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Internal Reference Compounds Available for the Determination of Binding Constants for Cyclodextrin Complexes by ^{1}H NMR Spectrometry

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The ^{1}H NMR chemical shifts (δ's) of the tetramethylammonium ion (TMA), methanol, and HDO, measured by using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an external reference, showed upfield shift with increasing concentration (c) of carbohydrates such as cyclodextrins (CD's) and methyl glucopyranoside. The plots of δ vs. c gave straight lines with slopes proportional to the number of glucopyranoside residues involved in the carbohydrates, indicating that the upfield shifts are not due to inclusion phenomena but to a change in water structure with the formation of hydrogen bonds between carbohydrates and D_2O. When an internal reference, TMA, was used instead, the ^{1}H NMR signals of such organic molecules as acetonitrile, ethanol, 1-propanol, and 1-butanol showed uniform downfield shifts upon adding α-CD. The curve-fitting analysis of the plots of δ vs. c gave binding constants in agreement with those determined by other methods. It is concluded that the internal reference method is more reliable than the external one.

Nuclear magnetic resonance (NMR) spectrometry has been widely used for the determination of binding constants (K_a) for cyclodextrin (CD) inclusion complexes with guest molecules or ions.[1—4] The complexation of CD with a guest often causes changes in the chemical shifts (δ's) of the ^{1}H's and ^{13}C's involved in the CD and/or guest. The K_a value is determined by the curve-fitting analysis of changes in δ's with the concentration of the CD or guest. External reference is preferable to internal reference in these NMR measurements, since the δ values of most internal reference compounds are significantly changed as a result of complexation with CD's.[5] This NMR method is based on an assumption that changes in δ are caused only by the complexation of CD with a guest: If no complexation occurs between CD and a guest, the δ values have to be constant, irrespective of any changes in concentration of CD or guest.

The present work was undertaken at the beginning to confirm whether the above assumption is valid or not. For this purpose, we investigated the effect of CD's on the δ's of ^{1}H's of the tetramethylammonium ion (TMA) and methanol, using a D_2O solution of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external reference. It was supposed that the K_a values for complexes of the positively charged TMA with CD's are small. It was also known that the K_a values for complexes of methanol with α- and β-CD's are very small.[6] Unexpectedly, we found that CD's, as well as methyl α-D-glucopyranoside (MG) and branched CD's, cause significant upfield shifts in δ's of TMA and methanol. This fact indicates that the above assumption is not valid, and the analysis of δ's determined with an external reference gives a wrong K_a value. A satisfactory K_a value was obtained by the use of TMA or methanol as an internal reference, instead of an external reference, in NMR measurement. K_a values for complexes of CD's with some organic molecules and DSS were determined by this method.

Experimental

Materials. The α-CD was supplied by Nihon Shokuhin Kako Co., Ltd. The β- and γ-CD's, 6-*O*-α-D-glucosyl-α- and -β-CD's (G1-α- and -β-CD's, respectively), and 6-*O*-α-maltosyl-β-CD (G2-β-CD) were supplied by Ensuiko Seito Co., Ltd. They were dried overnight in vacuo at 110 °C. MG, TMA chloride, and such organic solvents as methanol, ethanol, 1-propanol, 1-butanol, acetone, acetonitrile, dimethyl sulfoxide, and *N*,*N*-dimethylformamide were of reagent grade and commercially available.

Sample solutions for NMR measurements contained ca. 2 mmol dm^{-3} guest molecules in D_2O (Isotec, 99.8 atom% D), unless otherwise noted. The maximum concentrations (mmol dm^{-3}) of carbohydrates added to the sample solutions of TMA and methanol were 430 for MG, 70 for α-CD, 9 for β-CD, 32 for γ-CD, 100 for G1-α-CD, 93 for G1-β-CD, and 96 for G2-β-CD. ^{1}H NMR spectra were recorded using a JEOL Model JNM-A400 FT NMR spectrometer (400 MHz) at 25±0.1 °C. A D_2O solution of DSS ($\delta = 0.000$) was loaded into a glass capillary of 1.0 mm diameter and used as an external reference.

