Complexation of Inorganic Anions with Heptakis(6-butylamino-6-deoxy)-β-cyclodextrin and Its Analogs in Acidic Media

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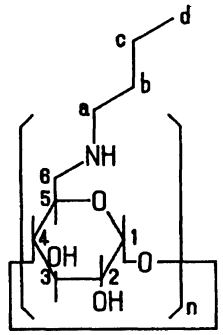
Heptakis(6-butylamino-6-deoxy)-β-cyclodextrin (**2**) forms 1:1 complexes with several inorganic anions in 0.10 mol dm^{-3} HCl or DCl at 25 °C. The binding constant for a complex of **2** with the SO_4^{2-} ion was remarkably larger than those for complexes of **2** with univalent anions and those for complexes of the α- (**1**) and γ-cyclodextrin (**3**) analogs with the SO_4^{2-} ion. The circular arrangement of the protonated amino groups in **2** may be advantageous for electrostatic interactions with the bivalent anion.

The complexation of host compounds with inorganic anions is interesting from the chemical and biological points of view.[1] Several of the hosts so far reported to recognize inorganic anions are spheroidal[2] and ellipsoidal[3] cryptands, heterocyclophane,[4] and macrocyclic polyamines,[1c,5] all of which bear four or more amino and/or ammonio groups with a circular or spherical arrangement in the molecule. The present study deals with the complexation of several inorganic anions with heptakis(6-butylamino-6-deoxy)-β-cyclodextrin (**2**) and its α- (**1**) and γ-cyclodextrin (**3**) analogs in acidic media. Since these compounds have six or more amino groups with a circular arrangement in the molecule, it is anticipated that they can recognize inorganic anions (Chart 1).

Experimental

Parent α-, β-, and γ-cyclodextrins were kindly supplied by Nihon Shokuhin Kako Co., Ltd. and Ensuiko Seito Co., Ltd. They were dried overnight in vacuo at 110 °C. The butylamine, dibutylamine, Methyl Orange (MO), and inorganic salts examined were of reagent grade and commercially available. D_2O (Merck, 99.75%) and DCl (Aldrich, 37 wt% solution in D_2O, 99 atom% D) were also commercially available for NMR use. The ^{1}H NMR spectra were recorded using a JEOL Model JNM-GX270 FT NMR spectrometer (270 MHz) at 25 °C. Acetonitrile (δ=2.00[6]) was used as an internal reference.



1: n = 6,
2: n = 7,
3: n = 8.

Chart 1. Structural formula.

Preparation of 1—3. Compounds **1**—**3** were prepared in a similar manner as reported by Takahashi, et al.[7] In a typical run, 1.88 g (0.869 mmol) of heptakis(2,3-di-*O*-acetyl-6-bromo-6-deoxy)-β-cyclodextrin[8] was dissolved in 30 cm^3 of butylamine; the resulting solution was refluxed for 20 h. After evaporation to dryness, the residue was dissolved in 10% (v/v) aqueous acetic acid, and the resulting solution was made basic by the addition of 10% aqueous ammonia to give a precipitate. After filtration, the residue was washed with water and dried in vacuo to give 1.17 g (0.769 mmol) of **2** in 88% yield; (Found for **1**: C, 51.86; H, 8.30; N, 5.78%. Calcd for $C_{60}H_{114}O_{24}N_6 \cdot 5H_2O$: C, 51.71; H, 8.97; N, 6.03%. Found for **2**: C, 52.98; H, 8.56; N, 5.88%. Calcd for $C_{70}H_{133}O_{28}N_7 \cdot 4H_2O$: C, 52.78; H, 8.92; N, 6.16%. Found for **3**: C, 51.78; H, 8.18; N, 5.78%. Calcd for $C_{80}H_{152}O_{32}N_8 \cdot 6H_2O$: C, 52.05; H, 8.95; N; 6.07%). The ^{1}H NMR signals of **1**—**3** in D_2O containing 0.10 mol dm^{-3} DCl were so simple as to be expected from their symmetrical structures (Table 1). The standard deviations of the chemical shifts for the signals shown were less than 0.002 ppm (n=16).

