Catalysis of the Hydrolysis of *p*-Nitrophenyl 1-Adamantaneacetate by Cyclodextrins

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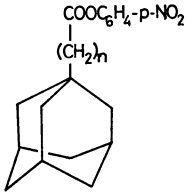
The cleavage of *p*-nitrophenyl 1-adamantaneacetate was accelerated by β- and γ-cyclodextrins, which was in contrast with their deceleration of the cleavage of a sterically analogous substrate, *p*-nitrophenyl 1-adamantanecarboxylate. The presence and absence of a methylene group in these substrates absolutely governed the directions of the rate effects of the cyclodextrins. The specificity, explicitly showing that cyclodextrins are quite good models of enzymes, was attributed to the difference of the structures of the inclusion complexes of cyclodextrins with these substrates.

Recently cyclodextrins (CDs) have been often used as an enzyme model. One of the important reasons for the increasing attention to CDs is the complex formation of these oligosaccharides with the substrates prior to their catalytic functions. Thus, the stereochemistry in the complex formation can result in the specificities in the subsequent reactions, which is similar to enzymatic reactions.[1)]

This paper describes a strict specificity in the catalyses by CDs. It will be shown that the cleavage of *p*-nitrophenyl 1-adamantaneacetate (**1**) is accelerated by β- and γ-cyclodextrins (β- and γ-CDs), in spite of the retardation of the cleavage of sterically a quite analogous substrate, *p*-nitrophenyl 1-adamantanecarboxylate (**2**) by CDs.[2)] The mechanism of the CD-catalyzed hydrolysis of **1** is kinetically examined. Furthermore, the difference in the effects of CDs on these two analogous substrates will be discussed in terms of the structures of the CD-substrate complexes by use of the results of absorption spectrometry.



1 : n = 1
2 : n = 0

Experimental

Materials. **1** was synthesized from 1-adamantaneacetic acid and *p*-nitrophenol by use of dicyclohexylcarbodiimide as the dehydration reagent and was purified by the recrystallization from ethanol; mp 114.0—114.5 °C; Found; C, 68.35; H, 6.75; N, 4.43%. Calcd for $C_{18}H_{21}NO_4$; C, 68.54; H, 6.72; N, 4.44%. Synthesis of **2** and its characterization were described in the previous paper.[2)] α-Cyclodextrin (α-CD) and β-CD were purified by the recrystallization from water. γ-CD was purchased from Nakarai Chemical Co. and was used without further purification. The 0.1 mol dm^{-3} NaOH and NaOD solutions had the factors of 1.00± 0.01 as shown by the titration with 0.1 mol dm^{-3} HCl standard solution.

Kinetics. Hydrolysis of **1** was carried out at 16 °C mostly in the 66:34 (v/v) mixture of 0.1 mol dm^{-3} NaOH aqueous solution and acetonitrile. The reaction was initiated by the addition of 30 μl stock solution of **1** in acetonitrile to the mixture of 2 ml 0.1 mol dm^{-3} NaOH aqueous solution and 1 ml acetonitrile. The initial concentration of **1** was approximately 2×10^{-5} mol dm^{-3}, and the cleavage was followed at 410 nm by the appearance of *p*-nitrophenol.

The cleavage of **1** was also examined in the 99:1 (v/v) mixture of 0.1 mol dm^{-3} NaOH aqueous solution and acetonitrile, which was prepared by the addition of 30 μl stock solution in acetonitrile to 3 ml 0.1 mol dm^{-3} NaOH aqueous solution. In this case, the initial concentration of **1** was kept as low as 5×10^{-6} mol dm^{-3}, because of the poor solubility of **1** in aqueous solution.

Absorption Spectrometry. Absorption spectra were measured at 16 °C in the 66:34 (v/v) mixture prepared similarly as described in the Kinetics section. The initial concentrations of **1** and **2** were 10^{-4} mol dm^{-3}, and that of β-CD was 3×10^{-2} mol dm^{-3}. The absorbance at a specific wavelength was determined by the extrapolation of the absorbance-time dependence to the time of the mixing (the addition of the stock solution).

