

Group-Inclusion Complex and Its External High Pressure Effect in *O*-Methylated β-Cyclodextrin as Compared with Unmodified β-Cyclodextrin

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High static pressure was applied to the inclusion complex of a modified β-cyclodextrin, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD), and the influence of *O*-methylation on the inclusion equilibrium was investigated. Guest molecules to be included by cyclodextrin were stable nitroxide radicals, α-substituted 2,4,6-trimethoxybenzyl(*t*-butyl)-nitroxides. Each guest molecule had more than one bulky group that can form ESR-spectroscopically separable isomeric inclusion complexes. Previously, using selected nitroxide probes, group-inclusion equilibrium in DM-β-CD has been shown to be significantly altered as compared with unmodified β-CD. Thus, we investigated group-inclusion equilibria in DM-β-CD under high static pressure by employing high pressure ESR spectroscopy, and compared the data with that for unmodified β-CD. The reaction volume (ΔV) of the inclusion equilibrium, a unique parameter which is calculated only from pressure dependence data, showed a drastic shift in some DM-β-CD group-in complexes, such as cyclohexyl- and *s*-butyl-in complex. We concluded that the *O*-methylation mediated changes in the hydrophobic void space and the number of residual water molecules in the CD cavity are causal factors for the difference.

β-Cyclodextrin (β-CD) is a cyclic molecule consisting of seven D-(+)-glucopyranose units that has a molecular void space capable of forming guest–host inclusion complex in aqueous media. Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) is a chemically modified β-CD arising from methylation of O(2)–H and O(6)–H groups on the end of β-CD (Fig. 1) and maintains inclusion capabilities similar to unmodified β-CD.[1,2] DM-β-CD is more solubility in water and organic solvents than β-CD;[3] thus, it is considered to be more practical pharmaceutical carrier molecules for drugs which are unstable at ambient conditions or poorly water-soluble.[4]

By using unique nitroxide radical probe that has more than one bulky functional group, we have shown that β-CD can recognize each individual functional group as a guest to be included in the cavity (Fig. 2).[5,6] For instance, when *t*-butyl(diphenylmethyl)nitroxide (DPBN) is included by β-CD, phenyl-in and *t*-butyl-in complexes can be identified with ESR spectroscopy. Such group-inclusion appears to be a universal phenomenon in all inclusion systems, because very recently, we have also shown that group-inclusion also occurs in calixarene inclusion systems.[7]

DM-β-CD also is able to form group-in inclusion complex with nitroxide radical probes, and its ESR study has been published.[8] Our recent investigations demonstrate that high static pressure influences the inclusion equilibrium, and thus, useful thermodynamic information can be obtained in β-CD inclusion system.[9,10] The objective of this study was: 1) to determine equilibrium constants of group-inclusion complexes of DM-β-CD, which have not been investigated yet, 2) to assess the difference of pressure effect in group-inclusion in β-CD and DM-β-CD systems, and 3) to reveal the role of *O*-methylation in the entrant group on the reaction volume ΔV, a unique parameter that can be calculated only with pressure dependence data.

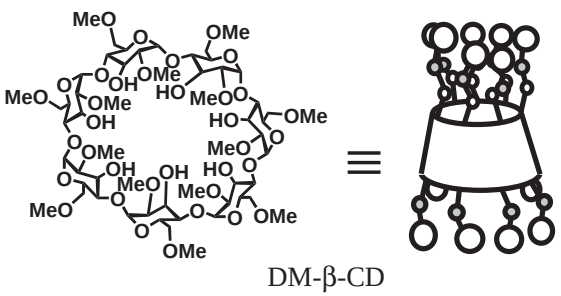

Fig. 1. Structure of DM-β-CD.

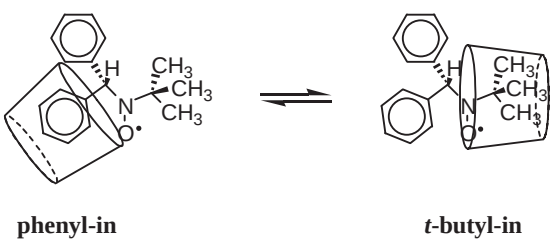

Fig. 2. Inclusion equilibrium between *t*-butyl-in and phenyl-in complexes of CD.

