^{1}H NMR Study of Intramolecular Hydrogen-Bonding Interaction in Cyclodextrins and Their Di-*O*-methylated Derivatives

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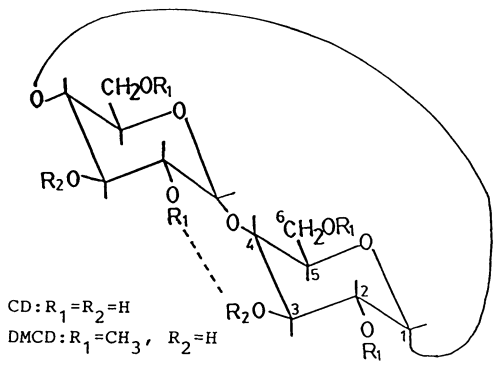
Intramolecular hydrogen bonding of α-, β-, and γ-cyclodextrins, and α- and β-dimethyl cyclodextrins has been investigated in dimethyl sulfoxide-d_6 by ^{1}H NMR spectroscopy. The temperature dependence of ^{1}H chemical shifts of secondary hydroxyl protons of cyclodextrins and dimethyl cyclodextrins reveals the existence of the intramolecular hydrogen bond. The strength of hydrogen bond becomes stronger as the ring size becomes smaller and by methylation. The intramolecular hydrogen bond in which the OH(3) is proton donor is preferred in α-cyclodextrin as well as in α- and β-dimethyl cyclodextrins. The orientation of secondary hydroxyl groups was estimated with the Karplus-type equation and the orientation is found to be suitable for making intramolecular hydrogen bond.

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six or more D-glucose units, which are connected by α-1,4-glucosidic linkages.[1] α-, β-, and γ-CDs are typical CDs composed of 6, 7, and 8 glucose units, respectively. Since CDs can accommodate guest molecules of suitable size in their cavities in both solid and solution states,[1-5] they have been paid great attention in various fields. The CD molecules have been modified chemically in attempts to improve properties such as the solubility and the complexation ability.[2,4,6-9] 2,6-Di-*O*-methyl-α- and β-CDs (α- and β-DMCDs, respectively) are typical examples of such modified CDs and they not only have higher solubility to both water and organic solvents but also appear to have significantly enhanced abilities of complexation and improved chiral recognition upon the inclusion processes.[2,10,11]

Hydrogen bonding is one of important interactions involved in determining the three-dimensional structures of biopolymers such as proteins, nucleic acids, and carbohydrates. The hydrogen bonds in these systems can be located by crystal structure analysis, especially by the neutron diffraction study which can directly prove the location of hydrogen atoms in the crystal lattice.[12,13] In solution state, on the contrary, it is not always easy to detect directly hydrogen-bond formation. Nuclear magnetic resonance spectroscopy (NMR) is one of potential physicochemical techniques which can provide the information about the hydrogen bonding in solution. Chemical shift or coupling constant, and temperature dependence of chemical shift, deuterium exchange and/or isotope effect, have been effectively used to study the hydrogen bonds, and in some case, to distinguish between the inter- and the intramolecular hydrogen bonds.[15-19]

X-Ray[20-24] and neutron diffraction[12,13] studies have shown that in solid state round but slightly conical form of CD macrocyclic ring structure is stabilized by the intramolecular hydrogen bonds between OH(2) and OH(3) groups of the adjacent glucose units. The existence of intramolecular hydrogen bonds of both O(3)–H···O(2) and O(3)···H–O(2) types in solid state have been suggested for α- and β-CDs. In DMCDs the formation of O(3)–H···O(2) type hydrogen bonds has also been suggested.[23,25]

There have been many attempts to determine the driving forces for complexation processes of CD and to make use of CDs inclusion ability in various field. Since CD inclusion complexation usually occurs in solution, the determination of CD conformation in solution could give some information on the complexation mechanism of CD inclusion complexes. The intramolecular hydrogen bond might play an important role for stabilizing the structure of CD macrocycle in solution. In the present work, the hydrogen bonding properties of CDs and DMCDs in DMSO-d_6 solution and orientation of their hydroxyl group were studied by the temperature dependence of chemical shifts and coupling constants of hydroxyl protons.



