DIFFERENCE IN GUEST BINDING CAPABILITY BETWEEN CYCLODEXTRINS HAVING AN ACETYLAMINO MOIETY AT PRIMARY- AND SECONDARY-HYDROXYL SIDES: THE EFFECT OF RING DISTORTION

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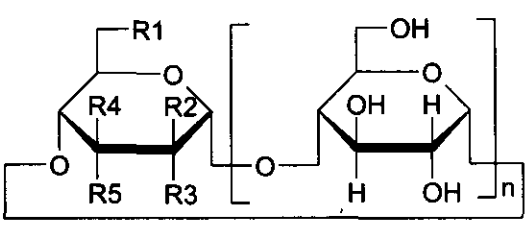
Abstract- A γ-cyclodextrin (γ-CyD) derivative possessing an acetylamino moiety at its secondary hydroxyl side can bind only one guest molecule into its distorted cavity. The γ-CyD derivative could discriminate the chirality of some terpene guests in binding strength.

There has been many reports on the complexation of cyclodextrins (CyDs) for organic compounds to improve their solubility in aqueous solutions and to raise their stability against oxidation and hydrolysis.[1,2] Chemically modified CyDs have also been investigated on the complexation behavior, especially as chromogenic chemosensors.[3] The major part of the studies on modified CyDs has been directed toward CyD derivatives modified at the primary-hydroxyl side, and the molecular recognition behavior of secondary-hydroxyl side-modified CyDs has not been studied enough.[4-6] Some modified CyDs bearing a pendant moiety at the secondary-hydroxyl side have a distorted cavity as a result of nucleophilic ring opening of the 2,3-mannoepoxide, which is converted from 2-*O*-tosylates of CyDs. We previously observed that on pyrene-modified γ-CyDs, modification at the secondary-hydroxyl side was favorable in binding guest compounds.[6] We considered that the strong guest binding property of the modified γ-CyD having a pyrene moiety at the secondary-hydroxyl side resulted from the distorted conformation as well as the hydrophobic cap effect. However, we were not able to separate the factors each other. In addition, Murakami *et al.* have presented that



Host	n	R1	R2	R3	R4	R5
β-CyD	6	OH	H	OH	OH	H
γ-CyD	7	OH	H	OH	OH	H
1	6	$NHCOCH_3$	H	OH	OH	H
2	6	OH	OH	H	H	$NHCOCH_3$
3	7	$NHCOCH_3$	H	OH	OH	H
4	7	OH	OH	H	H	$NHCOCH_3$

Structures of β-, γ-CyDs, and **1**-**4**.

the superiority of the secondary-hydroxyl side-modified β-CyD (2) on discriminating chirality of guests through ^{1}H-NMR spectroscopic studies,[5] although difference in binding strength for stereoisomers has still been unclear. Here, we wish to describe the effect of distorted conformation of the biding site upon the guest binding behavior of β- and γ-CyDs, using simply modified CyDs having an acetylamino group (1-4)[5] either at the primary- or secondary-hydroxyl side.

EXPERIMENTAL

^{1}H-NMR spectra were recorded on a JEOL GX-500 spectrometer in D_2O, using TMS as an external standard. UV-VIS spectra were recorded on a Jasco Ubset-30 spectrophotometer, the temperature of the cell holder being kept at 25.0 °C by circulating water during the measurements.

Binding constants of **1-4** for various guests were determined with a spectral displacement method refined by Eftink *et al.*,[7] using phenolphthalein (PP) as a primary guest. Briefly, PP was dissolved in buffered water (pH 10.1; [PP] = 1.85 x 10^{-5} M (=mol dm^{-3})), and changes in the absorbance at 550 nm were recorded, those which were induced by the stepwise addition of a concentrated aqueous solution of CyD derivatives. Curve fitting analyses based on simple 1:1 complexation were applied to estimating binding constants of **1-4**, β-, and γ-CyDs for PP. To determine binding constants for various guests, mixtures of PP and a guest (stereoisomers of borneol, menthol, fenchone, and camphor), and the CyD derivatives ([PP] = 1.85 x 10^{-5} M, [CyD] = 1.00 x 10^{-4} M, [guest] = 0 - 1.00 x 10^{-4} M; pH 10.1) were prepared and their UV-VIS spectra were measured. Curve fitting analyses assuming 1:1 complexation for both CyD-PP and CyD-guest were applied to estimating binding constants for the various guests, using the changes in absorbance at 550 nm.