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Probe	R
1	Cyclohexyl
2	Cyclopentyl
3	*s*-Butyl
4	Isobutyl
5	Butyl
6	Hexyl
7	Phenyl

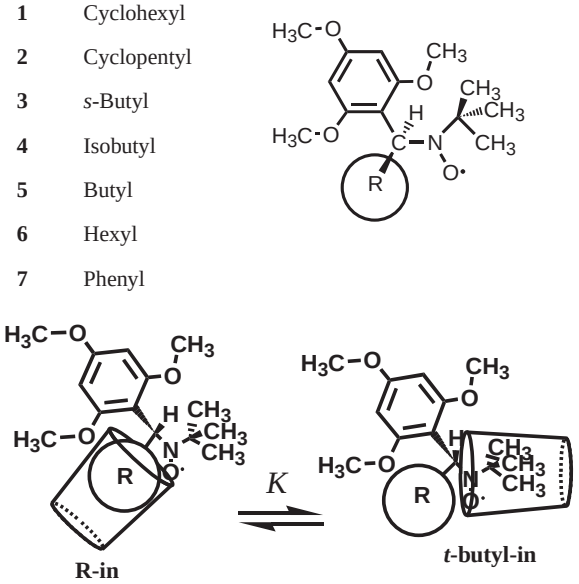

Fig. 3. Structures of nitroxide probes (**1**–**7**) and group-in complexation (R-in and *t*-butyl-in complexes) with DM-β-CD.

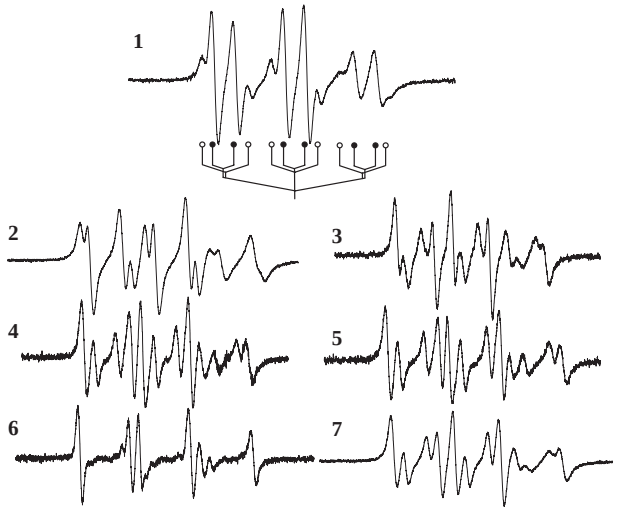

Fig. 4. ESR spectra of probe **1**–**7** in excess of DM-β-CD at 1×10^2 kPa. The probe and DM-β-CD concentrations were approximately 1×10^{-4} mol dm^{-3} and 2.5×10^{-2} mol dm^{-3}, respectively. In the ESR spectrum of **1**, the peaks marked with open circles (○) and closed circles (●) are assigned to the *t*-butyl-in and the R-in complexes, respectively.

Experimental

Nitroxide Probes. Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) was purchased from Aldrich Chemical Co. (Milwaukee WI, U.S.A.) and was used as received. Nitroxide spin probes **1**–**7** shown in Fig. 3 were prepared by the reaction of appropriate Grignard reagent or organolithium with *N*-(*t*-butyl)-2,4,6-trimethoxyaniline *N*-oxide (Aldrich Chemical Co.).[6] Water used as a solvent was distilled before use. The concentration of nitroxide radical was kept sufficiently low (1×10^{-4} mol dm^{-3}) so that the spin-exchange effect between nitroxide radicals could be neglected.

High-Pressure ESR. The high-pressure technique and procedures for ESR measurements were nearly the same as those described elsewhere.[10,11] Briefly, the sample solution was loaded into a pressure-proof quartz tube (6 mm o.d. and 1 mm i.d.), and after the required pressure had been attained with a plunger pump, the sample tube system was removed from the pump and inserted into the ESR spectrometer. ESR signals were recorded at room temperature in a JEOL JES-FE3XG ESR spectrometer (Akishima, Japan) with a 100 kHz field modulation. The ESR spectral simulation was carried out with computer software attached to the spectrometer operating software (WIN-RAD, Radical Research Inc., Hino, Japan).

Equilibrium Constants and Thermodynamic Parameters. In probes **1**–**7**, the DM-β-CD group-inclusion equilibrium between *t*-butyl-in and R-in complexes is shown in Fig. 3. The method of calculating equilibrium constants (K) and reaction volume (ΔV) are described in the following section.