Determination of K_a by NMR Spectrometry. On the assumption that CD forms a 1 : 1 inclusion complex with a guest molecule, K_a is represented by Eq. 1:

$$K_a = x/(c-x)(s-x), \quad (1)$$

where c and s are the initial concentrations of CD and a guest, respectively, and x is the equilibrium concentration of an inclusion complex. Then, x is given by

$$x = (1/2)\{(c+s+1/K_a) - [(c+s+1/K_a)^2 - 4c{\cdot}s]^{1/2}\}. \quad (2)$$

When a chemical exchange process in the complexation is rapid with reference to NMR time-scale, a change ($\Delta\delta$) in δ of a guest is represented by Eq. 3:

$$\Delta\delta = \Delta\delta_0 \cdot x/s, \tag{3}$$

where $\Delta\delta_0$ is a difference in δ between complexed and free guest molecules. If K_a and $\Delta\delta_0$ are given, $\Delta\delta$ is calculated for any c and s values by use of Eqs. 2 and 3. In practice, we determined K_a and $\Delta\delta_0$ by a nonlinear least-squares curve-fitting analysis of the change in $\Delta\delta$ with c at constant s.

Results and Discussion

Effect of CD's on δ's of TMA and Methanol. TMA gave a triplet ^{1}H NMR signal at δ=3.176 (J=0.6 Hz) upon using DSS as an external reference. The splitting may be due to spin-spin coupling of methyl ^{1}H's with ammonium ^{14}N. The signal did not overlap with ^{1}H NMR signals of carbohydrates such as CD's, MG, and branched CD's and showed upfield shifts with increasing concentrations (c) of the carbohydrates (Fig. 1). Plots of δ vs. c gave straight lines for all carbohydrates examined with correlation coefficients (r) of 0.999 for most carbohydrate systems, except r=0.97 for a β-CD system, in which only small changes in δ were observed because of the low solubility of β-CD in D_2O. The slopes of the straight lines are summarized in Table 1. Evidently, the magnitudes of the slopes were proportional to the number of glucopyranoside residues involved in the carbohydrates. Although MG has no cavity within which a guest molecule is to be included, it caused an upfield shift of TMA signal. These facts are new findings, which suggest that the upfield shifts caused by CD's are not due to inclusion of TMA within CD cavities.

Very similar phenomena were also observed for methanol/CD systems. Methanol gave a singlet ^{1}H NMR signal at δ=3.343 (with reference to external DSS), which did not overlap with signals of the carbohydrates examined. The signal also showed linear upfield shift with increasing c of the carbohydrates. The magnitudes of the slopes were roughly equal to those observed for TMA (Table 1). Methanol forms inclusion complexes with α- and β-CD's in crystalline states.[7,8] However, the K_a values in an aqueous solution are very small (0.9 and 0.3 mol^{-1} dm^3 for α- and β-CD's, respectively).[6] The amount of complexed methanol may not exceed 6% of total methanol for a α-CD system and 0.3% for a β-CD system under conditions examined. Thus, it is difficult to attribute the upfield shift to inclusion of methanol within CD cavities.

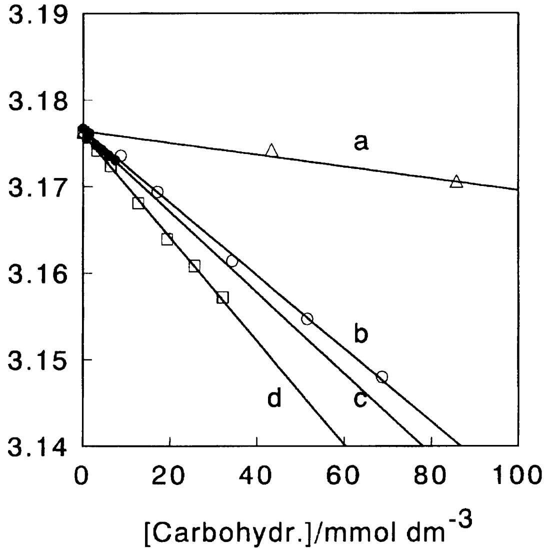

Fig. 1. Changes in δ of ^{1}H NMR signal of TMA (1.52 mmol dm^{-3}) with the addition of carbohydrates such as MG (a), α-CD (b), β-CD (c), and γ-CD (d). The straight lines are calculated by the least-squares analysis of measured data (△: MG, ○: α-CD, ●: β-CD, □: γ-CD). The data for [MG] > 100 mmol dm^{-3} are not shown.

