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α-Cyclodextrin-catalyzed Hydrolysis of 2,2,2-Trifluoroethyl 4-Nitrobenzoate

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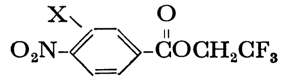
The hydrolysis of 2,2,2-trifluoroethyl 4-nitrobenzoate (**1**) was catalyzed by α-cyclodextrin at pH 8.6, 16 °C. The reaction, involving the complex formation of **1** with α-cyclodextrin, proceeded mostly (80%) *via* the general base catalysis by the secondary alkoxide ion of α-cyclodextrin with 20% contribution of the nucleophilic catalysis. This is the first finding of the general base catalysis of the hydrolyses of esters by cyclodextrins. The rate constants of the cleavage of **1** by the general base catalysis and the nucleophilic catalysis, respectively, of α-cyclodextrin were 5.3×10^{-2} and $1.5\times10^{-2}\ \text{min}^{-1}$, whereas the value in the absence of α-cyclodextrin was $1.2\times10^{-2}\ \text{min}^{-1}$. The hydrolysis of an analogous substrate, 2,2,2-trifluoroethyl 3-methyl-4-nitrobenzoate, however, was little affected by α-cyclodextrin, showing that **1** included the cavity of α-cyclodextrin with the nitro group first.

Recently the attention to the catalyses by cyclodextrins (CDs) as enzyme models has been increasing. This is mostly attributable to the fact that the reactions catalyzed by CDs proceed *via* the complex formation of the substrates with the catalysts preceding the catalytic functions, which is consistent with enzymatic reactions.[1)]

In spite of so many examples of nucleophilic catalyses by CDs,[1)] reports on general base catalyses, which are important in enzymatic reactions, by CDs have been scanty. In the previous paper,[2)] however, general base catalysis by CDs was found for the first time in the α-cyclodextrin (α-CD)-catalyzed hydrolysis of 2,2,2-trifluoroethyl 4-nitrobenzoate (**1**), which was accompanied by some contribution of nucleophilic catalysis by α-CD.

In the present study, both the rate constant of the general base catalysis and that of the nucleophilic catalysis for the α-CD-catalyzed hydrolysis of **1** will be determined. Furthermore, the effect of α-CD on the hydrolysis of 2,2,2-trifluoroethyl 3-methyl-4-nitrobenzoate (**2**), which is sterically analogous to **1** but has a methyl substituent in the *ortho* position to the nitro group, will be also shown. The comparison of the results on the reactions of **1** and **2** provides structural information on the binding of **1** with α-CD.



1: X=H
2: $X=CH_3$

Experimental

Materials. α-CD was purified by the recrystallization from water. The synthesis of **1** and its characterization were described previously.[2)] **2** was synthesized by the reaction of 3-methyl-4-nitrobenzoyl chloride, prepared from the corresponding acid and thionyl chloride, with 2,2,2-trifluoroethanol and was purified by the recrystallization from the ethanol-water solution; mp 54.0—54.2 °C.

Kinetics. The hydrolyses of **1** and **2** were carried out at 16 °C in a pH 8.6 borate buffer and in a pH 11.6 carbonate buffer, respectively. The initial concentrations of **1** and **2** were approximately $10^{-4}\ \text{mol dm}^{-3}$ and the appearance of the corresponding benzoate anions was followed at 296 nm.[3)]

The concentration of 4-nitrobenzoyl-α-CD (**4**), the intermediate of the nucleophilic catalysis of the hydrolysis of **1** by α-CD, was determined in the following way. At a predetermined time, sampling of an aliquot of the reaction solution was made, followed by its acidification with small amount of hydrochloric acid. Then, the aliquot was extracted three times with diethyl ether, and the aqueous layer containing **4** as well as α-CD was dried into a white powder *in vacuo*. The amount of **4** in the white powder was determined by measuring the absorbance at 270 nm, after dissolving it in pH 7 buffer.

The rate constant of the hydrolysis of **4** was determined to be $3.1\times10^{-3}\ \text{min}^{-1}$ by the measurement of the change of the absorbance at 260 nm (due to the liberation of 4-nitrobenzoate ion) in the α-CD-accelerated hydrolysis of 3-chlorophenyl 4-nitrobenzoate.[2)]

For the D_2O solvent isotope effect experiment, the pD was determined by using the equation: pD=pH meter reading +0.4.[4)]

Results

Figure 1 shows the time course of 4-nitrobenzoate ion (**3**) for the hydrolysis of **1** at pH 8.6 in the presence and the absence of $5.0\times10^{-2}\ \text{mol dm}^{-3}$ α-CD. Obviously α-CD showed acceleration of the hydrolysis of **1**.