Results and Discussion

Equilibrium Constants for Bimodal Inclusion in DM-β-CD and β-CD. Figure 4 shows the ESR spectra of nitroxide probes **1**–**7** in excess of DM-β-CD at room temperature. These ESR spectra exhibited separated ESR peaks for two kinds of radical complexes. In the complex formation of probes **1**–**7** with DM-β-CD, two isomeric complexes are formed, i.e., α-substituent (R)-in complex and *t*-butyl-in complex (bimodal or bidirectional inclusion).[6,8,12] The trimethoxyphenyl group does not form a complex because it has a larger radius than the wider end of the DM-β-CD cavity. Each ESR spectrum could be reproduced by computer simulation by superimposing two sets of triplet/doublet spectra. As an example, computer-simulated ESR spectrum for **2** is shown in Fig. 5. Table 1 lists all hyperfine coupling constants (hfsc) calculated from the simulated spectra for the group-in complexes of **1**–**7** for both DM-β-CD and unmodified β-CD. This table shows the similarity of nitrogen-hfsc's (A_N) of group-in complex in both CD complexes. The A_N's of the complex were smaller than those in water: $A_N = 1.632$ mT for **1**, 1.637 mT for **2**, and 1.631 mT for **3** in water.[6] The decrease in A_N in these inclusion complex indicated that the N–O group is surrounded by less polar environment than in water, suggesting that the N–O group is encapsulated in the CD cavity. The difference between $A_N^{\text{R-in}}$ and $A_N^{t\text{-butyl-in}}$ in DM-β-CD was 0.003 mT for **1**, 0.012 mT for **2**, and 0.048 mT for **3**. These differences are smaller than in unmodified β-CD complex (see Table 1).[10] Similar deviations from unmodified β-CD were obtained in γ-CD (cyclic octamer of glucose) group-inclusion complex,[6] suggesting that DM-β-CD has a wider opening than unmodified β-CD. These data are consistent with the fact that the methylation of the O(2)–H and O(6)–H groups in β-CD widens the radius of the hydrophobic torus, i.e., the radius estimated to be 11 Å as compared to 7.8 Å in β-CD,[13] indicating that DM-β-CD has a 40% wider opening than β-CD.

When a static pressure equivalent to 6.37×10^4 kPa was applied to the sample solution, the ESR spectrum was altered, as shown in Fig. 5. This ESR spectrum was reproduced with computer spectral simulation by adjusting the relative abun-

dance of the two complexes but keeping the hfsc the same. The values of K (=[*t*-butyl-in]/[R-in]) obtained at 1×10^2 and 6.37×10^4 kPa are listed in Table 1 for both DM-β-CD and β-CD. The results can be summarized as follows: (1) K values for DM-β-CD tended to decrease with an increase in the bulkiness of α-substituent groups, which are similar to the group-inclusion trend of β-CD[10] and (2) K values for **1** and **6** were much smaller than the others, i.e., K values for **1** and **6** with β-CD were 0.301 and 0.780, respectively,[10] while K values for DM-β-CD decreased to 0.224 and 0.192.

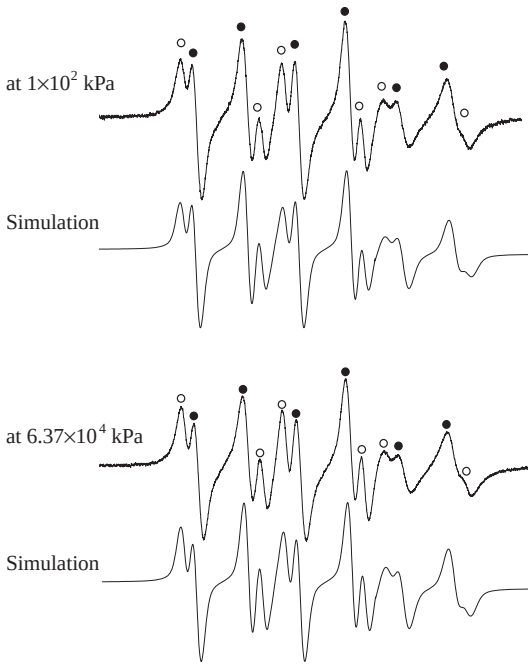

Fig. 5. ESR spectra and their simulated spectra of **2** in excess of DM-β-CD at 1×10^2 kPa and 6.37×10^4 kPa: [**2**] = 1×10^{-4} mol dm^{-3} and [DM-β-CD] = 2.5×10^{-2} mol dm^{-3}: (○): *t*-butyl-in complex and (●): cyclopentyl-in complex.