Product Analyses. The reactions were initiated by the addition of 23 mg of **1** (or 22 mg of **2**) in 2 ml acetonitrile to 4 ml of 0.1 mol dm^{-3} NaOH aqueous solution containing 51 mg of β-CD. The values of $[\beta\text{-CD}]_0$, $[\mathbf{1}]_0$, and $[\mathbf{2}]_0$ were 7.5×10^{-3}, 1.2×10^{-2}, and 1.2×10^{-2} mol dm^{-3}. Considering the K_d's of the β-CD–**1** and β-CD–**2** complexes in the 66:34 mixture shown in Table 1, about 77 and 61% of β-CD in the reaction solutions should form complexes with **1** and **2**, respectively. After 16 s for **1** and 500 s for **2** (the one half-life calculated from k_c's), the reactions were stopped by the addition of a small amount of 1 mol dm^{-3} HCl solution, followed by the extraction with 6 ml ether. The aqueous layer was dried *in vacuo* and the resulting white powder was analyzed by infrared spectrometry.

Results

Figure 1 shows the dependence of the rate constant of the cleavage of **1** (k_{obsd}) on the initial concentrations of CDs ($[CD]_0$). Obviously, both β- and γ-CDs showed acceleration effects of the cleavage of **1**. The data in Fig. 1 were analyzed according to Eq. 1, which is based on the scheme involving the complex formation of CDs with **1** prior to the chemical transformation:

$$(k_{obsd}-k_{un}) = -K_d(k_{obsd}-k_{un})/[CD]_0 + (k_c-k_{un}). \quad (1)$$

Here, k_{un} and k_c are the rate constant of the cleavage of **1** in the absence of CDs and that of **1** incorporated in the CD–**1** complex, respectively, and K_d is the equilibrium constant of the dissociation of the CD–**1** complex.[1)]

As shown in Fig. 2, the plots of $(k_{obsd}-k_{un})$ *vs.* $(k_{obsd}-$

k_{un})/[CD]$_0$ exhibited straight lines, from the slopes and the intercepts of which k_c and K_d were determined.

Table 1 lists the values of k_c and K_d for the CD-catalyzed cleavage of **1**. For the purpose of comparison, the values for the cleavage of an analogous substrate, **2**, determined in the previous paper,[2] are also shown.

β- and γ-CDs showed 3.0 and 2.1 fold *acceleration* of the cleavage of **1** in the 66:34 mixture of 0.1 mol dm^{-3} NaOH aqueous solution and acetonitrile, which is in contrast with the 3.3 and 1.4 fold *deceleration* of the cleavage of **2** by β- and γ-CDs. Still larger difference in the rate effects by CDs was found in the cleavages of **1** and **2** in the 99:1 mixture. Thus, the cleavage of **1** was *accelerated* by 1.8 and 1.7 fold by β- and γ-CDs, whereas the cleavage of **2** was *decelerated* by 28 and 13 fold by β- and γ-CDs. However, the effect of α-CD on the cleavages of **1** and **2** was much smaller.

Table 2 shows the D_2O solvent isotope effects on k_c and k_{un} in the 66:34 mixture. k_c's for **1** and **2** in the 66:34 D_2O–acetonitrile mixture were found to be 1.1—1.2 times those in the H_2O–acetonitrile mixture. These results indicated that the cleavages of **1** and **2** incorporated in the CD complexes proceed *via* nucleophilic catalysis by hydroxide ion, since the nucleophilicity of OD^- is 20—40% larger than that of OH^-.[3] Larger nucleophilicity of OD^- than OH^- in the present reactions is definitely shown by the 1.1 fold larger values of k_{un}'s in the D_2O–acetonitrile mixture than those in the H_2O–acetonitrile mixture.