Syntheses of **1-4** were followed by ref. 5 from the corresponding amino-CyDs.[5,8,9]

6^A-Deoxy-6^A-acetylamino-β-CyD (1). FABMS m/z 1176 ($[M+H]^+$); IR (KBr) 1645 cm^{-1} (amide C=O); ^{1}H-NMR (500 MHZ, D_2O) δ 2.03 (s, 3H), 3.57-3.67 (m, 14H), 3.82-3.98 (m, 28H), 5.05-5.08 (m, 7H); Anal. Calcd for $C_{44}H_{73}NO_{35}\cdot 5H_2O$: C, 41.74; H, 6.61; N, 1.11. Found: C, 41.60; H, 6.63; N, 1.23.

3^A-Deoxy-3^A-acetylamino-β-CyD (2). FABMS m/z 1176 ($[M+H]^+$), 1198 ($[M+Na]^+$); IR (KBr) 1645 cm^{-1} (amide C=O); ^{1}H-NMR (500 MHZ, D_2O) δ 2.02 (s, 3H), 3.58-3.72 (m, 14H), 3.80-4.02 (m, 28H), 4.23 (m, 2H), 4.94 (d, *J*=7.0 Hz, 1H), 4.99 (d, *J*=3.5 Hz, 1H), 5.04 (d, *J*=3.5 Hz, 1H), 5.06 (d, *J*=3.5 Hz, 1H), 5.08 (d, *J*=3.5 Hz, 1H), 5.14 (d, *J*=3.5 Hz, 2H); Anal. Calcd for $C_{44}H_{73}NO_{35}\cdot 2H_2O$: C, 43.60; H, 6.40; N, 1.16. Found: C, 43.48; H, 6.61; N, 1.26.

6^A-Deoxy-6^A-acetylamino-γ-CyD (3). FABMS m/z 1338 ($[M+H]^+$), 1360 ($[M+Na]^+$); IR (KBr) 1645 cm^{-1} (amide C=O); ^{1}H-NMR (500 MHZ, D_2O) δ 2.01 (s, 3H), 3.57-3.68 (m, 16H), 3.82-3.96 (m, 32H), 5.09-5.12 (m, 8H); Anal. Calcd for $C_{50}H_{83}NO_{40}\cdot 5H_2O$: C, 42.05; H, 6.56; N, 0.98. Found: C, 42.02; H, 6.57; N, 1.09.

3^A-Deoxy-3^A-acetylamino-γ-CyD (4). FABMS m/z 1338 ($[M+H]^+$), 1360 ($[M+Na]^+$); IR (KBr) 1645 cm^{-1} (amide C=O); ^{1}H-NMR (500 MHZ, D_2O) δ 2.03 (s, 3H), 3.58-3.72 (m, 16H), 3.82-3.98 (m, 28H), 4.21 (m, 2H), 4.96 (d, *J*=6.3 Hz, 1H), 5.00 (d, *J*=4.0 Hz, 1H), 5.07 (d, *J*=3.3 Hz, 1H), 5.09 (d,

J=3.3 Hz, 1H), 5.12 (d, J=3.7 Hz, 1H), 5.14 (d, J=3.7 Hz, 1H), 5.16 (d, J=4.0 Hz, 1H), 5.21 (d, J=3.0 Hz, 1H); Anal. Calcd for $C_{50}H_{83}NO_{40}\cdot 5H_2O$: C, 42.58; H, 6.50; N, 0.99. Found: C, 42.40; H, 6.72; N, 1.10.