Experimental

α-, β-, and γ-CDs were generous gifts from Nihon Shokuhin Kako Co. Ltd. and methyl α-D-glucoside (MG) was purchased from Nakarai Chemicals and were dried in vacua at 120 °C before use to remove most of the hydrated water. α-DMCD was purchased from Toshin Chemical Co., and was used after recrystallization from water. β-DMCD

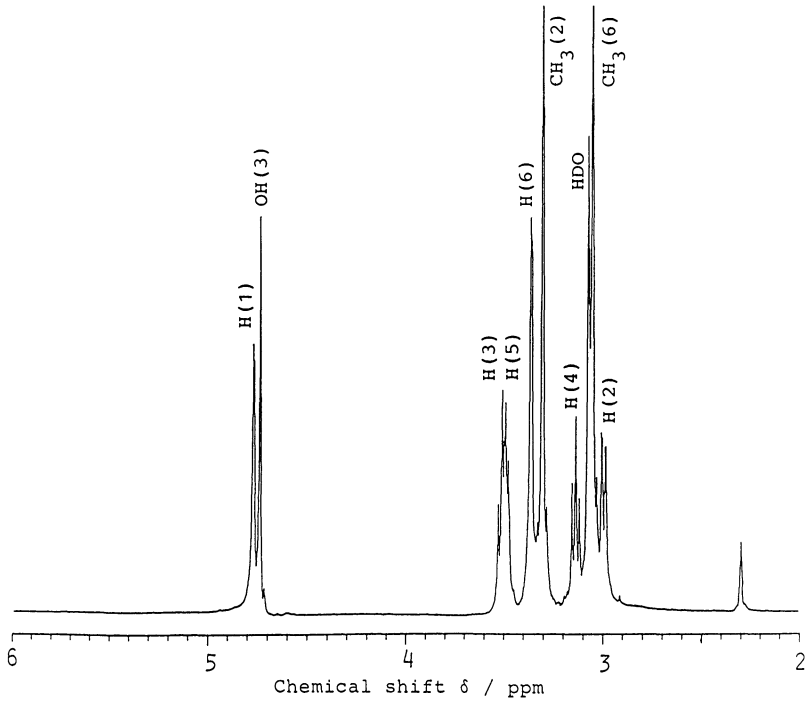


Fig. 1. The 500 MHz ^{1}H NMR spectrum of β-DMCD in DMSO-d_6 solution at 35 °C.

was obtained from Toshin Chemical Co., and was purified using the procedures reported by Koizumi et al.[26] The NMR solvent, deuterated dimethyl sulfoxide (DMSO-d_6), was purchased from Merck Co.

500 MHz ^{1}H NMR spectra were recorded on a JEOL GX-500 spectrometer in DMSO-d_6 at several temperatures from 25 to 75 °C. Concentration of all samples is 0.02 M (1 M= 1 mol dm^{-3}). Chemical shifts are given in parts per million (ppm) down field from tetramethylsilane (TMS). Digital resolution of the spectra is 0.0006 ppm (0.3 Hz).