The appearance of **3** in the absence of α-CD fol-

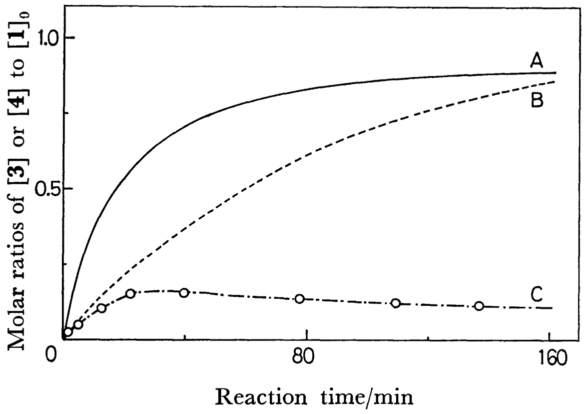


Fig. 1. Time courses of the hydrolysis of 2,2,2-trifluoroethyl 4-nitrobenzoate (**1**) in the presence and the absence of $5\times10^{-2}\ \text{mol dm}^{-3}$ α-CD at pH 8.6, 16 °C; A, ——, 4-nitrobenzoate ion (**3**) in the presence of α-CD; B, ----, **3** in the absence of α-CD; C, —·—, 4-nitrobenzoyl-α-CD (**4**) in the presence of α-CD, where the circles shows the experimental results; the amounts of **3** and **4** are represented by the molar ratios of their concentrations to the initial concentration of **1**.

lowed the first-order kinetics, the rate constant k_{un} being 1.2×10^{-2} min^{-1}. On the other hand, the reaction in the presence of α-CD did not follow the first-order kinetics. This deviation from the first-order kinetics is attributable, as shown later, to the small contribution of nucleophilic catalysis in addition to the major participation of the general base catalysis. Thus, the analysis of the major catalysis was first made by use of the initial slope of the plot of the concentration of **3** *vs.* reaction time, since the initial slope is essentially free from the contribution of the nucleophilic catalysis (see Fig. 4).

As depicted in Fig. 2, the rate constant of the appearance of **3** in the initial stage (k_{obsd}) asymptotically increased with the initial concentration of α-CD ([α-CD]$_0$), indicating the formation of the complex of **1** with α-CD prior to the catalysis. The equilibrium constant of the dissociation of the α-CD–**1** complex (K_d) and the rate constant of the hydrolysis of **1** incorporated in the α-CD–**1** complex (k_c) were determined to be 3.4×10^{-3} mol dm^{-3} and 5.0×10^{-2} min^{-1}, respectively, by plotting the date in Fig. 2 according to Eq. 1;[1]

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

The k_c (5.0×10^{-2} min^{-1}) is much larger than the rate constant of the hydrolysis of 4-nitrobenzoyl–α-CD (**4**) (3.1×10^{-3} min^{-1}), the reaction intermediate of the nucleophilic catalysis by the secondary alkoxide ion of α-CD. On the basis of this result, the nucleophilic catalysis by α-CD is definitely ruled out as the major catalysis associated with k_c.

The logarithm of k_{obsd} in the presence of 5×10^{-2} mol dm^{-3} α-CD, where about 94% of **1** form complexes with α-CD, increased linearly with pH (Fig. 3). This fact is consistent with participation of alkoxide ion of α-CD in the catalysis.

The k_{obsd} in the presence of 5×10^{-2} mol dm^{-3} α-CD in D_2O was 1.7 fold smaller than that in H_2O. Here, the difference between the pK_a of the secondary hydroxyl group of α-CD in H_2O and that in D_2O was taken as 0.6.[5] This D_2O solvent isotope effect, considerably different from unity, indicates the general base catalysis by α-CD. The alkaline hydrolysis assisted by α-CD, which is kinetically indistinguishable from the general base-catalyzed reaction, is unlikely, since the alkaline hydrolysis should show an inverse D_2O solvent isotope effect because of larger nucleophilicity of OD^- than OH^- by 20—40%.[6]

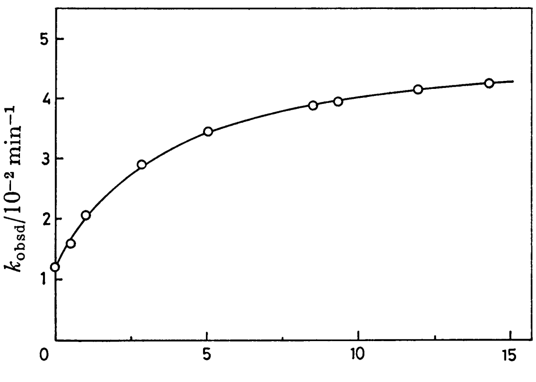


Fig. 2. Dependence of the rate constant of the hydrolysis of **1** (k_{obsd}) on the initial concentration of α-CD at pH 8.6, 16 °C; the k_{obsd}'s were determined from the rate of the appearance of 4-nitrobenzoate ion in the initial stage and are mostly associated with the general base catalysis by α-CD (see the text.).

