BINDING AND CATALYTIC BEHAVIOR OF MODIFIED γ-CYCLODEXTRINS

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ABSTRACT. Modified γ-cyclodextrins appended by an aromatic moiety show unique binding behavior, including a guest molecule together with the appended moiety in their γ-cyclodextrin cavities. Excimer formation, photoresponsive binding, association with β-cyclodextrin and enhanced catalytic activity have been explained on this basis.

1. INTRODUCTION

Cyclodextrins (CDs) are cyclic oligomers of D-glucose and extensive studies on binding and catalysis by them and their derivatives have been performed.[1] Most investigations had been confined to α-CD (cyclohexa-amylose) and β-CD (cyclohepta-amylose), which have suitable cavity sizes for many organic compounds, before we showed the phenomenon of inclusion of two guest molecules in the cavity of γ-CD (cycloocta-amylose).[2] The unique binding ability of γ-CD has excited much attention and the chemistry of γ-CD is now growing. We have shown that two types of complexes are possible, one (Type **A**) including a couple of same guests and another (Type **B**) including a couple of different guests as shown below.

A **B** **C** **D**

The enhanced excimer fluorescence observed for naphthalene, pyrene and their derivatives in the presence of γ-CD is evidence for the Type A complexation.[2~13] Acceleration of dimerization of anthracene derivatives by γ-CD is obviously related to this type of complexation.[14]

A phenomenon which suggests the Type **B** complex formation is the

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fluorescence of a chromophore (X) enhanced by an inert additive (Y). In this case, we called Y a spacer or a space-regulating molecule which narrows the large γ-CD cavity.[15] The charge-transfer complex formation enhanced by γ-CD is another evidence of the Type **B** complex formation.[16,17]

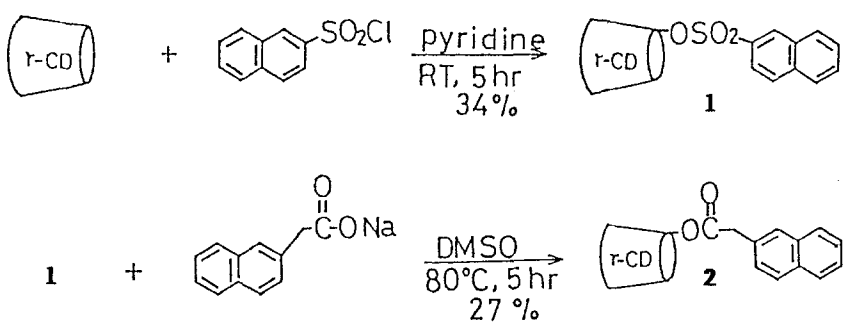
The γ-CD - pyrene (1:2) complex shows an exciton coupling type of circular dichroism in the pyrene absorption regions, which indicates that the two pyrene molecules included in the γ-CD cavity are facing each other, taking S-helical relationship.[3] On the other hand, designed guests which have two naphthyl moieties at both ends show enhanced excimer fluorescence in the presence of γ-CD, taking a folded form in the γ-CD cavity (Type **C**).[9~13] Circular dichroism spectrum of one of the systems was checked, but no exciton coupling type of dichroism band was observed, indicating the absence of chiral orientation of the chromophores in the γ-CD cavity.[11]

Guest molecules bearing different moieties at the opposite ends have been shown to be Type **D** complexes.[18] In the cases, the guest molecules are p-nitrophenyl esters bearing a long alkyl chain (tail) and enhanced acceleration in ester hydrolysis by γ-CD was observed, arising from the formation of the Type **D** complexes where X is a p-nitrophenyl moiety and Y is an alkyl chain.

All these experiments have been performed with native γ-CD. In this review we wish to describe the phenomena observed with modified γ-CDs.