Results and Discussion

Chemical Shift of Hydroxyl Proton Resonances in CDs. In Fig. 1 is shown the 500 MHz ^{1}H NMR spectrum of β-DMCD at 35 °C. In a previous paper,[27] we have assigned ^{1}H NMR spectra of some CDs and permethylated CDs by means of two-dimensional NMR techniques. ^{1}H NMR chemical shifts and coupling constants of hydroxyl groups and H(1) of α-, β-, and γ-CDs, and α- and β-DMCDs and MG in DMSO-d_6 at 35 °C are listed in Table 1. The resonances of the secondary hydroxyl protons of α-, β-, and γ-CDs (δ 5.2—5.6), and that of β-DMCD (δ 4.70) appear at lower field than those of the primary hydroxyl protons (δ 4.2—4.4) and the hydroxyl protons of MG (δ 4.1—4.6). These results suggest that the secondary hydroxyl groups form intramolecular hydrogen bond in α-, β-, and γ-CDs and β-DMCD. When the hydrogen bond is formed, polarization like $O^{\delta-}\cdots H^{\delta+}-O^{\delta-}$ along the hydrogen bond net work is enhanced and the proton participating in hydrogen bond becomes more positive in its electronic character. Therefore, such proton is deshielded compared to the proton that is not concerned with the hydrogen bond, i.e. its signal shifts to lower field. The resonance of secondary hydroxyl proton of α-DMCD appears at δ 4.45, which is clearly at higher field than the chemical shifts of other secondary proton resonances of CDs. But ^{1}H chemical shift data alone are not sufficient, and another supporting data are necessary to conclude that the intramolecular hydrogen bond is present in CDs and DMCDs (see below).

Table 1. Chemical Shifts[a] of H(1) and Hydroxyl Protons of Some CDs, DMCDs and MG in DMSO-d_6 at 35 °C

	H(1)	OH(2)	OH(3)	OH(6)	OH(4)
α-CD	4.60	5.32	5.26	4.28	
β-CD	4.68	5.52	5.48	4.26	
γ-CD	4.74	5.53	5.57	4.32	
α-DMCD	4.77		4.45		
β-DMCD	4.77		4.70		
MG	4.32	4.41	4.46	4.12	4.58

a) Chemical shifts are expressed in ppm from TMS.

Temperature Dependence of Hydroxyl Proton Chemical Shifts. Figure 2 shows the chemical shift changes of hydroxyl proton resonances of α-CD over the temperature range examined. Although the chemical shift of H(1) changes little with temperature, those of hydroxyl protons shift to higher field almost linearly with the increase of temperature (Fig. 3). Similar results are found for β- and γ-CDs, α- and

β-DMCDs, and MG.

For dilute solutions of CDs, DMCDs, and MG in DMSO-d_6, the following equilibrium of hydrogen bonding is present.

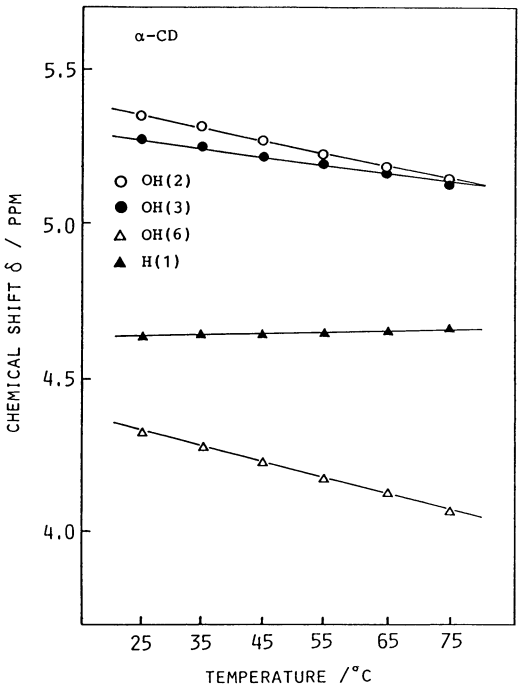


Fig. 2. Temperature dependence of the chemical shift of the hydroxyl and anomeric protons in α-CD.