Other interesting results include the following. Inspection of the Corey–Pauling–Koltun (CPK) space-filling model indicated that the hexyl group of probe **6** can penetrate the hydrophobic cavity of β-CD. Because *O*-methylation in DM-β-CD increases the depth of the cavity, complex formation with hexyl or cyclohexyl groups, rather than *t*-butyl group, is favored and results in smaller K values. Furthermore, in unmodified β-CD, the K value for butyl group is larger than that for the branched *s*- and *t*-butyl group (Table 1); however, the K values for DM-β-CD complex for **3**–**5** were similar each other. We speculate that this is also the outcome of the widening of the rim in DM-β-CD. The stability of an inclusion complex is determined by how well a guest fits in the CD cavity. The above findings suggested that the fit of *s*-butyl group in the cavity of unmodified β-CD is better than those of butyl and isobutyl groups. Selkti et al.[14] have suggested that in a modified β-CD, 6^A-boc-phenyl-alanylamino-6^A-deoxyl-β-CD, the hydrophobic *t*-butyl group is preferentially included as compared with the phenyl group, although the K value for **7** (1.93) of DM-β-CD is smaller than in β-CD (K(β-CD) = 4.50).[10] These results can be understood based on the difference in hydrophobicity of the modified group.

Pressure Effects on the Bimodal Inclusion Equilibrium. Figure 6 shows the pressure dependence on the bimodal inclusion equilibria of **1**–**7** with DM-β-CD. As the external pressure was increased, the DM-β-CD group-inclusion equilibrium for **1**–**7** shifted to the *t*-butyl side. Equilibrium constants under 6.37×10^4 kPa are shown in Table 1 for both DM-β-CD and β-CD. In unmodified β-CD, the same occurred for most group-in complexes, but not for **1** (cyclohexyl-in complex) (Table 1).

The slope in the plot of ln K against pressure can be used to determine ΔV between R-in and *t*-butyl-in complexes according to Eqs. 1 and 2.

Table 1. ESR Hyperfine Coupling Constants,[a] Equilibrium Constants,[b] and Reaction Volumes for Inclusion Complexes of Nitroxide Probes with DM-β-CD in Water at 298 K

Probe[c]	*t*-Butyl-in		R-in		K		ΔV	$-\Delta V_R$[d]	$\Delta\Delta V_{repel}$	
	A_H	A_N	A_H	A_N/mT	1×10^2 /kPa	6.37×10^4 /kPa	/cm^3 mol^{-1}	/cm^3 mol^{-1}	/cm^3 mol^{-1}	
1 (DM-β-CD)	0.972	1.574	0.504	1.577 (cyclohexyl-in)	0.224	0.248	-3.8 ± 0.1	113 ± 1	-11.8 ± 0.6	This work
(β-CD)	0.958	1.540	0.480	1.572	0.301	0.249	7.6 ± 0.6	113 ± 1	0	Ref. 10
2 (DM-β-CD)	1.221	1.620	0.823	1.634 (cyclopentyl-in)	0.531	0.622	-5.6 ± 0.3	101 ± 1	-1.6 ± 0.3	This work
(β-CD)	1.210	1.625	0.825	1.650	0.660	0.740	-4.0 ± 0.1	101 ± 1	0	Ref. 10
3 (DM-β-CD)	1.001	1.534	0.523	1.582 (*s*-butyl-in)	1.74	2.04	-5.8 ± 0.3	103 ± 1	-3.8 ± 0.3	This work
(β-CD)	0.992	1.522	0.710	1.601	0.522	0.554	-2.2 ± 0.1	103 ± 1	0	Ref. 10
4 (DM-β-CD)	1.271	1.572	0.608	1.607 (isobutyl-in)	1.94	2.36	-7.4 ± 0.2	97.9 ± 0.2	-0.3 ± 0.3	This work
(β-CD)	1.270	1.576	0.671	1.601	3.15	3.78	-7.1 ± 0.2	97.9 ± 0.2	0	Ref. 10
5 (DM-β-CD)	1.310	1.576	0.674	1.598 (butyl-in)	1.52	2.35	-16.6 ± 0.5	87.8 ± 0.9	0.6 ± 1.0	This work
(β-CD)	1.295	1.582	0.682	1.598	4.67	7.46	-17.2 ± 0.9	87.8 ± 0.9	0	Ref. 10
6 (DM-β-CD)	1.236	1.561	0.584	1.596 (hexyl-in)	0.192	0.311	-17.5 ± 1.1	88.3 ± 0.8	-0.8 ± 1.3	This work
(β-CD)	1.170	1.645	0.550	1.635	0.780	1.16	-16.7 ± 0.8	88.3 ± 0.8	0	Ref. 10
7 (DM-β-CD)	1.180	1.580	0.595	1.570 (phenyl-in)	1.93	2.79	-14.2 ± 0.3	91.0 ± 1.8	-0.2 ± 1.8	This work
(β-CD)	1.160	1.540	0.580	1.510	4.50	6.50	-14.0 ± 1.8	91.0 ± 1.8	0	Ref. 10

a) Error is ±0.005 mT. b) Precision within 5% error. c) The second line in each complex indicates the data for β-CD complex. d) Cited from Ref. 10.

