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Inclusion of Bisphenols by Cyclodextrin Derivatives *

HIROMI KITANO**, HIROSHI ENDO, MAKOTO GEMMEI-IDE and MAYUMI KYOGOKU
Department of Chemical and Biochemical Engineering, Toyama University, Toyama 930-8555, Japan

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Abstract

Complexation of various kinds of bisphenols (BPs) with cycloheptaamylose (β-cyclodextrin, β-CD) derivatives (β-CD, hydroxyethyl-β-CD (HE-β-CD), 2,6-di-*O*-methyl-β-CD (DM-β-CD) and polymerised β-CD (L-Poly-β-CD)) was examined fluorimetrically using 2-anilinonaphthalene-6-sulfonic acid (2,6-ANS) as a probe. From the inhibitory effect of BPs on the inclusion of 2,6-ANS by the β-CD derivatives, the association constants (K_{ass}) of BPs with the β-CD derivatives were determined. The K_{ass} values for bisphenol B (BPB) with β-cyclodextrin derivatives except for L-Poly-β-CD were always larger than those for other BPs including bisphenol A (BPA), due to the interaction between the non-polar cavity and hydrophobic BPB. Thermodynamic parameters indicated that the entropy change was always largely negative ($-90 \sim -120$ J/mol·K in the β-CD system, for example), and the inclusion of bisphenols into the CD cavity was completely enthalpy-driven. The very largely negative entropy change might be mainly due to the tight fixation of guest molecules in the CD cavity, resulting in the loss of freedom of both CD and guest molecules. The effect of the structure of guest and host molecules on the association was also examined.

Introduction

Bisphenol A (BPA, 2,2-bis-(4-hydroxyphenyl)propane, Figure 1) is widely used in manufacturing epoxy resins and polycarbonate and has been detected in waters and sediments almost everywhere on the earth. BPA is listed as one of the so-called "environmental estrogens" or "endocrine disrupters", and its toxicity (BPA is sometimes used as a fungicide) or biological effects have been very often reported [1]. Recently, the migration of BPA from epoxy can coatings to infant formula liquid concentrates has been observed [2]. Furthermore, it has been reported that bisphenol A diglycidylether methacrylate (Bis-GMA) leaches from Bis-GMA-based dental composites and sealants in concentrations at which biological effects have been demonstrated in *in vivo* experiments [3]. Therefore, detection and removal of BPA contained in foods, drinks, and wastewater are highly important and urgent.

We have been interested in constructing an electrochemical system for sensing various chemicals (azo dyes [4], environmental estrogens such as phthalic acid esters [5]) dissolved in water using a self-assembled monolayer of cycloamylose (cyclodextrin, CD). To design an effective CD-based sensing system for bisphenols, it is necessary to accumulate information about the interaction between free cyclodextrin derivatives and bisphenols including bisphenol A. In this report, therefore, the complexation of various kinds of bisphenols (BPs, Figure 1) with various cyclo-

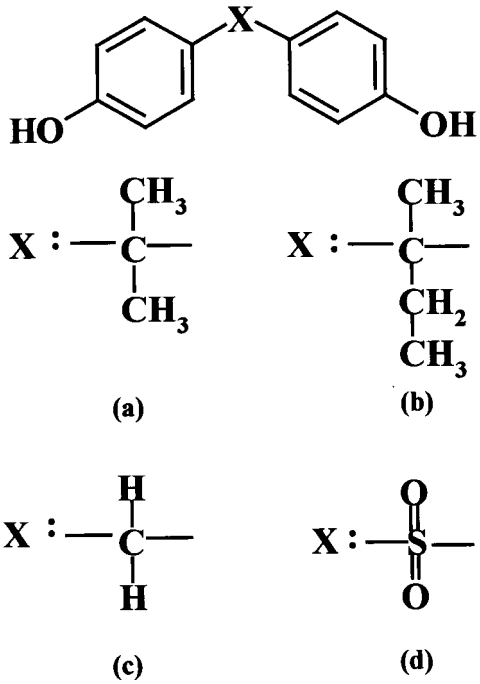

Figure 1. Chemical structure of (a) bisphenol A, (b) bisphenol B, (c) bisphenol F and (d) bisphenol S.

heptaamylose (β-cyclodextrin, β-CD) derivatives (β-CD, hydroxyethyl-β-cyclodextrin (HE-β-CD), 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD) and L-poly-β-cyclodextrin (L-Poly-β-CD), Figure 2) was investigated by using a fluorimetric technique. The effect of the temperature on the complexation was also examined.

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** Author for correspondence. E-mail: kitano@eng.toyama-u.ac.jp.