Nucleophilic attack by hydroxide ion indicated above for the CD-accelerated cleavage of **1** and the CD-decelerated cleavage of **2** was supported by the product analyses. After the reactions of β-CD with excess amounts of **1** and **2** (see Experimental), essentially

Table 1. Values of k_c, k_c/k_{un}, and K_d for the cleavages of **1** and **2** in the presence of CDs[a]

Substrate	CD	k_c / 10^{-3} s^{-1}	k_c/k_{un}	K_d / 10^{-3} mol dm^{-3}
1[b]	α-CD	1.6	1.0	—
	β-CD	4.6	3.0	1.8
	γ-CD	3.2	2.1	2.6
1[c]	α-CD	2.3[d]	1.1	—
	β-CD	3.9[d]	1.8	—
	γ-CD	3.6[d]	1.7	—
2[b]	α-CD	0.39[d]	0.82	—
	β-CD	0.14	0.30	4.7
	γ-CD	0.35[d]	0.74	—
2[c]	α-CD	0.60	0.31	13
	β-CD	0.069	0.036	0.36
	γ-CD	0.15	0.078	0.82

a) The data for **2** were taken from Ref. 2. b) In the 66:34 (v/v) 0.1 mol dm^{-3} NaOH-acetonitrile mixture at 16 °C; k_{un}'s of **1** and **2** under these conditions were 1.54×10^{-2} and 4.75×10^{-3} s^{-1}, respectively. c) In the 99:1 (v/v) 0.1 mol dm^{-3} NaOH-acetonitrile mixture at 16 °C; k_{un}'s of **1** and **2** under these conditions were 2.17×10^{-2} and 1.93×10^{-2} s^{-1}, respectively. d) k_{obsd}'s in the presence of 3×10^{-2} mol dm^{-3} CD. These values were taken as k_c here, since they were identical with those in the presence of 2×10^{-2} mol dm^{-3} CD within experimental error.

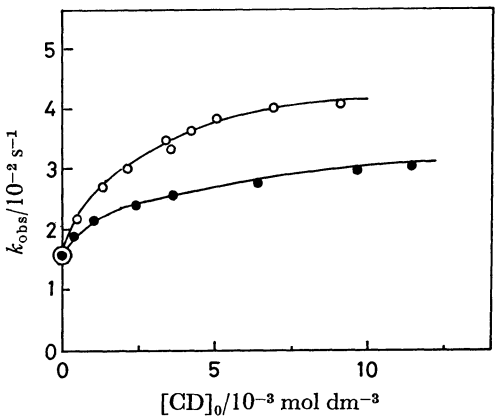


Fig. 1. Dependence of k_{obsd} of the cleavage of *p*-nitrophenyl 1-adamantaneacetate (**1**) on the concentration of cyclodextrins (CDs); ○, β-CD; ●, γ-CD; at 16 °C in the 66:34 mixture of 0.1 mol dm^{-3} NaOH aqueous solution and acetonitrile.

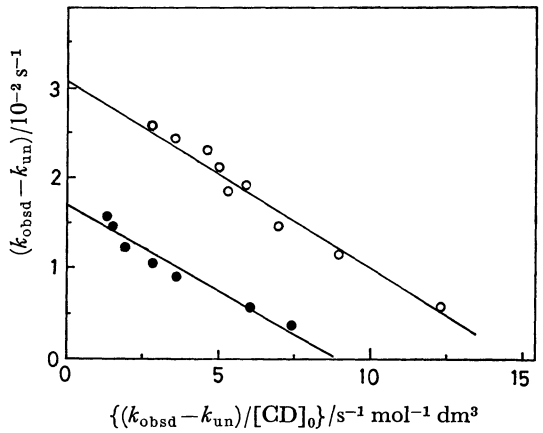


Fig. 2. Plots of $(k_{obsd}-k_{un})$ *vs.* $(k_{obsd}-k_{un})/[CD]_0$ for the β-CD (○)- and γ-CD (●)- catalyzed cleavage of **1**; at 16 °C in the 66:34 mixture of 0.1 mol dm^{-3} NaOH aqueous solution and acetonitrile; the data in Fig. 1 were used here.