The pH Titration of 1—3. Although compounds **1**—**3** were insoluble in neutral and basic aqueous solutions, they were soluble in an acidic aqueous solution and 50% (v/v) aqueous ethanol. Hence, about 22 mg of each host was dissolved in 50% ethanol; the resulting solution was

Table 1. ^{1}H NMR Chemical Shifts of **1**—**3** in 0.10 mol dm^{-3} DCl/D_2O at 25 °C.

Proton		1	2	3
C(1)–H[a)]	(1H,[b)] d[c)], J=3.3[d)])	5.126	5.160	5.259
C(2)–H	(1H, dd, J=3.3,10.1)	3.627	3.636	3.630
C(3)–H	(1H, t, J=9.4)	3.966	3.933	3.906
C(4)–H	(1H, t, J=9.4)	3.556	3.569	3.565
C(5)–H	(1H, m)	4.326	4.252	4.238
C(6)–H	(2H, m)	3.344	3.333	3.343
C(a)–H	(2H, t, J=7.9)	3.123	3.106	3.111
C(b)–H	(2H, m)	1.681	1.685	1.690
C(c)–H	(2H, m)	1.364	1.369	1.375
C(d)–H	(3H, q, J=7.3)	0.897	0.898	0.904

a) Numbers and letters in parentheses refer to the structural formula shown for **1**—**3**. b) Number of H per a glucopyranose residue. c) Multiplicity. d) J value in Hz for **2**.

titrated with 0.10 mol dm^{-3} HCl, HNO_3, or $HClO_4$ and 0.05 mol dm^{-3} H_2SO_4 in 50% ethanol under an atmosphere of N_2. The pH of the solution was measured by an Orion Model 810A digital pH/mV meter.

Spectrophotometry of the Complexation of 1—3 with MO. The absorption spectra were recorded for aqueous solutions containing 0.10 mol dm^{-3} HCl, 0.0186 mmol dm^{-3} MO, and 0.0—2.0 mmol dm^{-3} **1**, **2**, or **3** at 25 °C by means of a Shimadzu Model UV-2100 spectrophotometer. The dye gave an absorption maximum at 507 nm. Although absorbance was only little affected by the addition of **1** and **3**, it appreciably decreased and approached a constant value upon the addition of **2**. The binding constant (K_a) for a complex of **2** with MO was determined by a least-squares curve-fitting analysis of the changes in the absorbance at 510 nm with the concentration of **2**, based upon the assumption of 1 : 1 complexation. The thus-calculated curve was well-fitted to the observed data with a correlation coefficient greater than 0.999. The difference ($\Delta\varepsilon$) in the molar absorbance between free and complexed MO was simultaneously estimated.

Spectrophotometric Determination of K_a for Complexes of 2 with Inorganic Anions. The absorption spectra were recorded for 0.10 mol dm^{-3} HCl solutions containing 0.0186 mmol dm^{-3} MO, 2.0 mmol dm^{-3} **2**, and 0—150 mmol dm^{-3} inorganic salts at 25 °C. The absorbance at 510 nm increased and approached a constant value with increasing concentration of the inorganic salts examined (Fig. 1), suggesting that the salts competitively inhibit the complexation of **2** with MO. Based on the assumption that the inorganic anions of the salts form 1 : 1 complexes with **2**, the K_a values for the complexes were determined by a nonlinear least-squares curve-fitting analysis[9] of the changes in the absorbance with the concentrations of inorganic salts. The obtained curves (solid lines) were well-fitted to the observed data with correlation coefficients greater than 0.99.