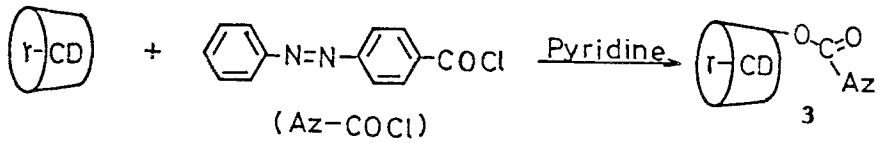
2. SYNTHESIS

There are two methods for preparation of modified γ-CDs, direct and indirect procedures. We usually used an indirect procedure, in which 2-naphthalenesulfonyl moiety was introduced into one of primary hydroxyls of γ-CD and then the moiety was substituted by other moiety with its sodium or pottasium salt in dimethyl sulfoxide (DMSO).



We found that 2-naphthalenesulfonyl chloride is better than p-toluenesulfonyl chloride in respect of lowering the amounts of di- and tri-substituted by-products. Purification of the crude products was performed by recrystallization from water or a mixed solvent of n-butanol, ethanol and water and/or chromatography with Sephadex G-15.

A direct procedure was used for preparation of azobenzene-appended γ-CD.



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The reaction also afforded the product modified at a primary hydroxyl of γ-CD.

3. INDUCED-FIT TYPE OF COMPLEXATION[19]

The naphthyl moiety of **2** can be used as a probe to monitor its environment in circular dichroism (c.d.) measurements. We observed that the molecular ellipticities of **2** in the absorption regions of the naphthyl moiety are remarkably enhanced by the addition of guests. Figure 1 shows the c.d. variations induced by cyclohexanol.

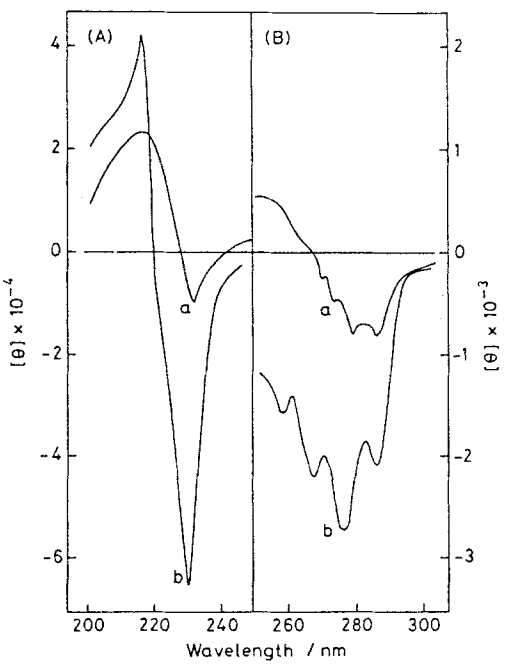
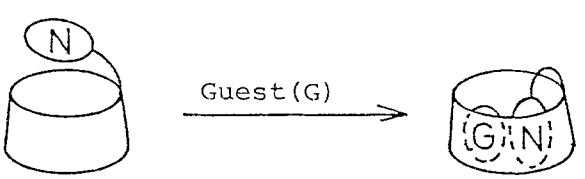


Figure 1. Circular dichroism spectra of **2** (A, 7.5×10^{-6} M; B, 1.5×10^{-4} M) in water at 25 °C, alone (a) or in the presence of cyclohexanol (b, 0.086 M).

The phenomenon may be ascribed to the limited mobility of the naphthyl moiety when involved together with a guest molecule in the chiral γ-CD cavity.

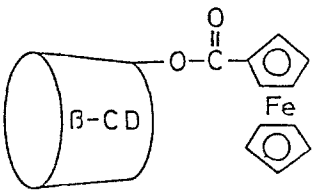


N: naphthyl moiety

This means that the host conformation changes so as to include a substrate tightly, the behavior being similar to the induced-fit binding of substrates proposed for enzymes. In this system, the appended naphthyl moiety acts as a spacer which narrows the large γ-CD cavity and allows the inclusion of the guest molecules.