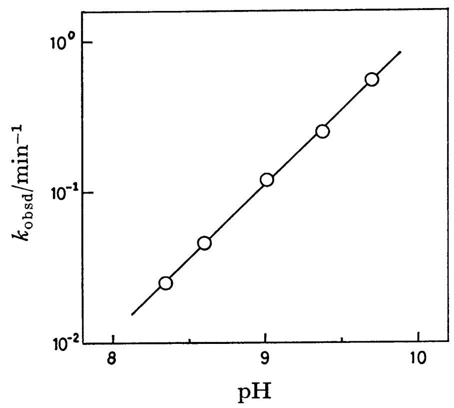


Fig. 3. pH-rate constant profile in the hydrolysis of **1** in the presence of the 5×10^{-2} mol dm^{-3} α-CD at 16 °C.

In Fig. 1, the time course of [**4**] in the α-CD-catalyzed hydrolysis of **1**, determined as described in the Experimental Section, is also shown (the C curve). [**4**] first increased with time, showing a maximum around 30 min. The rate constant of the subsequent decrease of the C curve was identical with the independently determined value of the hydrolysis of 4-nitrobenzoyl–α-CD (**4**), 3.1×10^{-3} min^{-1} (see Experimental).

The appearance of **3** due to the nucleophilic catalysis by α-CD was shown by the C curve in Fig. 4, which was estimated by using [**4**] in Fig. 1 and the rate constant of its hydrolysis (3.1×10^{-3} min^{-1}). The amount of **3** due to the nucleophilic catalysis gradually increased with time after being essentially zero for around 30 min.

The B curve in Fig. 4 corresponds to the appearance of **3** due to the general base catalysis by α-CD. This curve is determined by subtracting the appearance of **3** due to the nucleophilic catalysis (the C curve) from the total appearance of **3** measured photometrically (the A curve).

The B curve in Fig. 4 fits well with the first-order kinetics. Table 1 lists the rate constant of the general base-catalyzed hydrolysis of **1** by α-CD as well as that of the nucleophile-catalyzed hydrolysis by α-CD. The second value was determined by using the rate of the appearance of **4** (the C curve in Fig. 1) and the concentration of the residual **1** at a certain time, estimated from the total consumption of **1** (the sum of

Table 1. Rate constants of the cleavage of **1**[a]

Catalysis	Rate constant/10^{-2} min^{-1}
General base catalysis by α-CD	5.3
Nucleophilic catalysis by α-CD	1.5
Uncatalyzed	1.2

a) pH 8.6, 16 °C.

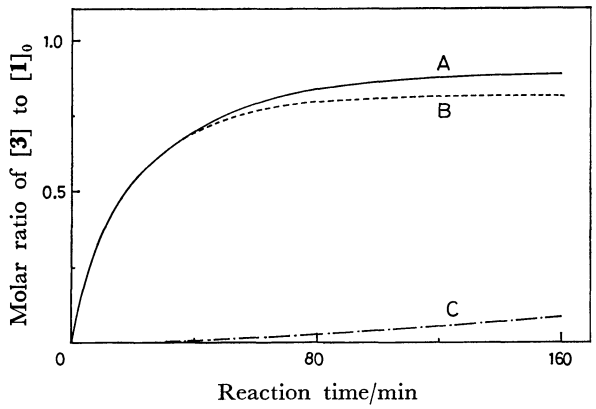


Fig. 4. Time courses of the hydrolysis of **1** in the presence of 5×10^{-2} mol dm^{-3} α-CD at pH 8.6, 16 °C; A, ——, the total amount of **3**; B, ----, **3** due to the general base catalysis by α-CD; C, —·—, **3** due to the nucleophilic catalysis by α-CD; the amount of **3** is represented by the molar ratio of its concentration to the initial concentration of **1**.

the B curve in Fig. 4 and the C curve in Fig. 1). The rate constant of the general base catalysis determined by the first-order plot of the curve B in Fig. 4 (5.3×10^{-2} min^{-1}) is almost identical with the value determined from the initial rate constants (k_{obsd}'s) in Fig. 2 (5.0×10^{-2} min^{-1}).

In contrast with the acceleration of the hydrolysis of **1** by α-CD, α-CD showed little effect on the hydrolysis of an analogous substrate, **2**. The rate constant of the hydrolysis of **2** at pH 11.5 (carbonate buffer), 16 °C in the presence of 5×10^{-2} mol dm^{-3} α-CD (1.4×10^{-2} min^{-1}) was virtually the same as that in its absence (1.3×10^{-2} min^{-1}). No deviation from the first-order kinetics was observed here.