Determination of K_a for Complexes of 1—3 with Inorganic Anions by ^{1}H NMR. ^{1}H NMR spectra were recorded at 25 °C for D_2O solutions containing 0.10 mol dm^{-3} DCl, 5—8 mmol dm^{-3} **1**—**3**, various concentrations of inorganic salts, and a trace amount of acetonitrile as an internal reference. Some protons involved in **1**—**3** showed significant changes in their chemical shifts upon the addition of inorganic salts. Figure 2 is an illustration of the changes ($\Delta\delta$) in the C(4)–H chemical shifts of **1**—**3** upon the addition of Na_2SO_4. The changes were analyzed by a nonlinear least-squares curve-fitting method to give the K_a values for the complexes of **1**—**3** with inorganic anions, based on the assumption of 1 : 1 complexation. The thus-calculated curves (solid lines) were well-fitted to the observed data with correlation coefficients greater than 0.99.

Results and Discussion

pH Titration. Compound **1**—**3** were titrated with HCl, HNO_3, $HClO_4$, and H_2SO_4 in 50% ethanol. Figure 3 shown typical curves for the titration of **2** with HCl and H_2SO_4. The curves for the titration with HNO_3 and $HClO_4$ were similar to that with HCl. All of the curves gave apparent inflection points at around $[H^+]/[\mathbf{2}]=6.0$, indicating that six of the seven amino groups in **2** were protonated in an acidic media below pH 3; the seventh was difficult to be protonated, probably due to electrostatic repulsive interactions. Another interesting observation was the pH difference between the curves of titration with H_2SO_4 and with HCl over the range of $[H^+]/[\mathbf{2}]$ from 1 to 5, where the pH for the

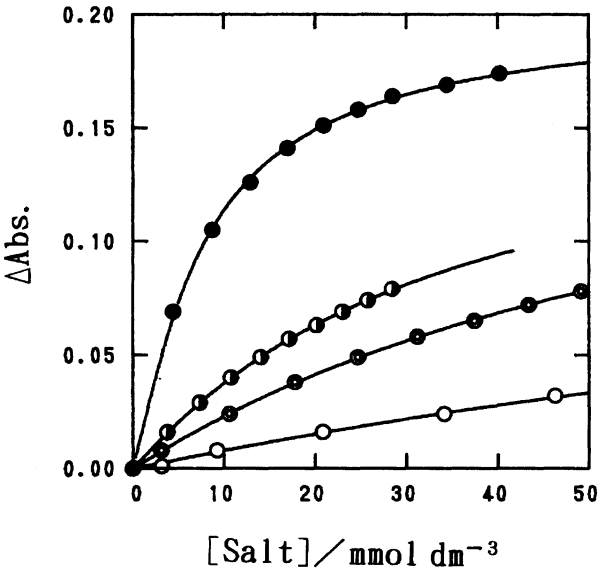


Fig. 1. Changes (ΔAbs.) in absorbance at 510 nm with the addition of KBr (○), KI (◎), KSCN (◑), and Na_2SO_4 (●) to 0.10 mol dm^{-3} HCl containing 0.0186 mmol dm^{-3} MO and 2.00 mmol dm^{-3} **2** at 25 °C. The solid lines were calculated by the curve-fitting analysis based on 1 : 1 complexation.