The c.d. behavior of **2** was analyzed by using an equation for 1:1 complexation,[20] the binding constants being 55 and 5670 l mol^{-1} at 25 °C for cyclohexanol and (-)-borneol, respectively.

It should be noted that the c.d. behavior of β-CD derivatives is usually quite different, showing a reduction in the c.d. intensity by guest addition; e.g. we observed the decrease in the c.d. intensity of ferrocene-appended β-CD **4** when guests are added.[21] The ferrocene moiety of **4** included in the β-CD cavity is replaced by added guests, getting out of the chiral β-CD cavity.



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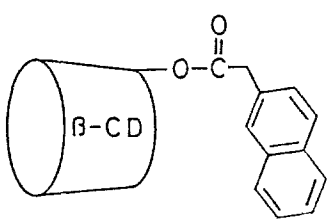
The spacer effect observed for **2** promises many γ-CD derivatives with a variety of cavity sizes by changing the size and shape of the appended moiety. Such cavity designing cannot be achieved by β-CD derivatives, since their cavity size is too small to include both an appended moiety and a guest molecule.

4. SPACER EFFECTS ON CATALYTIC ACTIVITY OF γ-CD DERIVATIVES[21]

Hydrolysis of esters by CD hydroxyls within complexes has intensively been studied as esterase models. Heretofore, much effort has been devoted by using α-CD, β-CD and their derivatives. γ-CD may be an unsuitable host for usual substrates because of the large cavity size. We have studied the spacer effect of the appended naphthalene moiety of **2** on its catalytic activity in ester hydrolysis of p- and m-nitropheny-acetates and compared the behavior with that of the corresponding β-CD derivative **5**.



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The reaction proceeds based on the following Scheme

$$\mathrm{CD} + \mathrm{S} \rightleftharpoons \mathrm{CD{:}S} \xrightarrow{k_2} \mathrm{CD'} + \mathrm{P}$$

$$\mathrm{CD{:}S} \xrightarrow{k_{un}} \mathrm{P}$$

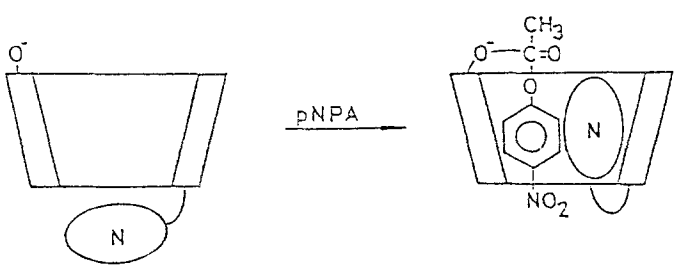
where k is the rate constant of hydrolysis, k_{un} in the absence of CD, k_2 in the complex, S is substrate and P is product. The kinetic data obtained in water (Tris buffer, pH 8.70) are listed in Table I.

Table I. Kinetic parameters for ester hydrolysis by native and modified cyclodextrins[a]

host	substrate[b]	solvent[c]	$k_2 \times 10^4$ s^{-1}	Km[d] mol l^{-1}	$(k_2/Km) \times 10^2$ l mol^{-1} s^{-1}
γ-CD	pNPA	H_2O	6.3	11.5	5.5
2	pNPA	H_2O	16.7	2.50	68.5
β-CD	pNPA	H_2O	9.9	8.27	11.9
γ-CD	mNPA	H_2O	13.4	32.9	4.1
2	mNPA	H_2O	8.6	1.95	44.4
β-CD	mNPA	H_2O	21.0	5.8	37.0
γ-CD	pNPA	30% DMSO	1.66	1.74	9.6
2	pNPA	30% DMSO	4.33	2.94	14.8
β-CD	pNPA	30% DMSO	1.90	4.09	4.7
5	pNPA	30% DMSO	1.80	1.46	12.4