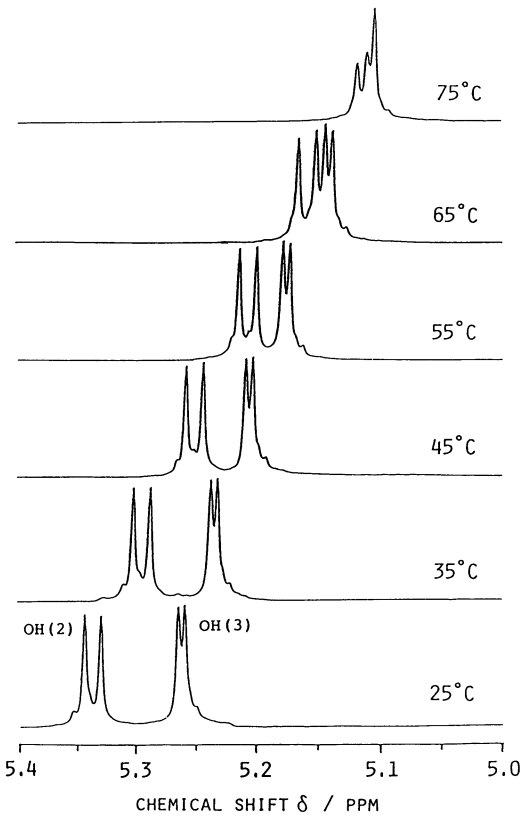


Fig. 3. The 500 MHz ^{1}H NMR spectrum of the secondary protons of α-CD at indicated temperature.

$$-OH\cdots HO- \rightleftarrows -OH\cdots O=S(CD_3)_2$$

The observed upfield shift of hydroxyl proton resonances with increase of temperature is attributed to decrease in the strength of the solvent–solute association. The smaller value of temperature coefficient of the chemical shift ($d\delta/dT$) means that the above equilibrium more prefers to lie to the left side i.e. hydroxyl groups preferentially form the intramolecular hydrogen bonds. The temperature dependent chemical shifts of hydroxyl protons of α-, β-, and γ-CDs and MG show that the $d\delta/dT$ values of all OH(6) proton signals are large and are nearly equal with each other (Fig. 4-A, Table 2). This observation suggests that all OH(6) protons are exposed to solvent DMSO-d_6 in a similar fashion. Since MG can't form intramolecular hydrogen bond, hydrogens of OH(6) of CDs do not participate in intramolecular hydrogen bonding. On the other hand, the $d\delta/dT$ values for OH(2) and OH(3) of CDs and DMCDs are significantly smaller than those of MG (Figs. 4-B and 4-C, respectively). This suggests that the secondary hydroxyl groups of α-, β-, and γ-CDs, and α- and β-DMCD prefer to form intramolecular hydrogen bond between OH(2) (in the case of DMCD, methoxy group) and OH(3) of the adjacent glucose units rather than to form a hydrogen bond with DMSO-d_6. The $d\delta/dT$ values for both OH(2) (α-, β-, and γ-CDs) and OH(3) increase in the following order: α-DMCD≒β-DMCD<α-CD<β-CD<γ-CD, indicating that the strength of the intramolecular hydrogen bond in DMSO-d_6 is in the following order: γ-CD<β-CD<α-CD<β-DMCD≒α-DMCD. The strength becomes weaker according to increase the ring size except α- and β-DMCDs. The macrocycle size of γ-CD might be

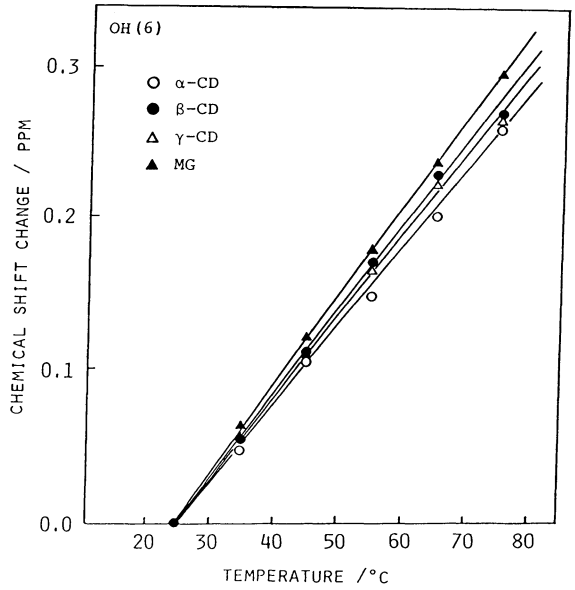
Table 2. Temperature Dependence ($d\delta/dT$) of the Hydroxyl Proton Resonances of Some CDs and MG in DMSO-d_6 at Concentration 0.02 M