RESULTS AND DISCUSSION

Ring distortion of **1**–**4** associated with the introduction of an acetylamino group was confirmed by ^{1}H-NMR spectra (Figure 1). The signals of anomeric protons of **1** and **3**, primary-hydroxyl side-modified CyDs, show severely overlapped signals, suggesting that the symmetric structure of CyDs were retained. On the other hand, the signals of **2** and **4**, secondary-hydroxyl side-modified CyDs, were widely spread and well separated. This indicates that symmetry of the CyD framework of **2** and **4** was reduced. In addition, an anomeric proton of the modified sugar unit had a larger J value (~7 Hz) than the others (3–4 Hz) having a $^4C_1$ conformation. According to the J values, the modified sugar unit (altrose) should have a $^1C_4$ or skew boat conformation.[5,10] CPK molecular model considerations suggested that CyDs possessing the sugar unit with either a $^1C_4$ or skew boat conformation should have distorted cavities which result in unsymmetrical oval or triangular shape.

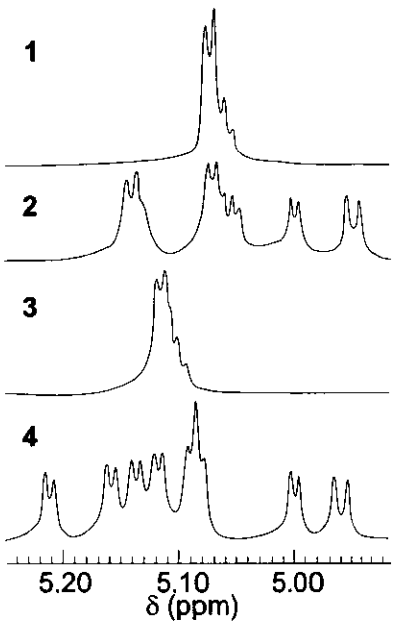


Figure 1. Partial ^{1}H-NMR spectra of **1**–**4**.

Figure 2 shows the results of curve fitting analyses upon binding processes of **1**–**4** with PP. No discrepancy between experimental data and theoretically generated fitting curves demonstrates that 1:1 binding occurs. From the curves, we obtained binding constants of **1**–**4** for PP (K_{PP}) as 18900 ± 1200, 6230 ± 380, 3270 ± 300, and 2300 ± 110 M^{-1}, respectively. K_{PP} values of native β- and γ-CyDs were also determined (35000 ± 2500 and 3270 ± 110 M^{-1}, respectively). In general, β-CyD derivatives have larger K_{PP} than γ-CyD derivatives, and the primary-hydroxyl side modification is favorable to binding PP than the secondary-hydroxyl side modification. As mentioned above, the modification at the secondary-hydroxyl side of CyDs created unsymmetrical hydrophobic cavities. Hence, this unsymmetrical shape of a hydrophobic cavity resulted in the smaller binding capability for PP. For γ-CyD series, the small modified group of acetylamino moiety did not perturb the binding capability for PP, while the group made K_{PP} values of β-CyD smaller. This may relate to the difference in efficiency of hydrogen bonding between the carboxylate group in PP and the CyD hydroxyl groups and/or the acetylamino group introduced.

Figure 3 shows the results of curve fitting analyses for systems containing **1**–**4**, PP, and *d*-camphor, as typical examples. Compounds (**1**, **2**, and **4**) bind the guest with 1:1 stoichiometry, as suggested