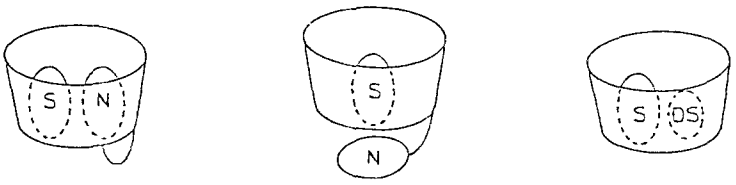
[a] Measurements were performed at 25 °C for H_2O solutions and 40 °C for DMSO solutions. [b] pNPA: p-nitrophenyl acetate; mNPA: m-nitrophenyl acetate. [c] H_2O: Tris buffer pH 8.70; 30% DMSO: 30% DMSO - 70% H_2O v/v (pH 8.92). [d] Dissociation constant of the substrate-cyclodextrin complex.

The γ-CD derivative **2** shows an increased k_2 value and a decreased dissociation constant, Km, for p-nitrophenyl acetate (pNPA), resulting in 12-fold increase in the apparent overall hydrolysis rate k_2/Km in comparison with the corresponding values for γ-CD. This result may arise from the spacer effect of the naphthyl moiety of **2** in the hydrolysis within the complex.



Comparative studies for **2** and **5** were done in 30% DMSO solution because of poor solubility of **5** in water. The kinetic data obtained in 30% DMSO solution (pH 8.92) at 40 °C were also listed in Table 1. Attachment of the naphthyl moiety to γ-CD accelerates the rate and decreases the binding. The attachment of the same moiety to β-CD slightly decreases the k_2 value and results in the stronger binding.

The data suggest that the binding behavior is different for **2** and **5**, the appending naphthyl moiety acting as a spacer for **2** and a simple floor for **5** as shown by **E** and **F**, respectively.



E **F** **G**

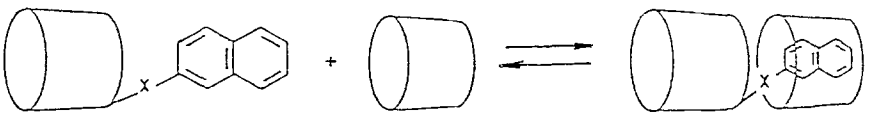
(S: substrate; DS: DMSO)

It should be noted that facilitated complex formation by DMSO is seen in the result that γ-CD shows 6.6 times stronger binding in 30% DMSO solution than in water, this behavior contrasting to the weakened binding of **2** in the presence of DMSO. Since DMSO weakens hydrophobic interactions because of its lipophylic nature, this improvement in binding is abnormal, and it might reflect the formation of a ternary complex **G** of γ-CD, substrate and DMSO.

As described above, the appending naphthyl moiety for **2** is likely to play a role as a spacer in ester hydrolysis. There remains a problem, however, whether the moiety acts also as a simple floor (**F**) to some extent or not.

5. ASSOCIATION OF NAPHTHALENE-APPENDED γ-CD WITH β-CD[23]

Naphthalene-appended γ-CDs **1** and **2** exhibit enhanced fluorescence on addition of β-CD, indicating a change in the environment around the naphthyl moiety from the polar medium to less polar one, and showing that the appending naphthyl moiety fits into the hydrophobic β-CD cavity. The association of different kinds of CD units is thus attained by using the naphthyl moiety of **1** and **2** as a connector.



γ-CD β-CD γ-CD β-CD

The analyses of the fluorescence data gave association constants of 98 and 217 mol^{-1} l for **1** and **2**, respectively. The smaller association constant for **1** may be related to the length of the naphthyl arm: i.e. the longer arm of **2** is better for such association. This association behavior can be a simple example for molecular organization.

