The Cyclodextrin-accelerated Cleavage of Thiocarboxylic *S*-Esters

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The effect of α- and β-cyclodextrins on the cleavage of the thiocarboxylic *S*-esters, *S*-*p*-nitrophenyl thioacetate (**1**) and *S*-ethyl *p*-nitrothiobenzoate (**3**), was compared with their effect on the cleavage of the corresponding oxygen esters, *p*-nitrophenyl acetate (**2**) and ethyl *p*-nitrobenzoate (**4**). The rate constants of α- or β-cyclodextrin-accelerated cleavage of **1** (1.5×10^{-2} or 4.8×10^{-2} s^{-1} at pH 10.5, 25 °C) were almost identical with the values of **2** (1.6×10^{-2} or 4.2×10^{-2} s^{-1}), definitely showing the rate-determining formation of tetrahedral intermediates. The cleavage of **3** was accelerated by α- and β-cyclodextrins, whereas the cleavage of **4** was decelerated by β-cyclodextrin but was not affected by α-cyclodextrin. The contrast in the effect of cyclodextrins on **3** and **4** was ascribed to the difference of the rate of breakdown of the tetrahedral intermediates to products, caused by different pK's of the leaving groups of **3** (10.5) and **4** (16.0). The formation of tetrahedral intermediates confirmed in the cyclodextrin-accelerated hydrolyses of esters further strengthened the previous proposal that cyclodextrin is an excellent model of serine proteases, since these intermediates are also formed in enzymatic reactions.

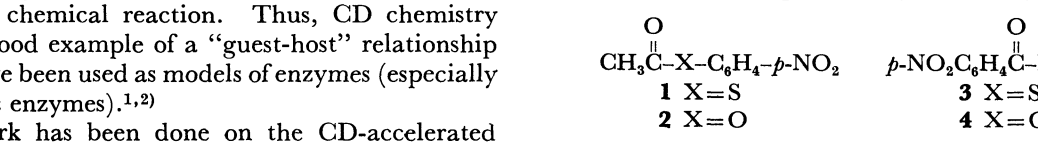
It is well known that cyclodextrins (CDs) affect the rates of many kinds of chemical reactions. The most important feature of reactions involving CDs as accelerating (or retarding) reagents is the formation of inclusion complexes of substrates with CD, which is followed by chemical reaction. Thus, CD chemistry provides a good example of a "guest-host" relationship and CDs have been used as models of enzymes (especially of hydrolytic enzymes).[1,2]

Much work has been done on the CD-accelerated cleavage of oxygen esters,[1-6] showing clear-cut differences of the effect of CD depending on the leaving groups. Thus, cleavage of phenyl esters are accelerated by CD, whereas cleavage of alkyl esters are decelerated by CD, which was attributed to either the high pK_a of the leaving group (alcohol) or non-productive binding.[1,4] Cleavage of phenyl esters is a nucleophilic reaction by the secondary hydroxyl group of CD, resulting in acyl-CD and phenol.

In spite of the multitude of information on the cleavage of oxygen esters, however, no report has been made about the effect of CD on the cleavage thiocarboxylic *S*-esters. This report describes the rate effect of α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) on the cleavage of *S*-*p*-nitrophenyl thioacetate (**1**), an *S*-phenyl thioester, and *S*-ethyl *p*-nitrothiobenzoate (**3**), an *S*-alkyl thioester. For purposes of comparison, the rate effect of α-CD and β-CD on the cleavage of the corresponding oxygen esters, *p*-nitrophenyl acetate (**2**) and ethyl *p*-nitrobenzoate (**4**) was also determined.

The comparison between **1** and **2** can be a good test to check whether or not the cleavage of these substrates proceeds *via* tetrahedral intermediates. If the cleavage involves formation of tetrahedral intermediates, the rate constant of the CD-accelerated cleavage of **1** should be (approximately) equal to that of **2**. Alternatively, in an S_N2 type reaction, an –SR group of **1** should be displaced at least 250 times faster than an –OR group of **2**.[7,8]

Furthermore, the effect of CD on the cleavage of **3** (whether acceleration or deceleration) can probe whether the high pK_a of the leaving group or non-productive binding is the important factor in the deceleration of the cleavage of alkyl esters by CD, since ethanethiol has smaller pK_a (10.5)[9] than the nucleophilic hydroxyl group of CD (around 12).[4,6]



Experimental

Materials. **1** was synthesized from acetyl chloride and thiophenol, and recrystallized from cyclohexane; mp 82 °C (lit, 82 °C,[10] 82.3—82.6 °C[11]). **2** was recrystallized from chloroform–hexane; mp 79.5 °C (lit, 79.5—80 °C[11]).

