Ester Hydrolysis by a 2-Naphthylacetyl-substituted γ-Cyclodextrin

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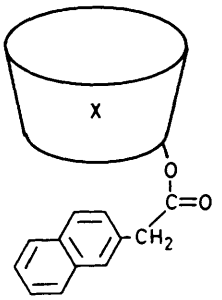
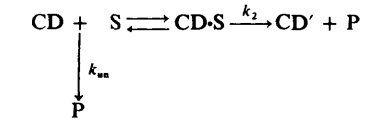
In comparison with native cyclo-octa-amylose (γ-cyclodextrin, γ-CD), a modified γ-CD (**1**), bearing an *O*-2-naphthylacetyl substituent, produced larger overall ester hydrolysis rates in water (pH 8.7). A 12-fold increase was observed for *p*-nitrophenyl acetate, arising from both an increased rate of intracomplex hydrolysis and stronger binding, and an 11-fold increase for *m*-nitrophenyl acetate, arising from stronger binding. The results were presumed to be due to spacer effects of the appended naphthalene moiety, which narrows the large γ-CD cavity. The behaviour of compound (**1**) was compared with that of cyclohepta-amylose (β-cyclodextrin, β-CD) bearing the same substituent.

Cycloamyloses (cyclodextrins) have the ability to bind a variety of guests in their molecular cavities. The observation of acceleration of chemical reactions by cyclodextrins has excited much interest as the basis for enzyme models.[1] In particular, hydrolysis of esters brought about by cyclodextrin hydroxy groups within complexes has been studied intensively in connection with esterase models. Previous work in this area has involved cyclohexa-amylose (α-cyclodextrin, α-CD), cyclohepta-amylose (β-cyclodextrin, β-CD), and their derivatives with appropriate functional groups.[2–7] Cyclo-octa-amylose (γ-cyclodextrin, γ-CD) was regarded as an unsuitable host for the usual substrates because of its larger cavity size. However, it has been reported recently that two guest molecules can be included in the large cavity of γ-CD.[8]

In an extension of this work, some modified γ-cyclodextrins have been prepared.[9] The γ-CD derivatives were found to undergo an induced-fit type of complexation by insertion of the appended moiety into the γ-CD cavity simultaneously with a guest molecule.[9a] The appended moiety, therefore, can act as a spacer which narrows the large γ-CD cavity. We now describe a study of ester hydrolysis by *O*-2-naphthylacetyl-γ-CD (**1**) which explores the roles of the appended moiety in substrate binding and subsequent reaction within the complex. The modified β-CD (**2**), which has the same appended moiety, was used as an alternative host for comparison.



(**1**) X = γ-CD
(**2**) X = β-CD



Scheme.

Results

In alkaline solution, the secondary hydroxy groups of cyclodextrin act as nucleophiles, and react with bound esters.[2,3] This reaction produces acylated cyclodextrin, so it is not catalytic. Nevertheless, it may be regarded as a partial enzyme model since it proceeds with Michaelis–Menten kinetics like most enzymic reactions (see Scheme, where k is the rate constant of hydrolysis, k_{un} that in the absence of cyclodextrin, and k_2 that in the complex; S is substrate and P is product).

The kinetic data obtained in water (Tris buffer, pH 8.70) at 25 °C are listed in the Table. The γ-CD derivative (**1**) shows an increased k_2 and a decreased dissociation constant, K_m, for *p*-nitrophenyl acetate (*p*NPA), resulting in a 12-fold increase in the apparent overall hydrolysis rate k_2/K_m in comparison with the corresponding values for γ-CD. A similar increase in k_2/K_m was observed when *m*-nitrophenyl acetate (*m*NPA) was used, but this increase is due to improved binding, which cancels the decreased k_2 value.

In this work, comparative studies with (**1**) and (**2**) were carried out in 30% Me_2SO solution because of poor solubility of (**2**) in water. Kinetic data obtained in 30% Me_2SO solution (pH 8.92) at 40 °C are also listed in the Table. Comparison of the values of k_2 and K_m for (**1**) and (**2**) with those of the native cyclodextrins showed opposite trends; both k_2 and K_m become larger for (**1**), but smaller for (**2**). The data suggest that the binding behaviour is different for (**1**) and (**2**), the appended naphthalene moiety acting as a spacer for (**1**) but a simple 'floor' for (**2**).