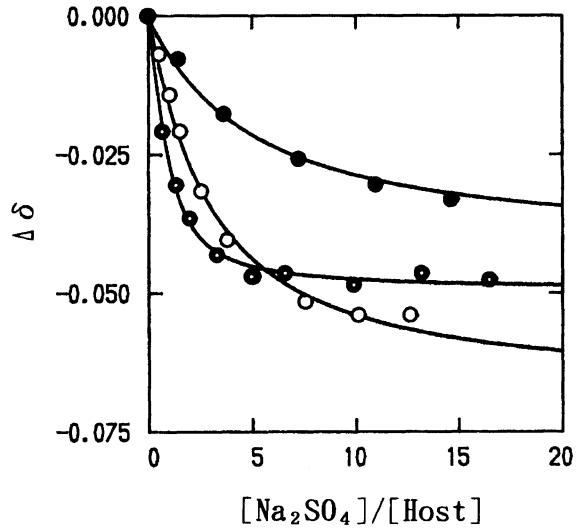


Fig. 2. Changes ($\Delta\delta$) in ^{1}H NMR chemical shifts of the C(4)–H of **1**—**3** with the addition of Na_2SO_4 to 0.10 mol dm^{-3} DCl in D_2O containing 7.96 mmol dm^{-3} **1** (○), 6.64 mmol dm^{-3} **2** (◎), or 5.79 mmol dm^{-3} **3** (●) at 25 °C. The solid lines were calculated by the curve-fitting analysis based on 1 : 1 complexation.

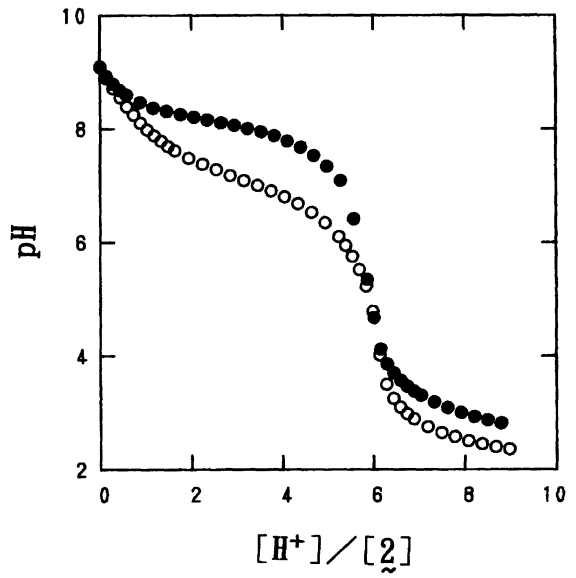


Fig. 3. pH titration of **2** with HCl (○) and H_2SO_4 (●) in 50% (v/v) aqueous ethanol.

former was remarkably higher than that for the latter. When dibutylamine was titrated in place of **2**, no such difference was observed. A possible explanation of the difference in the pH is that the SO_4^{2-} ion interacts with protonated **2** so strongly as to retard its deprotonation. Similar curves were obtained for the titration of **1** and **3** with HCl and H_2SO_4, except that the inflection points were observed at $[H^+]/[host]=5.0$ for **1** and 6.0 for **3**, suggesting that five of six amino groups in **1** and six of eight amino groups in **3** are protonated in acidic media below pH 3.

Spectrophotometry on Interactions of 1—3 with Inorganic Anions. In order to substantiate specific interactions between the protonated **1**—**3** with the SO_4^{2-} ion, we examined the effect of neutral salts on the complexation of the hosts with dyes. Among several dyes examined, only MO showed and appreciable decrease in absorbance at around 510 nm upon the addition of **2** in 0.10 mol dm^{-3} HCl, though **1** and **3** caused very small changes in the spectra of MO. The 1:1 complexation of **2** with MO was confirmed by an ordinary curve-fitting analysis of the spectrophotometric data obtained for 0.0186 mmol dm^{-3} MO at various concentrations of **2** in 0.10 mol dm^{-3} HCl at 25 °C. The K_a value (1.05×10^3 mol^{-1} dm^3) obtained for the system was abut 5-times larger than that (1.9×10^2 mol^{-1} dm^3) for a MO complex with a parent β-cyclodextrin. The $\Delta\varepsilon$ value (18.2×10^3 mol^{-1} dm^3 cm^{-1}) for the former was comparable to that (25.0×10^3 mol^{-1} dm^3 cm^{-1}) for the latter.