	$(d\delta/dT)/10^{-3}$ ppm deg^{-1}		
	OH(2)	OH(3)	OH(6)
α-CD	4.36 (0.60)[a]	2.67 (0.41)[a]	5.21 (0.87)[a]
β-CD	5.42 (0.74)[a]	4.16 (0.56)[a]	5.66 (0.95)[a]
γ-CD	5.59 (0.77)[a]	4.64 (0.72)[a]	5.59 (0.94)[a]
α-DMCD		1.61 (0.25)[a]	
β-DMCD		1.67 (0.26)[a]	
MG	7.25	6.36	5.93

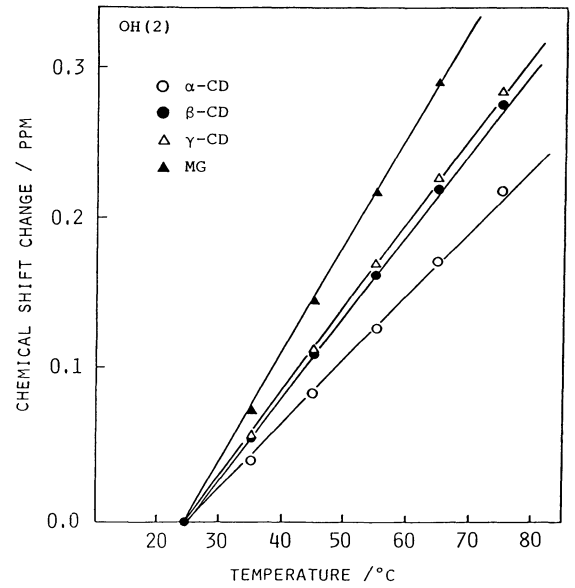
a) Data in parentheses represent the R valuse; where

$$R = \frac{d_j\delta/dT}{d_j^{MG}\delta/dT}$$

i = α-, β-, and γ-CDs, and α- and β-DMCDs
j = OH(2), OH(3), and OH(6).



(A)



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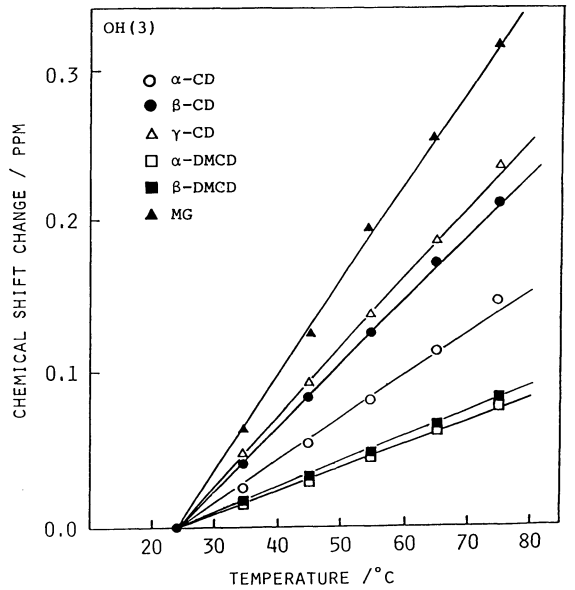


(C)

Fig. 4. Temperature dependence of the chemical shifts of the hydroxyl protons of α-, β-, and γ-CDs, and α- and β-DMCDs and MG; (A) OH(6), (B) OH(2), (C) OH(3). The ordinate refers to the magnitude of the upfield shift relative to the chemical shift observed at 25°C.

too large to form the tight intramolecular hydrogen bond, and so its macrocyclic ring might be flexible. The ring size could be an important factor to form the strong intramolecular hydrogen bond.