by the good correlation between experimental data and theoretically generated binding curves for which two independent guest binding processes (CyD:PP and CyD:guest) were postulated. Contrary, poor fit between experimental data and binding curve for **3** was obtained, indicating the presence of other stoichiometric complexes. γ-CyD itself exhibited a trend similar to **3** upon binding the guest. The cavity of γ-CyD is known to accommodate two guest species simultaneously into its wide cavity.[11] Therefore, **3** and γ-CyD might form ternary complexes with PP and the guest. We attempted to obtain binding constants (K_g) of γ-CyD and **3**, with assuming 1:1:1 ternary complexes for γ-CyD (or **3**), PP, and a guest, and 1:2 ternary complexes for γ-CyD (or **3**) and a guest. Unfortunately, these trials did not yield good correlation between the experimental data and theoretical binding curves. Presumably, complexation behavior of γ-CyD and **3** with a guest in the presence of PP would be more complicated. As a result, we could not obtain K_g values of γ-CyD and **3**. For **1**, **2**, and **4**, we were able to determine K_g values for four sets of stereoisomers of terpenes, and the values were summarized in Table 1.

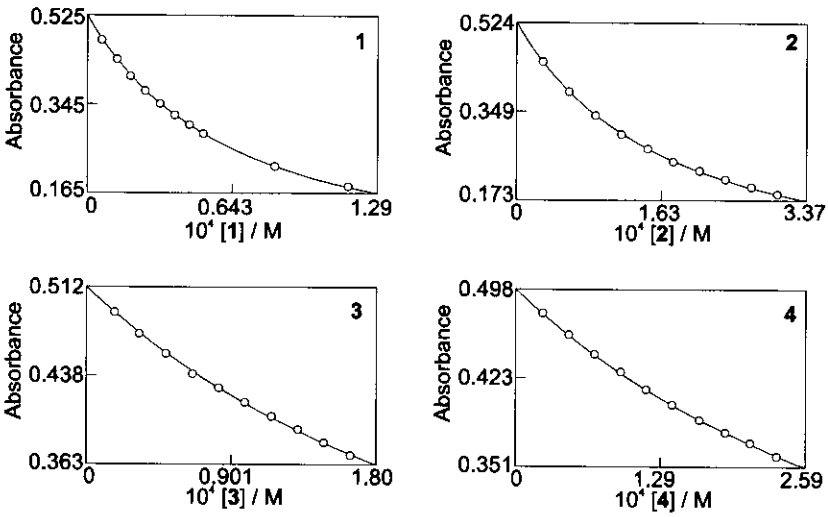


Figure 2. Plots of 550 nm absorbance of PP as a function of **1**–**4** concentrations. The solid lines are computer-generated best fit curves.

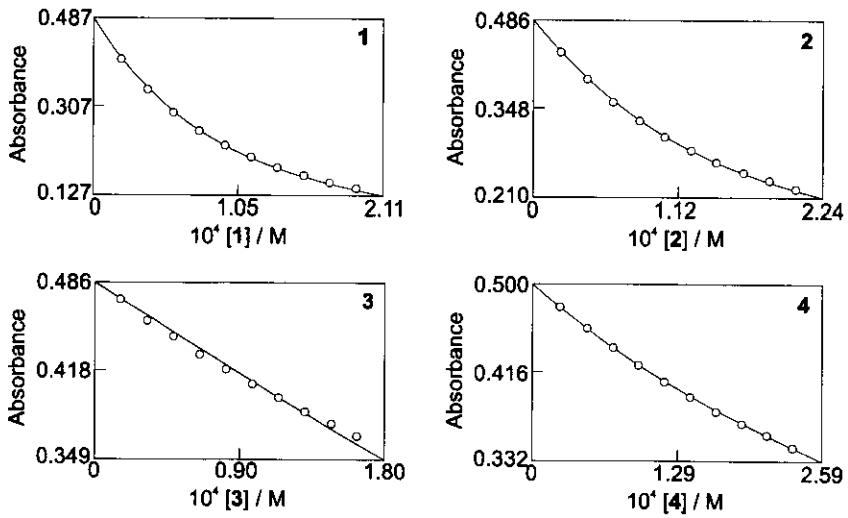


Figure 3. Plots of 550 nm absorbance of PP as a function of **1**–**4** concentrations in the presence of *d*-camphor. Solid lines are computer-generated best fit curves.