The addition of NaCl to a solution of **2** and MO had only a slight effect on the spectra of the solution. On the other hand, the absorbance at 510 nm increased and approached constant values upon the addition of Na^+ or K^+ salts of Br^-, I^-, NO_3^-, ClO_4^-, SCN^-, and SO_4^{2-} (Fig. 1). Based on the assumption that these anions form 1:1 complexes with **2** to competitively retard the complexation of **2** with MO, the K_a values for **2**-anion systems were determined by a curve-fitting analysis of the data (Table 2). The thus-calculated curves were well-fitted to the observed data, indicating that the above assumption is valid. The $\Delta\varepsilon$ value accompanied by the complexation of **2** with these anions was determined to be, on the average, $(16.3\pm0.9)\times10^3$ mol^{-1} dm^3 cm^{-1}, which was comparable to that for the above-mentioned **2**-MO system. The fact that the anions compete with MO for complexation with **2** suggests that the anions and MO are bound to nearly the same site of the host, probably the cyclodextrin cavity. The accompanying cations of NO_3^- and SO_4^{2-} had virtually no effect on the K_a values. Importantly, the K_a value for the SO_4^{2-} ion was much larger than those for the univalent anions. This fact is consistent with the presumption based on the pH titration mentioned above.

^{1}H NMR Spectrometry on the Complexation of 1—3 with Inorganic Anions. Complexation of **1**—**3** with inorganic anions was directly observed by ^{1}H NMR spectrometry in 0.10 mol dm^{-3} DCl/D_2O at 25 °C. The ^{1}H NMR signals of **1**—**3** were so simple as to be expected form their symmetrical structures (Table 1). Table 3 shows the changes ($\Delta\delta$) in the chemical shifts of **2** (6.6 mmol dm^{-3}) with the addition of ca. 200 mmol dm^{-3} Na salts of univalent anions. The addition of NaCl had virtually no effect on the chemical shifts of all the protons in **2**. On the other hand, the addition of NaBr, NaI, $NaNO_3$, and $NaClO_4$ caused significant changes in the chemical shifts of a part of the protons in **2**. It was estimated on the basis of the K_a values (as described below) that at the salt concentrations examined a large portion of **2** was bound to the inorganic anions (71% in NaBr, 79% in NaI, 82% in $NaNO_3$, and 84% in $NaClO_4$). $NaNO_3$ and $NaClO_4$ caused remarkable upfield shifts in C(5)–H located within the hydrophobic cavity of the cyclodextrin moiety, suggesting that the chaotropic anions of these salts are incorporated into the cavity by hydrophobic interactions. It may be impossible for **2** to accommodate two or more anions into the cavity, due to the

Table 2. The K_a Values Spectrophotometrically Determined for Complexes of **2** with Inorganic Anions in 0.10 mol dm^{-3} HCl at 25 °C

Anion	Cation	K_a/mol^{-1} dm^3
Br^-	K^+	17
I^-	K^+	46
NO_3^-	Na^+	41
NO_3^-	K^+	48
ClO_4^-	Na^+	40
SCN^-	K^+	93
SO_4^{2-}	Na^+	470
SO_4^{2-}	K^+	430

Table 3. Changes in ^{1}H NMR Chemical Shifts of **2** upon the Addition of Inorganic Salts in 0.10 mol dm^{-3} DCl/D_2O at 25 °C[a)]

Proton	NaCl	NaBr	NaI	$NaNO_3$	$NaClO_4$
C(1)–H	0.002	0.023	0.037	−0.015	−0.008
C(2)–H	0.001	0.007	0.014	−0.011	−0.008
C(3)–H	−0.001	0.004	0.034	−0.009	0.014
C(4)–H	0.005	0.028	0.058	0.000	0.006
C(5)–H	−0.001	0.046	0.115	−0.126	−0.065
C(6)–H	0.000	0.025	0.051	−0.001	−0.002
C(a)–H	0.002	0.021	0.045	−0.006	−0.010
C(b)–H	0.005	0.014	0.028	−0.033	−0.005
C(c)–H	−0.005	0.002	0.011	−0.025	−0.012
C(d)–H	−0.005	−0.002	0.003	−0.024	−0.012

a) [**2**] = 6.6 mmol dm^{-3} and [Inorganic Salt]=200 mmol dm^{-3}. Positive and negative values indicate downfield and upfield shifts, respectively.