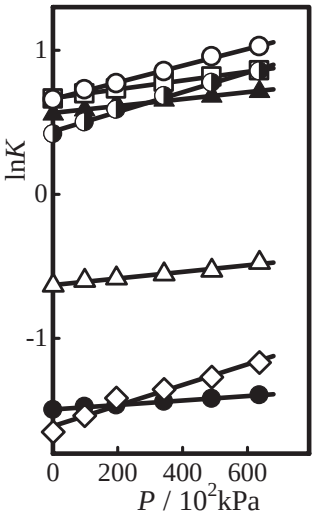

Fig. 6. Plots of $\ln K$ against external pressures; ●: **1**, △: **2**, ▲: **3**, □: **4**, ◑: **5**, ◇: **6**, and ○: **7**.

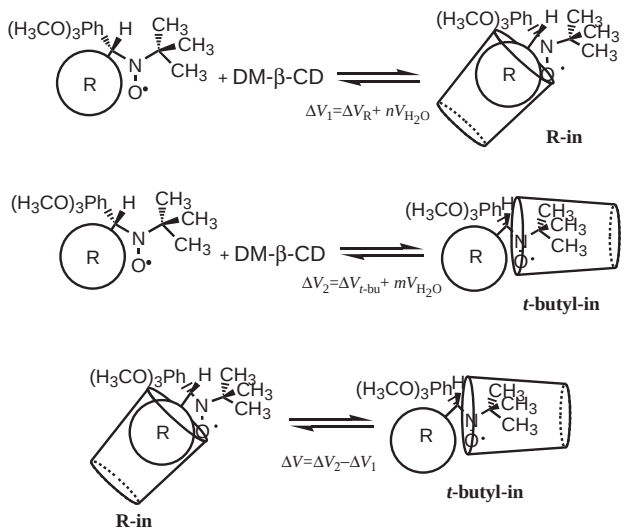

Fig. 7. Schematic illustration of group-in inclusion equilibrium from the R and *t*-butyl sides and the scheme that define reaction volumes, ΔV, ΔV_1, and ΔV_2.

$$\ln K = aP + b, \tag{1}$$

$$\Delta V = -RT\left(\frac{\partial \ln K}{\partial P}\right)_T. \tag{2}$$

As shown in Table 1, ΔV for group-in complexation of **1**–**7** with DM-β-CD had negative values, and the increasing volume of the group tended to make the absolute value of ΔV smaller. In contrast, in unmodified β-CD, most probes showed negative values ($\Delta V = -17.2 - -4.0\,\mathrm{cm^3\,mol^{-1}}$), while the bulky α-cyclohexyl probe showed positive one ($\Delta V = 7.6\,\mathrm{cm^3\,mol^{-1}}$) (Table 1).[10]

We evaluated the contents of ΔV. In the volume-change scheme in CD inclusion (Fig. 7), major contributions to the change in ΔV between *t*-butyl-in and R-in complexes can be expressed as follows[10,15,16]

$$\Delta V = \Delta V_2 - \Delta V_1 = \Delta V_{t\text{-bu}} - \Delta V_R + (m-n)V_{H_2O}, \tag{3}$$

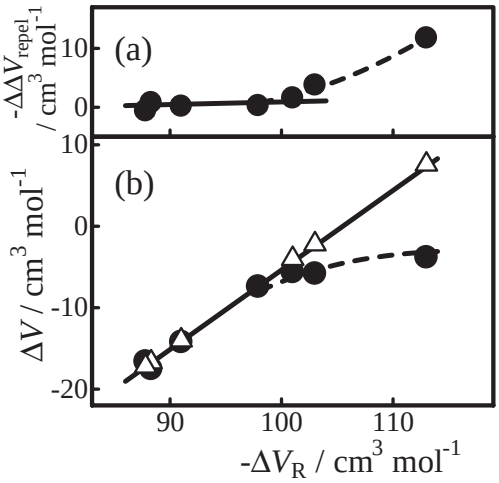

Fig. 8. Plots of ΔV and $-\Delta\Delta V_{repel}$ against $-\Delta V_R$: (●) the DM-β-CD group-inclusion and (△) the β-CD group-inclusion.