The solvent D_2O used contained ca. 0.4% (w/w) HDO, which gave a singlet signal at δ=4.770 with reference to external DSS. The signal also showed linear upfield shift with increasing c of the carbohydrates. The slopes were roughly equal to those observed for TMA and methanol (Table 1). Thus, we concluded that the observed upfield shifts are not due to specific inclusion phenomena. It is well known that carbohydrates form hydrogen bonds with water to cause a change in the structure of water clusters[9] and in water mobility.[10] The change in water structure may equally affect the magnetic susceptibilities of protons of TMA, methanol, and HDO to cause similar upfield shifts.

Determination of K_a's for Complexes of α-CD with Some Organic Solvent Molecules by ^{1}H NMR. Acetonitrile gave a singlet signal at δ=2.056 with reference to external DSS. Upon adding α-CD, the signal showed a downfield shift at lower α-CD concentrations and then an upfield shift at higher concentrations (Fig. 2a). The change in δ can not be explained in terms of simple 1 : 1 complexation of acetonitrile with α-CD. It is known that acetonitrile forms an inclusion complex with α-CD, the K_a value being equal to 5.6 mol^{-1} dm^3 in H_2O at 25 °C.[11] Thus, we assumed that the downfield shift is due to van der Waals contact of acetonitrile ^{1}H's with the α-CD cavity upon their complexation, and the upfield shift, to a change in water structure as described above. When TMA was used as an external reference instead of an internal reference of DSS, the upfield shift was compensated and changes ($\Delta\delta$'s) in δ of acetonitrile ^{1}H's showed uniform downfield shifts (Fig. 2b). The δ value of TMA in

Table 1. Slopes of Linear Regression Lines in the Plots of δ vs. c for TMA, Methanol, and HDO in D_2O at 25 °C

Sugar	TMA		Methanol		HDO	
	Slope	Ratio[a)]	Slope	Ratio[a)]	Slope	Ratio[a)]
MG	−0.068	1.0	−0.059	0.9	−0.065	0.8
α-CD	−0.421	6.0	−0.401	6.0	−0.485	6.0
β-CD	−0.467	6.7	−0.532	8.0	−0.664	8.2
γ-CD	−0.598	8.5	−0.574	8.6	−0.683	8.4
G1-α-CD	−0.487	6.9	−0.404	6.0	−0.549	6.8
G1-β-CD	−0.559	8.0	−0.534	8.0	−0.680	8.4
G2-β-CD	−0.612	8.7	−0.594	8.9	−0.745	9.2

a) Ratio of each slope to that of α-CD which is referred to 6.0.

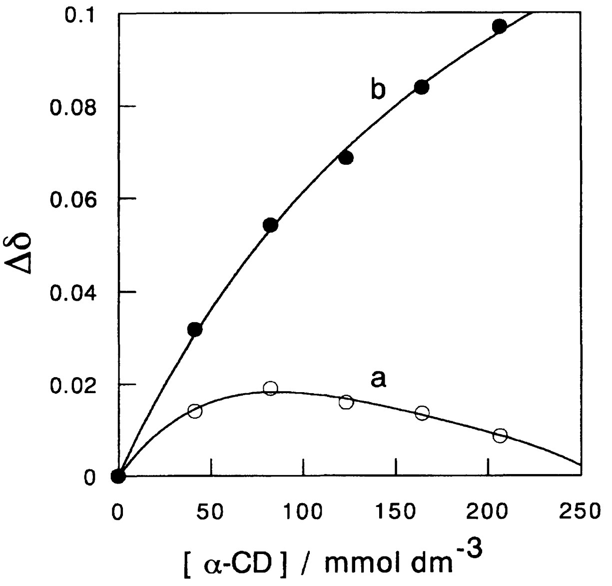

Fig. 2. Plots of $\Delta\delta$ vs. [α-CD] for acetonitrile (2.19 mmol dm^{-3}). The values of $\Delta\delta$ were measured by using external DSS (a) and internal TMA (b) as NMR references.