Discussion

The present paper showed that about 80% of the hydrolysis of **1** complexed with α-CD proceeded *via* the general base catalysis by the secondary alkoxide ion of α-CD. This is the first finding of the general base catalysis of the hydrolysis of an ester by CD.

On the complex formation of **1** with α-CD, the nitrophenyl group of **1** included in the cavity with the nitro group first, since no catalysis of α-CD was observed in the hydrolysis of **2** in contrast with the considerable acceleration of the hydrolysis of **1**. The predominant role of the steric hindrance by the adjacent methyl group in the complex formation of **2** with α-CD is consistent with the previous finding that the complex formation of 4-nitrophenol with α-CD is totally inhibited by the methyl substitution of 4-nitrophenol at the 3 position.[7] The structure of the α-CD–**1** complex, where the nitrobenzene group of **1** locates inside the cavity of α-CD, is further supported by the fact that the K_d of this complex (3.4×10^{-3} mol dm^{-3}) is close to that of α-CD–4-nitrophenol complex (5.2×10^{-3} mol dm^{-3}).[7]

The mechanism of the general base catalysis of the hydrolysis of **1** by α-CD is schematically depicted in Fig. 5. A secondary alkoxide ion of α-CD enhances the nucleophilic attack of a water molecule at the carbonyl carbon atom of **1** by partially abstracting a hydrogen atom from the water molecule.

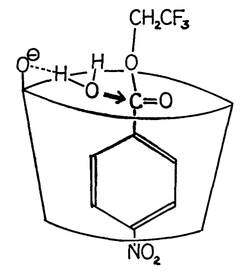


Fig. 5. Mechanism of the general base catalysis of the hydrolysis of **1** by α-CD.

The effectiveness of the general base catalysis by α-CD is attributable to the complex formation of **1** with α-CD, where the secondary alkoxide ion of α-CD functions as an intracomplex catalyst. The rate constant of the intermolecular general base catalysis of the hydrolysis of **1** by the alkoxide ion of α-CD, a base of pK_a 12,[1] was estimated as follows. First the rate constant of the general base-catalyzed hydrolysis of 2,2,2-trifluoroethyl acetate by *N*-methylimidazole (2.3×10^{-4} mol^{-1} dm^3 min^{-1})[8] was converted to the corresponding value of **1** (6.2×10^{-3} mol^{-1} dm^3 min^{-1}) by multiplying the ratio of the rate constant of the alkaline hydrolyses of these two substrates (2.7×10^2).[9] The conversion of the rate constant of the general base-catalyzed hydrolysis of **1** by *N*-methylimidazole (pK 7.0)[10] to that by the secondary alkoxide ion of α-CD (pK_a 12) was made by use of the Brønsted relationship, taking the α for this reaction as 0.5.[11] Consequently, the rate constant of the intermolecular general base-catalyzed hydrolysis of **1** by the secondary alkoxide ion of α-CD was estimated to be 2 mol^{-1} dm^3 min^{-1}. On the other hand, the rate constant of the general base-catalyzed hydrolysis of **1** in the α-CD–**1** complex, referring to the total ionization of α-CD, is calculated to be 1.3×10^2 mol dm^{-3} from the observed value at pH 8.6 (5.3×10^{-2} min^{-1}) and the pK_a of α-CD (12). Thus, the intracomplex general base catalysis of the secondary alkoxide ion of α-CD in the hydrolysis of **1** is equivalent to the catalysis by as large as 65 mol dm^{-3} [12] of the corresponding intermolecular catalyst.

Two reasons are responsible for the small contribution of the nucleophilic catalysis of α-CD in the hydrolysis of **1** in spite of effective nucleophilic catalyses in the hydrolyses of phenyl acetates[13] and *S*-ethyl thiobenzoate.[14] The first reason is that the pK_a of the leaving group, 2,2,2-trifluoroethanol (12.37)[15] is a little bit larger than the pK_a of the secondary hydroxyl group of α-CD (12). Thus, the tetrahedral intermediate, formed by the nucleophilic attack of the secondary alkoxide ion of α-CD at the carbonyl carbon atom of **1**, should return to α-CD and **1** more favorably than produce the products, **3** and **4**. The second reason comes from the structure of the α-CD–**1** complex, in which the nitrobenzene group is located inside the cavity of α-CD. In order for the nucleophilic catalysis to take place, the nitrobenzene group must be taken out of the cavity in the course of the catalysis, since 4-nitrobenzoyl–α-CD with the nitrobenzene group inside the cavity is too much sterically strained to be formed. This structural change should reduce the rate constant.

In conclusion, the hydrolysis of 2,2,2-trifluoroethyl 4-nitrobenzoate (**1**) catalyzed by α-cyclodextrin (α-CD) proceeds mostly (80%) *via* general base catalysis with 20% contribution of nucleophilic catalysis. The complex formation of **1** with α-CD involves the inclusion of the nitrobenzene group with the nitro group first.

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