where ΔV_1 and ΔV_2 denote the volume change accompanying the inclusion from R and *t*-butyl sides, respectively. $\Delta V_{t\text{-bu}}$ and ΔV_R are the intrinsic volume changes related to inclusion of *t*-butyl and R groups, respectively. *m* and *n* denote the number of water molecules repelled out from the DM-β-CD cavity, and $(m-n)V_{H_2O} = \Delta\Delta V_{repel}$. V_{H_2O} is the molar volume of a water molecule. Using known ΔV, $\Delta V_{t\text{-bu}}$ (=−105 $\mathrm{cm^3\,mol^{-1}}$),[9] and ΔV_R values,[10] $\Delta\Delta V_{repel}$ could be calculated from Eq. 3 and are given in Table 1. $\Delta\Delta V_{repel}$'s for DM-β-CD were near zero for probes **4**–**7** (Table 1 and Fig. 8a). Figure 8b shows the plots of ΔV against $-\Delta V_R$ for β-CD and DM-β-CD. The plots for probes **1**–**7** showed linear relationship for unmodified β-CD; however, the plots of probes **1**–**3** for DM-β-CD showed downward deviation from a straight line with increasing bulkiness of R group. Judging from the CPK space-filling model inspection, we assumed that all water molecules situated in β-CD are repelled out by the included group, leading to the linear relationship between ΔV and $-\Delta V_R$. In β-CD complexation, the reaction volume corresponds to the difference in the inclusion volumes between *t*-butyl-in and R-in complexes, i.e.,

$$\Delta V = \Delta V_{t\text{-bu}} - \Delta V_R. \tag{4}$$

The pressure results in β-CD complex of probe **1** suggested that the decrease in volume for the inclusion from the cyclohexyl side is large compared to that from the *t*-butyl side; thus, the group-inclusion equilibrium shifted to the cyclohexyl side with increasing pressure. On the contrary, in probes **2**–**7**, the decrease in volume for the R-in complex formation was smaller than that for the *t*-butyl-in complex formation. Study of the CPK model showed that the vacancy of the DM-β-CD cavity is not fully occupied by the included groups of probes **1**–**7**. Therefore, all water molecules involved in the DM-β-CD cavity might not have been repelled out upon inclusion. In DM-β-CD complex of probes **4**–**7**, the linear relationship between ΔV and $-\Delta V_R$ suggested that the number of water molecules repelled for R-in inclusion is compared with that for *t*-butyl-in inclusion, i.e., $n \approx m$ ($\Delta\Delta V_{repel} \approx 0$). In contrast, in DM-$\beta$-CD complex of probe **1**–**3**, because of the large vacant space,

t-butyl-in and R-in complexes did show the difference in the number of water molecules repelled. A value of 0.65 for $n - m$ was obtained from the $\Delta\Delta V_{repel}$ value for probe **1**. About 0.7 water molecule was repelled out by inclusion of the bulky cyclohexyl group as compared with *t*-butyl-in inclusion.

As the driving forces for the inclusion complex formation with CD, several intermolecular interactions have been proposed: (1) hydrophobic interaction, (2) van der Waals interaction, (3) relief of high-energy water from the CD cavity upon inclusion of a guest, etc.[17] VanEtten et al. indicated that in phenyl acetate β-CD inclusion complex, exclusion of water molecules from the CD cavity is an important factor for the formation of stable inclusion complex.[18] Most of the water molecules in the β- and γ-CD cavities are situated through hydrogen bond with the O–H group of CD's rim.[19,20] Although the number of water molecules in the DM-β-CD cavity is yet unknown, we speculate that water molecules in the DM-β-CD cavity are not fully hydrogen-bonded and may be in a high-energy state.

Water molecules in the CD cavity play an important role for the formation and stability of inclusion complexes. The above result obtained from this pressure dependence study on bimodal inclusion equilibrium were indicative of the existence of high-energy water molecules in the DM-β-CD cavity in water. It should be pointed out that this is not the case in unmodified β-CD. In DM-β-CD, the methylation of OH groups on the CD rim enhances the inclusion ability of whole molecule,[21] while we found that the functional group recognition by DM-β-CD is not as sensitive as whole molecule recognition.

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