D_2O was not affected by the addition of acetonitrile, indicating that no interaction occurs between TMA and acetonitrile. Thus, it is reasonable to consider that the uniform downfield shifts correspond to net shifts due to the complexation of acetonitrile with α-CD. The K_a value, as well as $\Delta\delta_0$ value, for a complex of acetonitrile with α-CD was determined by a nonlinear least-squares curve-fitting analysis of the change in $\Delta\delta$ with c, as described in Experimental, on the basis of the assumption that acetonitrile forms a 1 : 1 complex with α-CD (Table 1). The calculated curve was well-fitted to the observed data with a correlation coefficient of 0.999. The obtained K_a value was 4.6 mol^{-1} dm^3, being in good agreement with that previously reported.[11)] This fact suggests that a satisfactory K_a value is obtained in NMR measurements by the use of TMA as an internal reference, instead of by the use of DSS as an external reference.

In order to confirm the validity of this method, the K_a values for α-CD complexes with ethanol, 1-propanol, and 1-butanol were determined and compared with those determined by other methods (Table 2). The NMR signals due to the alcohol ^{1}H's, except for those due to the hydroxymethyl ^{1}H's, were well separated from those of α-CD ^{1}H's and showed downfield shifts with increasing α-CD concentration. The curve-fitting analysis of the plots of $\Delta\delta$ with reference to the TMA signal vs. c gave correlation coefficients larger than 0.998. The analysis of $\Delta\delta$'s for the β-methylene, γ-methylene, and methyl ^{1}H's of 1-butanol afforded virtually the same K_a values of 75, 75, and 74 mol^{-1} dm^3, respectively. Similarly, the β-methylene and methyl ^{1}H's of 1-propanol resulted in virtually the same K_a values. Furthermore, the obtained K_a values for these alcohols were in good agreement with those reported. These facts support the validity of this method. K_a values for α-CD complexes with acetone, dimethyl sulfoxide (DMSO), and *N*, *N*-dimethylformamide (DMF) were also determined by the same method and are shown in Table 2. The ^{1}H NMR signals of these organic solvent molecules also showed downfield shifts, with reference to the TMA signal, upon adding α-CD. Acetone and DMF gave satisfactory K_a values. However, in the case of DMSO, the change in $\Delta\delta$ was too small, and virtually linear with c, to give a K_a value.

Table 2. Binding Constants (K_a/mol^{-1} dm^3) for α-CD Complexes with Some Organic Solvent Molecules in D_2O at 25 °C

Guest	K_a/mol^{-1} dm^3		$\Delta\delta_0$	
	Obsd.	Lit.	ppm	
Acetonitrile	4.6	5.6[a)]	0.19	(CH_3)
Ethanol	2.8	4.1,[a)] 5.6[b)]	0.24	(CH_3)
1-Propanol	19	23[b)]	0.13	(CH_3),
			0.20	(β-CH_2)
1-Butanol	75	89[b)]	0.13	(CH_3),
			0.19	(γ-CH_2),
			0.18	(β-CH_2)
Acetone	2.0		0.22	(CH_3)
DMF	3.1		0.20	(CH_3[c)]),
			0.14	(CH_3[d)])
DMSO	<1.0	0.41[a)]		

a) Ref. 11. b) Ref. 6. c) Methyl protons at higher field. d) Methyl protons at lower field.