Table 4. Changes in ^{1}H NMR Chemical Shifts of **1**—**3** upon the Addition of Na_2SO_4 in 0.10 mol dm^{-3} DCl/D_2O at 25 °C[a)]

Proton	**1**[b)]	**2**[c)]	**3**[d)]
C(1)–H	−0.038	−0.017	−0.084
C(2)–H	−0.017	−0.011	−0.007
C(3)–H	0.006	−0.020	0.004
C(4)–H	−0.054	−0.048	−0.037
C(5)–H	−0.025	0.017	0.000
C(6)–H	−0.087	−0.049	−0.037
C(a)–H	−0.087	−0.040	−0.049
C(b)–H	−0.021	−0.017	−0.025
C(c)–H	−0.020	−0.014	−0.021
C(d)–H	−0.014	−0.005	−0.014

a) Positive and negative values indicate downfield and upfield shifts, respectively. b) [**1**]=7.7 mmol dm^{-3} and [Na_2SO_4]=100 mmol dm^{-3}. c) [**2**]=6.6 mmol dm^{-3} and [Na_2SO_4] = 110 mmol dm^{-3}. d) [**3**] = 5.8 mmol dm^{-3} and [Na_2SO_4]=210 mmol dm^{-3}.

limited cavity size and/or strong electrostatic repulsion between the anions. This restriction in the complexation may be related to the 1 : 1 stoichiometry observed by spectrophotometry for complexes of the chaotropic univalent anions with the polycationic species of protonated **2** in an acidic media. The addition of NaBr and NaI caused significant downfield shifts not only in the C(5)–H, but also in the C(1)–H, C(4)–H, and C(6)–H of the cyclodextrin moiety and the C(a)–H of the butylamino moiety. The C(6)–H and C(a)–H are located close to the positively charged ammonium ions in the protonated **2**. The C(1)–H and C(4)–H are attached to carbons which constitute the glucoside linkages of cyclodextrin; the chemical shifts of these protons are sensitive to any conformational change in the macrocycle of cyclodextrin.[10] Thus, the chaotropic Br^- and I^- ions may be incorporated into the cavity of cyclodextrin by hydrophobic interactions, and also located in the vicinity of the ammonium ions, accompanied by significant conformational changes in the cyclodextrin macrocycle. Similar changes in the chemical shifts were observed for complexes of **1** and **3** with the chaotropic univalent anions.

Table 4 shows the $\Delta\delta$ values for **1**—**3** upon the addition of Na_2SO_4 in 0.10 mol dm^{-3} DCl/D_2O at 25 °C. At the SO_4^{2-} concentrations examined, most of the hosts were bound to SO_4^{2-} (83% of **1**, 98% of **2**, and 89% of **3**, estimated by the K_a values shown below). Interestingly, the addition of the salt caused significant upfield shifts in C(1)–H, C(4)–H, C(6)–H, and C(a)–H, but no, or slight, shifts in C(5)–H. The interactions of the antichaotropic SO_4^{2-} ion with the hydrophobic cavity of cyclodextrin may be weak, if any. Instead, the SO_4^{2-} ion may be electrostatically bound to two or more ammonio groups of protonated **2** so as to form a bridge-like linkage. The linkage may reduce the repulsive interactions between the positively charged ammonium ions, make the ammonio groups closer together, and cause a conformational change in the cyclodextrin macrocycle. The $\Delta\delta$ value for C(1)–H of **2** was significantly smaller than those of **1** and **3**, suggesting that the conformational change with the complexation is smaller in **2** than in **1** and **3**. The 1 : 1 stoichiometry for a complexe of **2** with SO_4^{2-} was confirmed based on a change in the C(4)–H chemical shift of **2** upon the addition of Na_2SO_4 in D_2O containing 0.05 mol dm^{-3} DCl at 25 °C. Under these conditions, the interactions between **2** and SO_4^{2-} were so strong that a clear inflection point was observed at the point of 1 : 1 stoichiometry (Fig. 4). The effect of the DCl concentration on the K_a value is now more closely under investigation.