Discussion

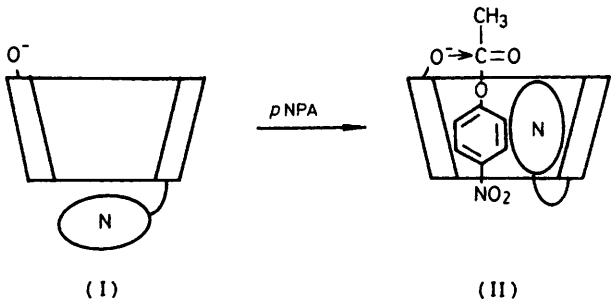
Hydrolysis of *p*NPA by (**1**) proceeds in water with 4.6-fold stronger binding and 2.7-fold larger k_2 than with γ-CD. Such behaviour would be expected to result from the spacer effect of the appended moiety; the acceleration in the reaction would be caused by fixing the substrate in the cavity at a position close to the rim of the γ-CD part of (**1**) (Figure 1). In the complex, the secondary hydroxy groups seem easily able to attack the carbonyl of the bound substrate.

Hydrolysis of *m*NPA by (**1**) proceeds differently under the same conditions, with k_2 only 64% of the value when γ-CD is used, but with 17-fold stronger binding. The cavity of γ-CD (internal diameter 8.5 Å) is too large for *p*NPA or *m*NPA alone, as shown by the fact that either substrate can be included in a α-CD, which has an internal diameter of 4.5 Å.[1] In spite of the lack of fit, however, the reaction was accelerated by γ-CD with values of 6.6 and 26.8 for k_2/k_{un} for *p*NPA and *m*NPA, respectively. Molecular models (CPK) reveal that the substrates should be

Table. Kinetic parameters for ester hydrolysis by native and modified cyclodextrins

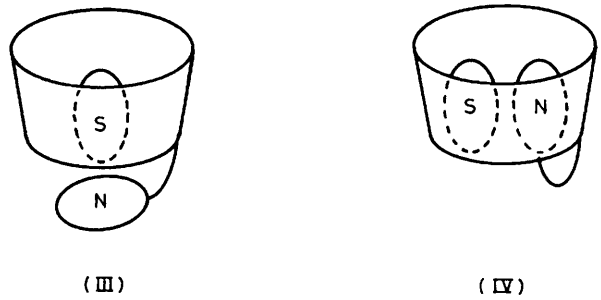
Host	Substrate[a]	Solvent[b]	T/°C	10^4k_2/s^{-1}[c]	K_m/ mmol l^{-1}[d]	$10^2(k_2/K_m)$/ l mol^{-1} s^{-1}
γ-CD	*p*NPA	H_2O	25	6.3 ± 0.5	11.5 ± 1.4	5.5 ± 0.6
(1)	*p*NPA	H_2O	25	16.7 ± 0.9	2.5 ± 0.3	68.5 ± 3.7
β-CD	*p*NPA	H_2O	25	9.9 ± 0.3[e]	8.27 ± 0.05[e]	11.9 ± 0.5[e]
γ-CD	*m*NPA	H_2O	25	13.4 ± 1.5	32.9 ± 4.1	4.1 ± 0.1
(1)	*m*NPA	H_2O	25	8.6 ± 0.1	1.95 ± 0.17	44.4 ± 3.6
β-CD	*m*NPA	H_2O	25	21.0 ± 2.4	5.8 ± 1.2	37.0 ± 3.7
γ-CD	*p*NPA	30% Me_2SO	40	1.66 ± 0.25	1.74 ± 0.29	9.6 ± 0.5
(1)	*p*NPA	30% Me_2SO	40	4.33 ± 0.04	2.94 ± 0.22	14.8 ± 1.0
β-CD	*p*NPA	30% Me_2SO	40	1.90 ± 0.23	4.09 ± 0.67	4.7 ± 0.3
(2)	*p*NPA	30% Me_2SO	40	1.80 ± 0.19	1.46 ± 0.19	12.4 ± 1.3

[a] *p*NPA = *p*-nitrophenyl acetate; *m*NPA = *m*-nitrophenyl acetate. [b] H_2O represents Tris buffer pH 8.70; 30% Me_2SO, 3:7 Me_2SO–H_2O v/v (pH 8.92). [c] Rate constant for intracomplex hydrolysis. [d] Dissociation constant of the substrate–cyclodextrin complex. [e] Reported values in ref. 11 are 11.5 × 10^{-4} s^{-1} for k_2, 8.3 mmol l^{-1} for K_m, and 14 × 10^{-2} l mol^{-1} s^{-1} for k_2/K_m.