Harata[22] has found from the X-ray study that in the hydrated crystal state the OH(2)···OH(3′) distance of adjacent glucose units is in order of α-CD>β-CD>γ-CD, indicating that the OH(2)···OH(3′) intramolecular hydrated crystal state the OH(2)···OH(3′) distance of adjacent glucose units is in order of α-CD>β-CD>γ-CD, indicating that the OH(2)···OH(3′) intramolecular remarkably distorted and two intramolecular hydrogen bonds are disrupted because of the sharp inclination of one glucose unit.[20,24] This can make weak the intramolecular hydrogen bond of α-CD. In solution, the flexibility of α-CD macrocyclic ring is sufficiently high to compensate for the disruption of intramolecular hydrogen bond. On occasion of crystallization, CDs may take energetically stable conformations, which are not necessarily suitable to form a strong hydrogen bond. Thus, it is concluded that not all of the results for the solids necessarily correspond with those for solution. For solutions, the role of solvent molecules must be considered also. The methylation of OH(2) groups are found to lead the formation of stronger intramolecular hydrogen bond. This point will be discussed in a following section.

Type of Intramolecular Hydrogen Bond. Three different modes (Fig. 5) are possible for the intramolecular hydrogen bonds of CD in DMSO-d_6, i.e. in types 1 and 2, OH(2) and OH(3) groups are main proton donors of the intramolecular hydrogen bond, respectively, and in type 3 (flip-flop type) the rapid exchange is assumed to occur between type 1 and type 2. St-Jacques et al.[17] have reported from the analysis of coupling constant $^3J_{HOCH}$ that α- and β-CDs form type 2 intramolecular hydrogen bond and Christofides et al.[18,19] have also reported from the studies of ^2H isotope effect on ^1H and ^{13}C chemical shifts that α- and β-CDs form type 2 hydrogen bond, but there is no description about the difference between α- and β-CDs. For DMCDs the type of intramolecular hydrogen bond to be considered is only type 4, because there is only one type of secondary hydroxyl group in each glucose constituent. To identify the type of

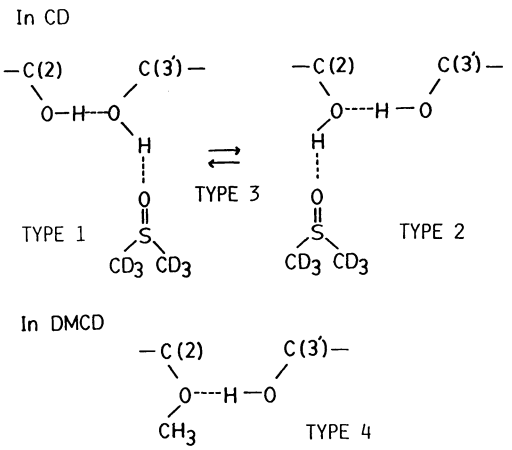


Fig. 5. The schematic representation of the intramolecular hydrogen bonds in CDs and DMCDs.

intramolecular hydrogen bond, we have tried to measure deuterium exchange rates of CD hydroxyl protons upon an addition of small amounts of deuterium oxide. If the hydrogen bond is type 1 or 2, ^1H-^2H exchange rate of hydrogen-donating hydroxyl group is slower than that of free hydroxyl group. But, ^1H-^2H exchange rate could not be estimated, since it was too rapid as compared to the NMR time scale. Then, the type of intramolecular hydrogen bond is identified based on R values (Table 2), defined as follows,

$$R = \frac{d_j^i \delta/dT}{d_j^{MG}\delta/dT}$$

where $d_j^i\delta/dT$ and $d_j^{MG}\delta/dT$ indicate the temperature coefficients of chemical shifts of j-th hydroxyl group of CD and MG, respectively, and superscript i and subscript j are,

i = α-, β-, and γ-CDs, and α- and β-DMCDs
j = OH(2), OH(3), and OH(6).