Complexation of DSS with *α*-, *β*-, and *γ*-CD's. Li et al.[5)] showed by NMR spectrometry that DSS forms inclusion complexes with β- and γ-CD's, but not with α-CD, in a D_2O solution. However, their work was somewhat qualitative, and no K_a values for these systems were given. Thus, we investigated the effect of CD's on the ^{1}H NMR signals of DSS in D_2O in a quantitative manner. Different from the conclusion by Li et al., we found that DSS interacts not only with β- and γ-CD's but also with α-CD. The methyl ^{1}H's of DSS gave a singlet signal at the same δ as an external DSS reference (δ=0.000) in the absence of CD's (Fig. 3a). Upon adding α-CD to the DSS solution, the singlet signal markedly broadened and shifted to downfield with reference to the external DSS (Fig. 3b). Further addition of α-CD resulted in narrowing of the signal width and further downfield shift (Figs. 3c and 3d). The downfield shift may be caused by complexation of DSS with α-CD in a similar manner as the ^{1}H's of organic solvent molecules. The marked broadening of the signal may be due to acceleration of magnetic relaxation by van der Waals contact of the DSS methyl ^{1}H's with the α-CD cavity. At higher α-CD concentrations, a chemical exchange reaction of DSS with α-CD may be enhanced to narrow the signal width. The ^{1}H's of methylenes involved in a DSS molecule also showed similar downfield shifts, though the extent of broadening was much less than that in the methyl ^{1}H's. Upon adding MG, instead of CD's, to a DSS solution, the signal of the DSS methyl ^{1}H's shifted upfield, very similarly to the signals of TMA and methanol. Then, we analyzed the change in $\Delta\delta$ with reference to the TMA signal, instead of the external DSS signal, to give the

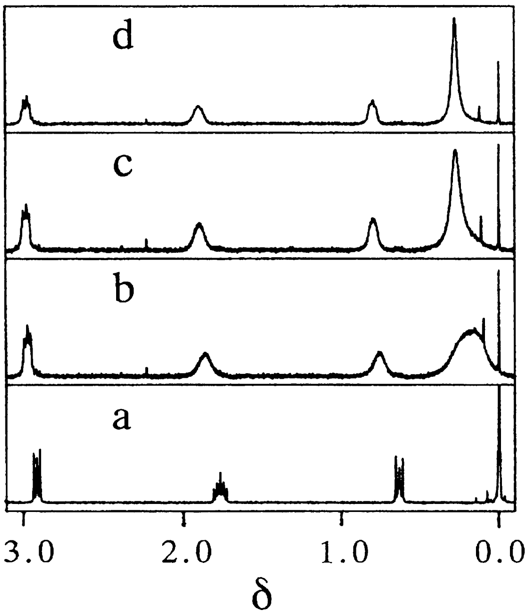

Fig. 3. Effect of α-CD on the ^{1}HNMR spectra of DSS (5.00 mmol dm^{-3}) measured by using DSS as an external reference. [α-CD]/mmol dm^{-3}=0 (a), 20.8 (b), 41.4 (c), and 61.9 (d).

K_a value of 64±2 mol^{-1} dm^3 for a 1 : 1 complex of DSS with α-CD. The analysis of $\Delta\delta$'s for four kinds of ^{1}H's in a DSS molecule gave K_a values which were in good agreement with one another. The $\Delta\delta_0$ values were determined to be 0.39 ppm for the methyl protons of DSS, and 0.25, 0.20, and 0.12 ppm for the 3-, 4-, and 5-methylene protons of DSS, respectively. The same method was applied to the determination of K_a for complexes of DSS with β- and γ-CD's. The obtained K_a values were 3000±400 and 58±6 mol^{-1} dm^3 for β- and γ-CD complexes, respectively. The K_a value for β-CD complex was much larger than those for α- and γ-CD complexes. The size of the trimethylsilyl group of DSS may be suitable for close van der Waals contact with the β-CD cavity. The $\Delta\delta_0$ values for a β-CD–DSS complex were 0.15, 0.04, 0.03, and −0.10 ppm for the methyl and 3-, 4-, and 5-methylene protons of DSS, respectively. Corresponding $\Delta\delta_0$ values for a γ-CD–DSS complex were 0.04, −0.02, 0.05, and −0.03 ppm, respectively.

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