD cavity, seems hard to form 1:1 complex with *p*-nitrophenol. In other words, **1** is likely to be good host for the substrate and poor host for the product. Although the present study gave no data indicative of the formation of the ternary complex of **1**, substrate and *p*-nitrophenol, it is noted that *p*-nitrophenol may act as a spacer in the catalytic system of **1** as **4** does if its concentration is appropriate. The spacer-assisted or product-assisted binding of substrate cannot be expected to occur in α- and β-CD systems.

The present γ-CD system **1** was not a better catalyst than the β-CD system **2** even in the presence of spacers, but the results obtained here present guideline for designing effective catalytic systems of γ-CD. As an extension of this study, construction of some γ-CD systems having both spacer and catalytic moiety are now under way.

Experimental

Materials. β-CD (Tokyo Kasei) was recrystallized from water and dried in vacuo. γ-CD was kindly gifted from Nihon Shokuhin Kako Ltd., and was used without further purification. *p*-Nitrophenyl acetate (Tokyo Kasei) was recrystallized from ethanol. 4-(Hydroxymethyl)imidazole (Aldrich) and sodium 1-naphthaleneacetate (Tokyo Kasei) were used without further purification. Aqueous buffers were prepared with deionized water.

Tosyl-β-CD was prepared in pyridine, so that one of primary hydroxyls of β-CD was sulfonated.[15,16] The crude product was purified by repeated recrystallization from the mixed solvent of 1-butanol, ethanol, and water (5:4:3 by volume). The TLC of the obtained crystals showed a single spot on the plate (Merck, Kieselgel 60 F_{254}) when the plate was treated with *p*-anisaldehyde solution and then heated: R_f 0.49 (1-butanol : ethanol : water=5 : 4 : 3).

6-*O*-(2-naphthylsulfonyl)-γ-CD was prepared from 2-naphthalenesulfonyl chloride and γ-CD by the method previously reported.[17]

Measurements. The kinetic runs were performed with a Shimadzu UV-360 spectrophotometer. Reaction temperature was kept at 25±0.1 °C by using a Haake water circulation instrument. A kinetic run was initiated by injecting a solution of substrate in acetonitrile into the catalyst solution. The absorbance at 400 nm of *p*-nitrophenol was monitored as a function of time. The data were fitted to a simple exponential curve for a first-order reaction. The pseudo-first-order rate constants in Tables 1 and 3 were averages of at least two runs. The kinetic parameters shown in Table 2 represent the averages of three runs of Lineweaver-Burk plots. Figure 1 shows the results of one run of the plots for **1** and **2**.

6-Deoxy-6-(4-imidazolylmethylthio)-γ-cyclodextrin (1). 4-(Mercaptomethyl)imidazole hydrochloride (430 mg) and 6-*O*-(2-naphthylsulfonyl)-γ-CD (1 g) were dissolved in 200 ml of 30% aqueous ethanol solution. After adding sodium carbonate to adjust pH of the solution at 10.0, the solution was stirred 4 d under nitrogen. The reaction mixture was neutralized with 1M HCl (1M=1 mol dm^{-3}) and concentrated to 15 ml. Purification was performed by desalting with Bio-Bead ™SM-4 and SP-Sephadex C-25 resins, followed by column chromatography with Sephadex G-15 (yield 0.25 g (25%)). R_f=0.21 (28% aqueous NH_3 : H_2O : AcOEt : 2-PrOH= 1 : 4 : 3 : 5); ^{1}H NMR (DMSO-d_6, 20 °C) δ=3.09—3.94 (br,

50H, CH_2 protons of mercaptomethyl-4-imidazole and CD protons other than C_1H and OH), 4.54 (7H, O_6H), 4.88 (8H, C_1H), 5.79 (16H, O_2H and O_3H), 6.63—7.17 (1H, aromatic), 7.52 (1H, aromatic), 11.90 (1H, NH); Found: C, 41.51; H, 6.51; N, 1.93; S, 2.19%. Calcd for $C_{52}H_{84}N_2O_{39}S \cdot 6H_2O$: C, 41.60; H, 6.45; N, 1.87; S, 2.14%.

6-Deoxy-6-(4-imidazolylmethylthio)-β-cyclodextrin (2). This compound was prepared previously.[18] In the present work, **2** was prepared according to the procedure described in the synthesis of **1** (yield 27%): R_f=0.28 (28% aqueous NH_3 : H_2O : AcOEt : 2-PrOH=1 : 4 : 3 : 5). Found: C, 43.56; H, 6.31; N, 1.95; S, 2.80. Calcd for $C_{46}H_{74}N_2O_{34}S \cdot 2H_2O$: C, 43.60; H, 6.20; N, 2.21; S, 2.53%.

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