6. PHOTORESPONSIVE γ-CD[24]

The cis-trans isomerism of azobenzene can be used as a photoresponsive "switch" to regulate binding ability of CD when an azobenzene moiety is attached to CD. The photoresponsive CD reported first is azobenzene-capped β-CD in which the cap structure changes, reversibly enlarging the hydrophobic cavity around substrate in its cis form.[25]

In the case of azobenzene-appended γ-CD (**3**), we observed different c.d. behavior depending on the size of substrate in both trans and cis forms. Compound **3** before ph otoirradiation represents induced c.d. bands in the azobenzene π-π* (332 nm) and n-π*(410 nm) regions. The π-π* c.d. band changes remarkably on addition of guest molecules; it is enhanced with a wavelength shift to 343 nm by cyclohexanol, whereas it nearly vanishes in the presence of excess (-)-borneol. These results suggest that two kinds of complexes **H** and **I** are formed depending on the guest size, **H** for smaller guests and **I** for larger guests.

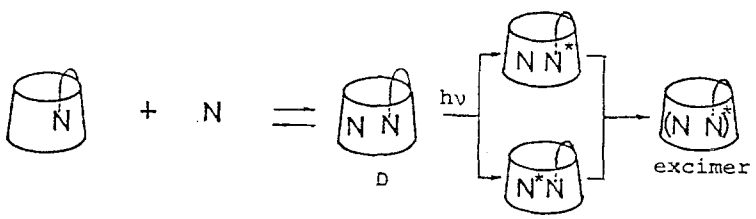
H

I

The binding constants of the trans form are 78, 105, 144, 733 and 2870 mol^{-1} l for cyclohexanol, anisol, benzyl alcohol, (+)-fenchone and (-)-borneol, respectively. After photoirradiation, the c.d. pattern of **3** is changed to the one which has a decreased positive c.d. in the π-π* region and an enhanced negative c.d. in the n-π* region. The analyses of the variations of the n-π* c.d. band induced by guest addition gave binding constants of the cis form , 1630 and 5790 mol^{-1} l for (+)-fenchone and (-)-borneol, respectively. These values of the cis form are 2-fold larger than those of the trans form. Thus, photo-regulation in complex formation has been shown to be possible with this system. It should be noted that such analysis based on 1:1 complexation was not successful for smaller guests probably owing to the mixing of 1:2 host-guest complexation.

7. EXCIMER FORMATION OF NAPHTHALENE-APPENDED γ-CD[26]

The naphthyl moiety of **2** forms an excimer with naphthalene as shown in the following scheme.



When (-)-borneol was added to the system, excimer fluorescence was depressed but normal fluorescence was enhanced, this behavior indicating that (-)-borneol was involved in the cavity of **2** in place of naphthalene.

8. ENERGY TRANSFER IN THE CAVITY OF MODIFIED γ-CD[27]

The fluorescence intensity of **2** around 335 nm was observed to diminish when ketones were added to aqueous solutions of **2.** If the fluorescence quenching occurs according to the static process, that is, it occurs only within the complexes **J**, binding constants (K) can be equated to the slopes of the Stern-Volmer plots

$$I_o/I = 1 + K[Q]$$

J

where I_o and I are fluorescence intensities in the absence and presence of ketone and [Q] is the concentration of ketone. The order of the slopes, (-)-fenchone > di-isopropyl ketone > di-n-propyl ketone > diethyl ketone > acetone, parallels to the order of the bulkiness of the ketones. On the other hand, the association constants of (-)-fenchone and di-isopropyl ketone obtained by c.d. measurements are close to the values of the slopes of the Stern-Volmer plots, this suggesting that the slopes are related to the binding behavior of **2** for the ketones.

In the case of complete static quenching, the ratio of lifetimes in the absence and presence of ketones, τ_o/τ, should not be dependent on the ketone concentration. The Stern-Volmer plots obtained by lifetime measurements gave slopes which are smaller than those obtained by intensity measurements but cannot be negligible. Thus, both static and dynamic processes are mixed in this system. It should be noted however that the static quenching is predominant for the large guests.

9. FUTURE ASPECTS

In these works, we have shown that both appending moiety and guest molecule can be included in the same cavity. This phenomenon may enable us to construct the unique catalytic system **K** in which the appended moiety is catalytic one and both substrate and catalyst are included in the same cavity and catalytic action can proceed in the hydrophobic γ-CD cavity. Studies on such systems are now under way in our laboratory.

K

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