A hydroxyl group with R value of 1 is considered to be forming the intermolecular hydrogen bond with DMSO-d_6 in a similar way as that of MG. The R value smaller than 1 means that a hydroxyl group prefers to form the intramolecular hydrogen bond. The R values for the OH(6) groups of all CDs are nearly equal to 1, indicating they form the intermolecular hydrogen bond with DMSO-d_6. The OH(3) groups of α- and β-DMCDs have the smallest R values of 0.25 and 0.26, which are strongly supporting the conclusion obtained from the $d\delta/dT$ values that α- and β-DMCDs form the strongest intramolecular hydrogen bond. In α-CD the R value for the OH(3) groups (0.41) is much smaller than that for the OH(2) groups (0.60). So the OH(3) group is considered to be a dominant proton donor (type 2) for the intramolecular hydrogen bond between it and the OH(2) group of the adjacent glucose unit. Similar tendency is also observed for β-CD, though the absolute R values for OH(2) and OH(3) become larger. Thus in β-CD the type 2 intramolecular hydrogen bond is dominant, but the type 3 intramolecular hydrogen bond and the intermolecular hydrogen bond with solvent DMSO-d_6 are also formed more frequently than in α-CD. In γ-CD the R value for OH(2) is nearly equal to that for OH(3) and both are relatively large, although they are clearly less than 1. So the preferred intramolecular hydrogen bond in γ-CD is type 3, and the extent of hydrogen bond with DMSO-d_6 is not negligibly small. Therefore the character of intramolecular hydrogen bond is found to change from type 2 to type 3 with increasing the ring size of CD. The ring size of CD is considered to be an important factor determining not only the strength but also the type of the intramolecular hydrogen bond.

Orientation of Secondary Hydroxyl Groups. The values of vicinal coupling constants $^3J_{HOCH}$ for OH(2) and OH(3) groups of α-, β-, and γ-CDs didn't change within experimental error over the temperature ranged from 25 to 75 °C. This result indicates that the orientation of hydroxyl group is independent of the temperature range examined here, because the $^3J_{HOCH}$ value can be related to the dihedral angle Φ around the HO-CH bond through the Karplus type equation;[28]

$$^3J_{HOCH} = 10.4\cos^2\Phi - 1.5\cos\Phi + 0.2. \quad (1)$$

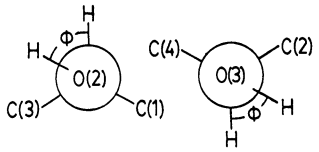
The values of coupling constants for the secondary hydroxyl groups and the estimated dihedral angles Φ are listed in Table 3. Some sets of values of dihedral angles Φ for α-, β-, and γ-CDs appear to be reasonable for making an intramolecular hydrogen bond. From the CPK molecular model study, in the case of combination of $\Phi_{OH(2)}$ and $\Phi_{OH(3)}$ values both of which are positive, formation of the intramolecular hydrogen bond between adjacent glucose units is impossible.