Table 2. D_2O solvent isotope effects for the cleavages of **1** and **2** in the presence or absence of β- and γ-CDs[a,b]

Substrate	$k_{un}(D_2O)/k_{un}(H_2O)$	$k_c^{\beta\text{-CD}}(D_2O)/k_c^{\beta\text{-CD}}(H_2O)$	$k_c^{\gamma\text{-CD}}(D_2O)/k_c^{\gamma\text{-CD}}(H_2O)$
1	1.14±0.02	1.17±0.04	1.16±0.04
2	1.12±0.02	1.11±0.03	—

a) In a 66:34 mixture of 0.1 mol dm^{-3} NaOH H_2O solution (or 0.1 mol dm^{-3} NaOD D_2O solution) and acetonitrile. b) The k_{obsd}'s in the presence of 3×10^{-2} mol dm^{-3} CDs were taken as k_c's here. See the footnote d of Table 1.

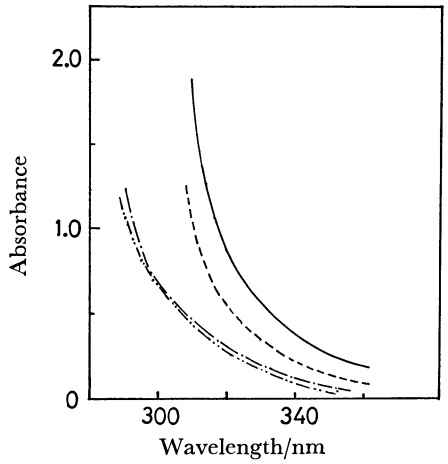


Fig. 3. Absorption spectra of **1** and **2** in the presence and absence of β-CD; —·—, **1**+β-CD; —··—, **1** only; ——, **2**+β-CD; ----, **2** only; [β-CD]=3×10^{-2} and [**1**]=[**2**]=10^{-4} mol dm^{-3} in the 66:34 mixture of 0.1 mol dm^{-3} NaOH aqueous solution and acetonitrile.

all of β-CD was recovered in its intact form as characterized by infrared spectrometry. No carbonyl stretching band due to the β-CD ester of 1-adamantanecarboxylic acid or 1-adamantaneacetic acid, the possible intermediate of nucleophilic catalysis by β-CD, was detected at all. At least some of these intermediates should be trapped by the present procedure (after one half-life of the substrates), if the reaction were involving the nucleophilic attack by β-CD, since the rates of the hydrolyses of the intermediates, which have rather poor leaving groups of pK_a 12 (β-CD),[1] should be smaller than those of **1** and **2**, which have good leaving groups of pK_a 7.1 (*p*-nitrophenol).[4] These arguments are supported by the fact that the rate constant of the alkaline hydrolysis of *p*-nitrophenyl acetate is five fold larger than that of trifluoroethyl acetate, the pK_a of the leaving group of which (12.4) is similar to that of β-CD,[4] and the rate constant of the alkaline hydrolysis of α-CD ester of *p*-nitrobenzoic acid ester is about two fold larger than that of its trifluoroethyl ester.[5]

Figure 3 shows the change in the absorption spectra of **1** and **2** due to the addition of 3×10^{-2} mol dm^{-3} β-CD. Almost all of **1** and **2** should form complexes with β-CD under the present conditions considering the K_d values shown in Table 1. Considerable change was found in the 300—360 nm region in the case of **2**, whereas much smaller change, if any, was observed in the case of **1**. These results indicated that the *p*-nitrophenyl portion of **2** is included in the cavity of β-CD to a greater extent than that of **1** is, since the change of absorption spectrum of *p*-nitrophenol on the complex formation with CDs was well known.[1] The change in the molar absorption coefficient observed for the complex formation between **2** and β-CD is a little bit smaller than the value reported for the complex formation between *p*-nitrophenol and α-CD (2.9×10^3 at 354 nm).[6]