3 was synthesized from *p*-nitrobenzoyl chloride and ethanethiol, and purified by recrystallization from acidified aqueous ethanol; mp 70.0—70.2 °C (lit, 69.5—70 °C,[7] 67—68 °C[12]). **4** was recrystallized from aqueous ethanol; mp 56.5—57 °C (lit, 56.5—57 °C). α-CD and β-CD were purified by recrystallization from water. All water used for kinetic studies was doubly distilled.

Kinetics. The cleavage of **1**—**4** was carried out at 25 °C and was followed at 410 nm (**1** and **2**) and 300 nm (**3** and **4**) by a Cary 14 spectrophotometer. Reactions were initiated by addition of stock solution of **1**—**4** in acetonitrile to a thermostatted buffer solution. In the reactions of **3**, buffer solution was flushed with nitrogen before the addition of the sample stock solution, since an accelerating effect by oxygen was reported.[7]

The kinetic data (k_{obsd}) were treated based on the following reaction scheme and the corresponding Lineweaver-Burk plots:[1]

$$\text{Ester} + \text{CD} \underset{K_d}{\rightleftharpoons} \text{Complex} \xrightarrow{k_c} \text{Products};$$

(k_{un}: Ester + CD → Products)

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

Results

Both α-CD and β-CD showed acceleration of the cleavage of **1** and **2**. The rate constants of the cleavage of **1** and **2** in the presence of CD (in large excess),

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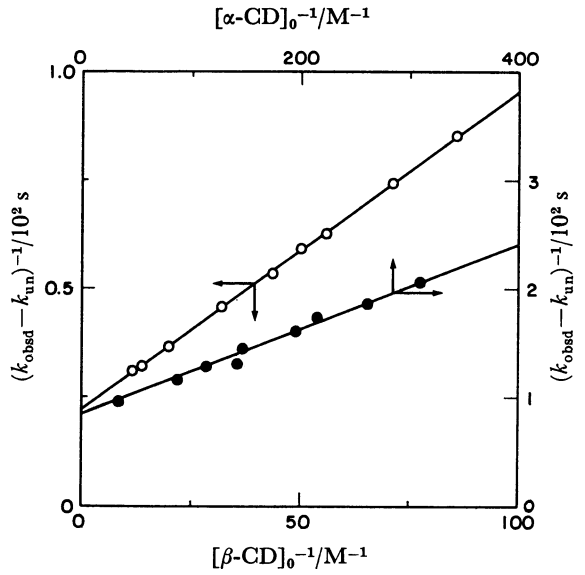

Fig. 1. Plot of $1/(k_{obsd}-k_{un})$ *versus* $1/[C]_0$ of the α-CD (●)- and β-CD (○)-accelerated cleavage of **1**. pH 10.5, I=0.2 M, 25 °C.

k_{obsd}, asymptotically approached a maximum value as $[CD]_0$ increased, showing the complex formation of CD with **1** and **2**. Thus, the rate constant of the cleavage of **1** and **2** complexed with CD, k_c, and the dissociation constant of the CD-**1** (or **2**) complex, K_d, were determined by plotting $1/(k_{obsd}-k_{un})$ *vs.* $1/[CD]_0$ as shown in Fig. 1. Here k_{un} is the rate constant in the absence of CD (see Experimental).

TABLE 1. VALUES OF k_c AND K_d FOR THE α-CD AND β-CD ACCELERATED CLEAVAGE OF **1** AND **2**[a,b]

Cyclodextrin	Substrate	$\frac{k_c}{10^{-3}\,s^{-1}}$	$\frac{K_d}{10^{-3}M}$
α-CD	**1**	15±1	4.8±0.3
	2	16±1	12±2
β-CD	**1**	48±3	3.0±0.4
	2	42±3	6.1±0.7

a) pH 10.5, I=0.2 M. b) k_{un}'s are 2.8×10^{-3} s^{-1} for **1** and 4.6×10^{-3} s^{-1} for **2**.

Table 1 lists the values of k_c and K_d for the CD-accelerated cleavage of **1** as well as those of **2**.

Significantly, k_c of **1** accelerated by α-CD is almost equal to that of **2** accelerated by α-CD. β-CD also gave almost identical values of k_c of **1** and **2**. Thus, *S*-phenyl thioester and phenyl ester are cleaved by CD at similar rates. This result shows that a rate-limiting step occurs before the hydroxyl or mercapto group partitions, since the cleavage of the *S*-phenyl thioester should be faster than that of the phenyl ester by at least 250 fold if breaking of the bond between the carbonyl atom and the sulfur atom (or oxygen atom) were rate-limiting.