(I) (II)

Figure 1. Induced-fit location of substrate in the modified γ-CD; N = naphthalene moiety



(III) (IV)

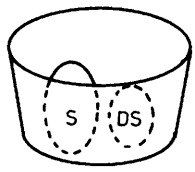
Figure 2. Schematic representations of the roles of the appended naphthalene moiety (N) as a simple 'floor' (III) or a spacer (IV) in the complexes with substrate (S)

tilted to approach the secondary hydroxy groups of γ-CD, that is, the carbonyl group of the substrate is too far from the rim hydroxy groups if the substrate is positioned in the centre of γ-CD with its axis parallel to the axis of γ-CD. The models also show that the substrate fits snugly in the cavity together with the appended naphthalene moiety [(II) in Figure 1].

The marked *meta* selectivity reported[3] for ester hydrolysis by α-CD and β-CD was not observed in the case of γ-CD, as shown by the almost equal values of k_2/K_m for *p*NPA and *m*NPA. Thus, we presume that the substrates do not take any specific orientation in the complexes of γ-CD. Detailed examination of the data, however, reveals that k_2 and K_m for *m*NPA are 2.1 and 2.9 times larger than those for *p*NPA, respectively. In contrast to the data obtained with γ-CD, the γ-CD derivative (**1**) shows opposite trends: k_2 and K_m for *m*NPA are only 51% and 78% of the corresponding values for *p*NPA. This case in *meta–para* selectivity may be ascribed to the spacer effect, although it is not clear why the reversion in substrate selectivity occurs.

Different effects of the appended naphthalene moiety are seen in the hydrolysis of *p*NPA in 30% Me_2SO solution. Attachment of the naphthalene moiety to γ-CD accelerates the reaction and decreases the binding. However, the attachment of the same moiety to β-CD slightly decreases k_2 and increases the binding. This difference is presumably related to the geometrical features of the complexes of (**1**) and (**2**). Since the cavity of (**2**) is not large enough to include both substrate and the appended naphthalene moiety, the geometry of the complex must be that shown as (III) in Figure 2. On the other hand, (**1**) can assume a different geometry (IV) because of its large cavity.

The reaction is expected to be accelerated if the position of the carbonyl group of substrate in the complexes is the required one for conversion into the tetrahedral intermediate by addition of



(V)

Figure 3. A plausible ternary complex in which both substrate (S) and dimethyl sulphoxide (DS) are included in the cyclodextrin cavity

hydroxy to the carbonyl group. Thus, the observed decrease in k_2 and increase in the binding for (**2**) suggest that the position of *p*NPA deviates from that required by the flexible 'floor,' probably with deeper binding. Similar situations have been observed in complexes of some β-CD derivatives with geometry (III).[10] In contrast with the case of (**2**), a shallower involvement, which enables the carbonyl group of the substrate to come closer to a cyclodextrin hydroxy group, seems to be attained in the complexes (IV). It should be noted, however, that the behaviour of (**1**) in 30% Me_2SO is different from that in water, since both increased rate and strong binding were achieved in water. Some participation of Me_2SO in the reaction might occur in the mixed solution.

Facilitated complex formation by Me_2SO is reflected in the result that γ-CD shows 6.6 times stronger binding in 30% Me_2SO solution than in water; this behaviour contrasts with the weakened binding of (**1**) in the presence of Me_2SO. Since Me_2SO is lipophilic and weakens hydrophobic interactions, this improvement in binding is abnormal; it might reflect the formation of a ternary complex (V) (Figure 3) of γ-CD,

substrate, and Me_2SO. A similar ternary complex seems to be formed with β-CD, though to a lesser extent because of its smaller cavity size, as shown by the 2-fold stronger binding in 30% Me_2SO solution. The weakened binding of (**1**) in 30% Me_2SO solution is consistent with the postulated complex (IV) in which the cavity is fully occupied by substrate and the appended naphthalene.

In conclusion, the appended naphthalene moiety in (**1**) is likely to play a role as a spacer in ester hydrolysis. There remains a problem, however, as to whether the moiety acts also as a simple 'floor' (III) to any extent. In connection with this study, we are now constructing some γ-CD systems which have incorporated catalytic moieties.