Discussion

This paper shows that catalyses by CDs are quite specific, which is characteristic of enzymatic catalyses. β-CD and γ-CD showed deceleration of the cleavage of **2**, whereas they showed acceleration of the cleavage of **1**. Thus, the presence or absence of a methylene group in the substrate predominantly governs the catalyses by CDs. There have been few reports showing the different directions of the rate effects of CDs on the reactions of analogous substrates,[7] although the magnitudes of the rate effects of CDs (in the same directions) were in some cases strongly dependent on the structures of the substrates.[8] The specificity observed here may be associated with the specific catalysis by β-CD for the selective preparation of vitamin K analogs.[9]

In the previous paper,[2] the deceleration of the alkaline hydrolysis of **2** by β- and γ-CDs was attributed to the steric hindrance by the wall of CDs as well as the electrostatic repulsion between the negative charges of the secondary alkoxide anions of CDs and that of hydroxide ion. The steric hindrance was so important in the hydrolysis of **2** because the adamantyl portion and the carbonyl group are located in a close proximity. Therefore, both of them should be included in the cavity. In the hydrolysis of **1**, however, the methylene group between the adamantyl group and the carbonyl group makes it possible for the carbonyl group to be kept outside the cavity, when the apolar adamantyl group is included in the cavity. Thus, the steric and electrostatic hindrance by CDs is not operative so much in the hydrolysis of **1**.

Absorption spectrometry shown in Fig. 3 is consistent with the above arguments. The *p*-nitrophenyl group of **2**, which is adjacent to the adamantyl group, is partly located inside the cavity (the considerable change in the absorption spectrum), whereas that of **1**, which is located at a larger distance from the adamantyl group, is hardly included in the cavity (the negligible change in the absorption spectrum).

The acceleration of the cleavage of **1** by β- and γ-CDs, which proceeds *via* nucleophilic catalysis by hydroxide ion, is probably attributed to the functions of the primary hydroxyl group(s) of CDs. It is difficult to figure out any mechanism where the secondary hydroxyl groups, which are present in their anionic forms under the present conditions, accelerate the alkaline reactions. Rather, the electrostatic repulsion between the secondary alkoxide anion and hydroxide ion should retard the reactions. The primary hydroxyl group(s) of CDs can alter the microscopic surroundings of the ester group, resulting in the acceleration of the attack by hydroxide ion. Alternatively, the hydrogen bonding of the primary hydroxyl group(s) with the substrate in the transition state can facilitate the alkaline reaction.[10–13] In the hydrolysis of **2**, however, the attack of the hydroxide ion at the carbonyl carbon atom as well as the assistance of the primary hydroxyl groups described above should be unfavorable, since the carbonyl carbon atom is deeply included in the cavity of CDs.

The magnitude of the acceleration of the cleavage of **1** by β- and γ-CDs in the 66:34 mixture of 0.1 mol dm^{-3} NaOH aqueous solution and acetonitrile was larger than that in the 99:1 mixture. This result

is probably associated with the shallower inclusion of the carbonyl carbon atom of **1** in the cavity in the 66:34 mixture than in the 99:1 mixture, resulting in the more efficient nucleophilic attack by hydroxide ion assisted by the primary hydroxyl groups of CDs. This interpretation is supported by the fact that the magnitude of the effect of the deceleration of the cleavage of **2** by CDs was much larger in the 99:1 mixture than in the 66:34 mixture. Here, the shallower inclusion of the carbonyl carbon atom of **2** should enhance the alkaline reaction.

In conclusion, cyclodextrins showed marked specificity in the hydrolyses of analogous substrates, *p*-nitrophenyl 1-adamantaneacetate (acceleration) and *p*-nitrophenyl 1-adamantanecarboxylate (deceleration). This specificity is similar to that of the enzymatic reactions. Present finding confirmed that CDs are quite good models of enzymes.

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