It is noteworthy that the $^3J_{HOCH}$ values for OH(2) of α-, β-, and γ-CDs are almost the same with that of MG, while those for OH(3) of all CDs are quite different from that of MG. This result implies that significant conformational change occurs not at OH(2) but at OH-(3) group by cyclization. Needless to say that MG is monosaccharide, so it does not form the intramolecular hydrogen bond like CDs. Thus, such conformational change of the secondary hydroxyl groups of CDs might be attributed to the formation of the intramolecular hydrogen bond. The change of orientation of OH(3) group implies again that OH(3) is a major proton donor of the hydrogen bond, i.e., the predominant type of the hydrogen bond in all CDs is type 2. For these cases, the negative values of $\Phi_{OH(3)}$ are adequate for α- and β-CDs. The OH(3) group is directed to opposite direction of OH(2′) of the adjacent glucose unit, when $\Phi_{OH(3)}$ value is positive.

Table 3. The Observed Vicinal Coupling Constants $^3J_{HCOH}$ and the Calculated Dihedral Angles of Secondary Hydroxyl Groups of Some CDs in DMSO-d_6 at 35°C

	OH(2)			OH(3)		
	J/Hz	Φ/deg[a]		J/Hz	Φ/deg[a]	
α-CD	7.0	±27.9,	±137.7	2.8	±54.7,	±115.7
β-CD	6.7	±30.0,	±136.1	2.5	±56.8,	±113.8
γ-CD	7.0	±27.9,	±137.7	2.5	±56.8,	±113.8
α-DMCD				ca. 0.0	ca. ±85.9	
β-DMCD				ca. 0.0	ca. ±85.9	
MG	6.6	±30.7,	±135.7	5.1	±40.3,	±135.7

a) Φ's were computed based on the following Karplus type equation,
$^3J_{HCOH} = 10.4\cos^2\Phi - 1.5\cos\Phi + 0.2$.



For β-DMCD we can not determine the exact value of coupling constant $^3J_{HOCH}$, since the splitting of the OH(3) resonance is too small even at 500 MHz. If $^3J_{HOCH}$ value for OH(3) of β-DMCD is assumed to be 0.0 Hz, the dihedral angles estimated from Eq. 1 are ±85°. When the type 4 hydrogen bond is formed, the value of +85° is ruled out. Making CPK model with the value of −85°, the distance between OH(3′) and O(2) is found to become the shortest. This results corresponds well with small temperature coefficient of OH(3) proton chemical shift, indicating the formation of very strong intramolecular hydrogen bond in β-DMCD. It is also found from the CPK model study that the rim of β-DMCD made by the secondary hydroxyl and the methoxyl groups is slightly wider than that of β-CD made by the secondary hydroxyl groups. For 2,3,6-tri-*O*-methyl-β-CD with no intramolecular hydrogen bond, some conformational changes around the glucosidic linkage have been detected from ^{13}C NMR study,[27] but for β-DMCD any obvious changes have not been detected. The secondary hydroxyl groups of β-DMCD form the intramolecular hydrogen-bond network which maintains the stable macrocyclic structure in DMSO-d_6. For α-DMCD we can not also determine the exact value of coupling constant $^3J_{HOCH}$, because the line width of the secondary hydroxyl proton is too large in this system. The value of the coupling constant of α-DMCD is expected to be the same order as that of β-DMCD. In such case, the distance between OH(3′) and O(2) of α-DMCD becomes nearly the same as that of β-DMCD. Hence it is reasonably expected that the strength of the intramolecular hydrogen bond of α-DMCD is about the same as that of β-DMCD. This expectation is also derived from the values of $d\delta/dT$ and R.

Conclusion

In α-, β-, and γ-CDs and α- and β-DMCDs the intramolecular hydrogen bond is formed between OH(2) and OH(3′) groups of adjacent glucose units in DMSO-d_6. The strength of the intramolecular hydrogen bond of CDs in DMSO-d_6 is in the following order: α-DMCD≒β-DMCD>α-CD>β-CD>γ-CD, and this order is opposite to that found in solid. For solutions, the role of solvent molecules must be considered. α- and β-CDs prefer to form the intramolecular hydrogen bond in which OH(3) is the donor of proton. The intramolecular hydrogen bond plays an important role to maintain the stable macrocyclic structure of CDs and DMCDs investigated here.

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