As for PP, in guest binding capability the primary-hydroxyl side modification seems to be superior to

Table 1. K_g Values for Various Guests in Aqueous Solutions (pH 10.1)[a]

Guest	K_g / M^{-1} (mol^{-1} dm^3) [b]					
	β-CyD	1	2	γ-CyD[c]	3[c]	4
d-borneol	19000	16500	3200	–	–	700
l-borneol	20000	18000	3350	–	–	900
d-menthol	3700	4000	1300	–	–	450
l-menthol	4100	3400	1350	–	–	700
d-fenchone	2200	2000	600	–	–	400
l-fenchone	2400	2350	650	–	–	150
d-camphor	6500	6200	900	–	–	1550
l-camphor	7000	6600	1050	–	–	1350

a) Determined by spectral displacement method at 25.0 °C, using PP as a ghost guest.

b) Errors were estimated as ± 20%.

c) K_g values could not be obtained.

the secondary-hydroxyl side modification, when comparison is made for 1 *and* 2. *Also, such a small* substituent of acetylamino group exerted slight effect on guest binding behavior of β-CyD when it was attached at the primary-hydroxyl side. The reduction in the guest binding capability of **2** may be attributable to the reduction in size effective to accommodate the guests. It is known that CyD can be discriminate the chirality of a guest upon binding, although the binding strength difference for a set of stereoisomers is usually small. This situation was retained for the primary-hydroxyl side-modified β-CyD, **1**. We anticipated that **2** would be able to bind one stereoisomer stronger than the other one. However, the chiral recognition capability of **2** was limited. Murakami *et al.* reported[5] that **2** was excellent host in discriminating the chirality of aromatic compounds through ^{1}H-NMR studies. This difference may arise from shape and size difference between aromatic compounds and terpenes. A benzene ring is small in size and planar in shape as compared to terpenes, and is suitable to being bound by **2** having a distorted cavity whose apparent size is smaller than that of β-CyD (and **1**). The chiral discriminating capability previously reported on **2** for aromatic compounds,[5] thus, may result from difference in conformations of complexes rather than binding strength.

The reduction in the cavity size provided **4** with capability to bind one guest molecule into its distorted γ-CyD cavity. This is sharp contrast to the primary-hydroxyl side-modified γ-CyD (**3**) and γ-CyD itself, which form more intricate complexes. Although **4** exhibited small binding capability for the terpenes, its chiral discrimination for menthol and fenchone was noticeable. Namely, the K_g values for *l*-menthol and *d*-fenchone were 1.56- and 2.67-times as strong as those for *d*-menthol and *l*-fenchone, respectively. These results may relate to the large difference in

binding conformations. Since γ-CyD is more flexible than smaller β-CyD, γ-CyD can adapt its conformation to guest shape during binding process (induced-fit binding). This flexibility should be retained in **4**. Therefore, **4** can take suitable conformations to the each stereoisomer of menthol and fenchone, this resulting in the large difference in K_g values. However, this induced-fit process should be unfavorable with respect to an entropy term in ΔG° ($= -RTlnK_g$) because the process accompanies a large change in γ-CyD ring shape. This may result in the small binding capability of **4** together with the larger ring size.

CONCLUSION

Secondary-hydroxyl side-modified γ-CyD, **4**, which had unsymmetrical hydrophobic cavity was found to bind terpene guests in 1:1 fashion despite of the presence of PP. In the same condition, parent γ-CyD and its primary-hydroxyl side-modified one, **3**, could not form pure 1:1 host-guest complexes with the terpene guests. In addition, **4** could discriminate the chilarity of the guests in binding strength. The results obtained in this study may be useful in constructing a sophisticated molecular recognition system toward a compound without a strong ionic group. The construction of the molecular recognition system based on a secondary-hydroxyl side-modified CyDs is now underway.

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