The change of absorbance at 300 nm in the cleavage of **3** by CD did not follow first-order kinetics (Fig. 2). This result indicates a stepwise pathway (acylation of CD by **3**, followed by hydrolysis of acyl-CD, as shown

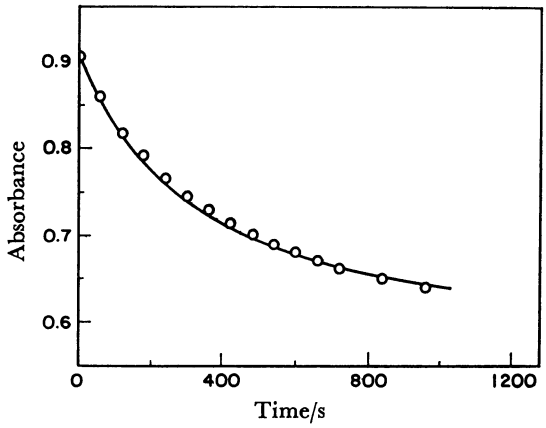

Fig. 2. Change of absorbance at 300 nm for the hydrolysis of **3** in the presence of 0.02 M α-CD at pH 10.5, 25 °C; blank circles are experimentally obtained, whereas the solid line is the theoretical line calculated by using $k_1=1.5\times10^{-3}$ s^{-1} and $k_2=7.5\times10^{-3}$ s^{-1} in Eq. 2.

by Eq. 1 which is consistent with CD-accelerated hydrolyses of phenyl esters.

$$\mathbf{3} + \mathrm{CD} \xrightarrow{k_1} p\text{-nitrobenzoyl-CD (}\mathbf{5}\text{)} + \text{ethanethiol}$$
$$\downarrow k_2 \qquad (1)$$
$$p\text{-nitrobenzoate (}\mathbf{6}\text{)} + \mathrm{CD} + \text{ethanethiol}$$

Of the species in Eq. 1, **3**, **5**, and **6** all absorb at 300 nm (molar absorption coefficients ε_3, ε_5,[13] and ε_6= 8.97×10^3, 2.04×10^3, and 5.84×10^3 M^{-1} cm^{-1}). When the rate constant of the hydrolysis of **5** (k_2) is comparable to that of the cleavage of **3** in the presence of CD (k_1) in magnitude, the intermediate **5** accumulates. Thus, k_1 was determined by fitting the observed absorbance at 300 nm (A) to the theoretical values calculated by Eq. 2.

$$A=\frac{A_0}{\varepsilon_3}\left[\varepsilon_3\exp(-k_1t)+\varepsilon_5\frac{k_1}{k_2-k_1}\{\exp(-k_1t)-\exp(-k_2t)\}\right.$$
$$\left.+\varepsilon_6\left\{1+\frac{k_2\exp(-k_1t)-k_1\exp(-k_2t)}{k_1-k_2}\right\}\right], \qquad (2)$$

where A_0 is the initial absorbance, and the first, second, and third terms of Eq. 2 correspond to the absorbance due to **3**, **5**, and **6**, respectively, in the reaction solution. k_2 was independently determined to be 7.5×10^{-3} s^{-1} at pH 10.5 using the change of absorbance at 260 nm in the hydrolysis of *m*-chlorophenyl *p*-nitrobenzoate in the presence of 0.02 M α-CD according to the previous paper.[4]

As shown in Fig. 2, the change of the absorbance at 300 nm in the hydrolysis of **3** in the presence of 0.02 M α-CD (blank circles) satisfactorily fits the theoretical line calculated from Eq. 2 by using $k_1=1.5\times10^{-3}$ s^{-1}.

Small deviations of the experimental results from the theoretical line are attributable to **3** alone not complexed with α-CD, being hydrolyzed by hydroxide ion without forming **5** as an intermediate. A rough estimate of the amount of complexed **3** was made by using the dependence of the initial slope of the absorbance change upon $[\alpha\text{-CD}]_0$, indicating that about 70% of **3** is complexed with α-CD when $[\alpha\text{-CD}]_0$ is 0.02 M. Similarly, k_1 of **3**

in the presence of 0.015 M β-CD, where about 80% of **3** is complexed with β-CD, was determined to be 5×10^{-4} s^{-1}.

On the other hand, the absorbance change at 300 nm in the hydrolysis of **4** in the presence of CD did not show any measurable deviation from first-order kinetics and the rate constant of its cleavage was determined by the usual first-order equation. This fact indicates that the cleavage of **4** does not follow Eq. 1 but rather proceeds by alkaline reaction, which is discussed later.

Table 2. Effects of α-CD and β-CD on the cleavage of **3** and **4**[a]

CD	Substrate	Observed rate constant (10^{-4} s^{-1})	Rate effect by CD[d]
α-CD[b]	**3**	15±2	6.5
	4	2.6±0.05	1.0
β-CD[c]	**3**	5±1	2.2
	4	1.5±0.03	0.57

a) pH 10.5, I=0.2 M, and 25 °C. b) 0.02 M. c) 0.015 M. d) The ratio of the rate of cleavage of **3** and **4** in the presence of CD to that in its absence.