The K_a values for 1 : 1 complexes of **1**—**3** with several inorganic anions were determined by a nonlinear least-squares curve-fitting analysis of the changes in the

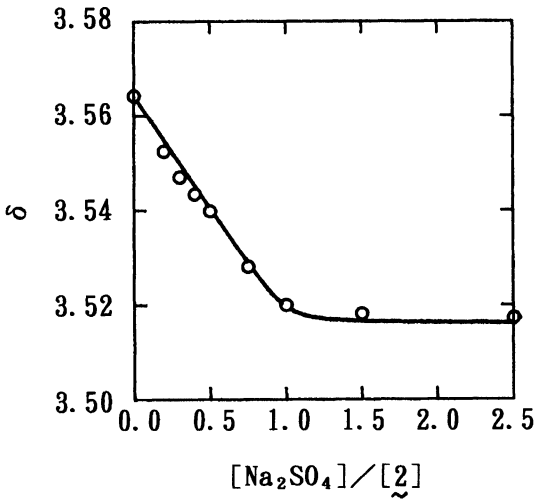


Fig. 4. Plot of ^{1}H NMR chemical shift for the C(4)–H of 6.72 mmol dm^{-3} **2** vs. Na_2SO_4 added to 0.05 mol dm^{-3} DCl/D_2O at 25 °C. The solid line was calculated by the curve-fitting analysis based on 1 : 1 complexation.

Table 5. The $K_a/mol^{-1}\,dm^3$ Values Determined by ^1H NMR for Complexes of **1**—**3** with Inorganic Anions in 0.10 $mol\,dm^{-3}$ DCl/D_2O at 25 °C

Anion[a)]	**1**	**2**	**3**
SO_4^{2-}	52	382	40
HSO_3^-	2	4	5
Br^-	11	13	10
I^-	16	20	15
NO_3^-	10	23	23
ClO_4^-	64	27	42
SCN^-	44	10	19

a) Sodium or potassium salts.

^1H NMR chemical shifts of the hosts with increasing concentrations of the guests in 0.10 $mol\,dm^{-3}$ DCl/D_2O at 25 °C (Table 5). The thus-calculated curves were well-fitted to the observed data, indicating that the assumption of 1 : 1 complexation is valid. The K_a values obtained for **2** roughly agreed with those determined by spectrophotometry (Table 2), though some differences were found between them, probably due either to a solvent deuterium effect or to an experimental error. In any event, the K_a value for a complex of **2** with SO_4^{2-} was remarkably larger than those for complexes of **2** with univalent anions, in a similar manner as observed by spectrophotometry in H_2O. Moreover, the K_a value was 7- and 10-times larger than those for the corresponding complexes of **1** and **3**, respectively. This fact indicates that the size of cyclodextrin macrocycle is also essential for the formation of a bridge-like linkage between the protonated **2** and SO_4^{2-} and, thus, for stabilization of a complex between them. This presumption is also supported by the fact mentioned above that a conformational change in the cyclodextrin macrocycle of the host accompanied by complexation with SO_4^{2-} is smaller in **2** than those in **1** and **3**.

The SO_4^{2-} ion is one of the most ubiquitous inorganic anions in nature, and plays important roles in chemistry and biology. However, no organic host compound has been reported to recognize the SO_4^{2-} ion, so far as we know. The present study showed that **2** forms a relatively stable complex with SO_4^{2-} with noticeable selectivity. However, complexation occurs only in an acidic media. It is expected that the replacement of all the primary hydroxyl groups of β-cyclodextrin by any quarternary ammonium ions gives a host compound which recognizes the SO_4^{2-} ion, even in neutral or basic media. We are now working on the preparation of such a host compound.

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