Experimental

β-CD (Tokyo Kasei) was recrystallized from water and dried *in vacuo*. γ-CD was a gift from Nihon Shokuhin Kako and used without further purification. Water content in the samples of β-CD and γ-CD was determined by elemental analysis (nine and three water molecules per CD unit for β-CD and γ-CD, respectively) to enable solutions to be prepared with accurately known CD concentrations.

Compound (**1**), which has an *O*-naphthylacetyl moiety at one of the primary hydroxy groups of the CD unit, was prepared by the procedure reported previously.[9b] Compound (**2**) was synthesized in the same manner from sodium 2-naphthylacetate and 6-*O*-tosyl-β-CD; R_F 0.59 (butan-1-ol–ethanol–water, 5:4:3 v/v); $\nu_{max.}$ 1 720 cm^{-1}; δ [$(CD_3)_2SO$; 20 °C] 3.0—3.8 (br, 42 H, CD protons other than H-1 and OH) and 7.3—8.0 (7 H, aromatic); $\lambda_{max.}$ (50% Me_2SO) 278 nm (ε 4 600). The samples of (**1**) and (**2**) are tetra- and penta-hydrated, respectively, as determined by elemental analysis.

The dimethyl sulphoxide (Me_2SO) used in the kinetic runs was Dotite Spectrosol grade. A Tris buffer (0.05 mol l^{-1}) with pH 8.70 was used directly or to prepare a mixed 7:3 buffer–Me_2SO system (30% Me_2SO solution). The mixed solvent had pH 8.92.

The kinetic runs were performed with a Shimadzu UV-360 spectrophotometer. Reaction temperature was kept at 25 ± 0.1 °C for water solutions and 40 ± 0.1 °C for 30% Me_2SO solutions by using a Haake water circulation instrument.

A kinetic run for *p*NPA was initiated by injecting a solution (10 μl) of substrate in acetonitrile into the catalyst solution (2.5 ml; 0.4—4.0 mmol l^{-1} in CD) to make the solution 25 μmol l^{-1} in substrate; the absorbance at 410 nm was monitored as a function of time. In the case of *m*NPA, the absorbance at 390 nm was monitored for a solution 50 μmol l^{-1} in substrate.

The data were fitted to a simple exponential curve for a first-order reaction. Analyses of the data were performed by using Lineweaver–Burk plots. The rate constants shown in the Table represent the averages of three runs. The k_{un} values in H_2O were 9.50×10^{-5} s^{-1} for *p*NPA and 5.00×10^{-5} s^{-1} for *m*NPA. The value in 30% Me_2SO was 6.80×10^{-5} s^{-1} for *p*NPA.

Acknowledgements

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References

1 M. L. Bender and M. Komiyama, 'Cyclodextrin Chemistry,' Springer-Verlag, Berlin, 1978.
2 N. Heinrich and F. Cramer, *J. Am. Chem. Soc.*, 1965, **87**, 1121.
3 R. L. van Etten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Am. Chem. Soc.*, 1967, **89**, 3242, 3253.
4 R. Breslow, M. F. Czarniecki, J. Emert, and H. Hamaguchi, *J. Am. Chem. Soc.*, 1980, **102**, 762.
5 G. Trainor and R. Breslow, *J. Am. Chem. Soc.*, 1981, **103**, 154.
6 R. Breslow, G. Trainor, and A. Ueno, *J. Am. Chem. Soc.*, 1983, **105**, 2739.
7 Y. Kitaura and M. L. Bender, *Bioorg. Chem.*, 1975, **4**, 237.
8 (*a*) A. Ueno, K. Takahashi, and T. Osa, *J. Chem. Soc., Chem. Commun.*, 1980, 921; (*b*) N. Kobayashi, Y. Hino, A. Ueno, and T. Osa, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1849, and references therein.
9 A. Ueno, Y. Tomita, and T. Osa, (*a*) *J. Chem. Soc., Chem. Commun.*, 1983, 976; (*b*) *Chem. Lett.*, 1983, 1635; (*c*) *Tetrahedron Lett.*, 1983, **24**, 5245.
10 K. Fujita, A. Shinoda, and T. Imoto, *J. Am. Chem. Soc.*, 1980, **102**, 1161.
11 A. Harada, M. Furue, and S. Nozakura, *Macromolecules*, 1976, **9**, 705.

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