Table 2 lists the rate constants of the cleavage of **3** and **4** in the presence of CD determined by the above method. It was found that 0.02 M α-CD and 0.015 M β-CD show 6.5 and 2.2 fold acceleration of the cleavage of **3**, an *S*-alkyl thioester. This is in contrast to (about 2 fold) deceleration by β-CD and virtually no effect by α-CD in the cleavage of **4**, an alkyl ester.

Discussion

The almost identical k_c's of **1** and **2** definitely showed the formation of tetrahedral intermediates in the CD-accelerated cleavage of esters. The change of leaving group from *p*-nitrophenol to *p*-nitrothiophenol does not produce a significant effect on k_c, since the formation of tetrahedral intermediates (rather than their breakdown to products) is rate-determining. This occurs because of the larger pK_a of the attacking secondary hydroxyl groups of the CD (around 12) than the pK_a's of the leaving groups (the pK_a's of *p*-nitrophenol and *p*-nitrothiophenol are 7.15[15] and 4.47[11]).

The formation of tetrahedral intermediates was also confirmed in the CD-accelerated cleavage of **3** by the deviation of the absorbance at 300 nm from first-order kinetics (Fig. 2). In the CD-accelerated cleavage of **1** and **2**, however, no deviation from first-order kinetics was observed in spite of the formation of tetrahedral intermediates, since spectroscopy directly followed the cleavage of **1** and **2** by release of *p*-nitrothiophenol and *p*-nitrophenol.

Now it is evident that formation of a tetrahedral intermediate is a common feature among serine protease-catalyzed[11,16] and CD-accelerated hydrolyses of esters (as well as alkaline hydrolyses of esters[17]).

The large difference of the effect of CD on the cleavage of **3** (acceleration by both α-CD and β-CD) from the effect on the cleavage of **4** (retardation by β-CD and no effect by α-CD) is mainly attributable to the difference of pK_a's of the leaving groups. Ethanethiol and ethanol have pK_a's of 10.5[9] and 16.0.[18] As the secondary hydroxyl group of CD has a pK_a around 12,[4,6] the formation of tetrahedral intermediate is rate-determining in the CD-accelerated cleavage of **3**, which can be facilitated by complexation. In the cleavage of **4**, however, reaction involving a tetrahedral intermediate produced by nucleophilic attack of CD is unlikely, since its breakdown to product is energetically unfavorable. Thus, the cleavage of **4** in the presence of CD proceeds largely *via* an alkaline reaction. Here, CD shows retardation because of steric hindrance (protection of the substrate by the wall of the CD) and electrostatic repulsion between two anions (the secondary hydroxide anion of CD and OH^-); with α-CD this effect is very small.

The above argument is consistent with the results of the aminolysis of **3** and **4**, showing no reaction of *n*-butylamine (pK_a 10.6[19]) with **4** as opposed to its considerable reaction with **3**.[7]

Non-productive binding can be partly responsible for the different effect of CD on **3** and **4**. Two structures of inclusion complexes are possible, *i.e.* structure A in which the *p*-nitrophenyl portion of **3** (or **4**) is included in the cavity of CD and the ethanol (or ethanethiol) portion protrudes from the secondary hydroxyl side of the cavity, and structure B in which the ethanol (or ethanethiol) portion is located inside the cavity and the rest is located outside. Of these two structures, only structure B is productive, whereas structure A is non-productive since cleavage the carbonyl carbon atom between and the ether oxygen (or sulfur) atom bond through nucleophilic attack by CD is sterically impossible as shown by CPK molecular models.

Since the sulfur atom is larger in size than the oxygen atom and is more apolar, thioester **3** assumes a structure (where the ethylthio portion is included in the apolar cavity of CD) more favorably than the oxygen ester **4** does (structure B). In other words, the proportion of structure B in the CD-**3** complex is larger than that in the CD-**4** complex. Since structure A is nonproductive and structure B is productive, as described above, the CD-**3** complex is more reactive than the CD-**4** complex.

In conclusion, it was found that CD accelerates the cleavage of *S*-phenyl thioester (**1**), *S*-alkyl thioester (**3**), and phenyl ester (**2**). However, the cleavage of the alkyl ester (**4**) was retarded by β-CD but was not affected by α-CD. The CD-accelerated cleavage of **1**—**3** proceeds *via* tetrahedral intermediates, which is consistent with serine protease-catalyzed reactions. Thus, the similarity between CD reactions and enzymatic reactions conforms to not only the overall pathway, as proposed before, but also the more detailed mechanism. The present finding shows that CD is an excellent model of serine proteases.

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14) Similar treatment of data at 320 nm gave k_1 of 1.4×10^{-3} s^{-1}, which is identical with the result obtained at 300 nm within experimental error.

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