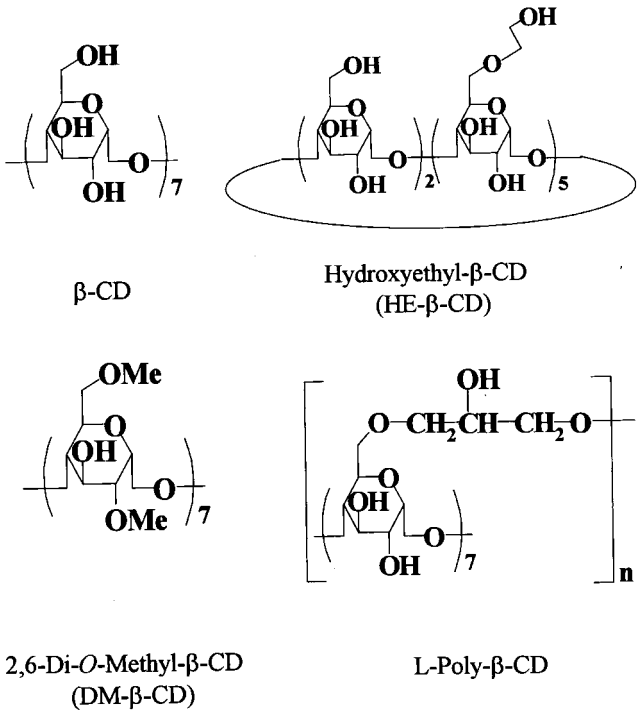


Figure 2. Chemical structure of β-cyclodextrin derivatives.

Experimental

(a) Materials

Bisphenol A (BPA) was obtained from Nacalai Tesque, Kyoto, Japan. Bisphenol B (BPB), bisphenol S (BPS) and bisphenol F (BPF) were from Tokyo Kasei, Tokyo, Japan. Hydroxyethyl-β-cyclodextrin (HE-β-CD) and 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD) were from Aldrich, Milwaukee, WI, and Wako Pure Chemicals, Osaka, Japan, respectively. L-Poly-β-cyclodextrin (L-Poly-β-CD, β-CD cross-linked with epichlorohydrin (water soluble); $M_w = 8.9 \times 10^3$ determined by GPC) was from Funakoshi, Tokyo, Japan. All other reagents used were commercially available. A Milli-Q grade water was used for the preparation of sample solutions.

(b) Characterization of β-CD derivatives

For the characterization of β-CD derivatives, a matrix-assisted laser desorption-ionization time-of-flight mass-spectrometer (MALDI-TOF-Mass, Voyager RP^{TM}, PerSeptive Biosystems) was used. The molecular weight of L-Poly-β-CD was determined by GPC (Waters HPLC system; column, Wako Gel G-30, Wako Pure Chemicals; mobile phase, aqueous 0.1 M NaBr; standard sample, pullulan, Showa Denko, Tokyo, Japan).

(c) Estimation of association constants of β-CD derivatives with bisphenols

At first, it was tried to estimate the stoichiometry and association constants of bisphenols with β-CD derivatives (K_{ass}) by a direct method using a UV-vis spectrophotometer. However, remarkable absorbance and λ_{max} changes of BPs were not observed. Therefore, the K_{ass} values in 10 (v/v)% methanol-phosphate buffer (pH 7.0, 9 mM) were estimated fluorimetrically using 2-anilinonaphthalene-6-sulfonic acid (2,6-ANS) as a probe with a fluorescence spectrophotometer (Model FP-777, Japan Spectroscopic Co., Tokyo, Japan). For the measurements at high pressures, a high-pressure observation cell (Teramecs, Kyoto, Japan) was placed in the fluorescence spectrophotometer. Because the pK_a of phosphate is largely influenced by the pressure (volume change upon the ionization (ΔV), -23 mL/mol) [6], a *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES, $\Delta V = +3$ mL/mol) [6] buffer (pH 7.0, 9 mM, 10 (v/v)% of methanol was added) was used in place of phosphate buffer at high pressures. The quartz observation cells were thermostated using a circulating water bath (RM-6, Lauda).

2,6-ANS, which is well known as a "hydrophobicity" probe, showed a much stronger fluorescence in the CD cavity than in the free state (Figure 3a) [7, 8]. The association constant for β-CD with 2,6-ANS (K_1) was estimated by the double reciprocal plots of the increase in fluorescence intensity of 2,6-ANS (ΔF) and the total concentration of β-CD ([CD]$_0$).

With the co-existence of bisphenols, the increase in the ΔF value was diminished (Figure 3b), suggesting that the bisphenols competitively inhibited the inclusion of 2,6-ANS in the CD cavity (Scheme 1). Using Equation (1), the K_{ass} value for β-CD - bisphenol complex was determined by the curve-fitting method.

$$K_{ass} = \frac{\{[CD]_0 - [X/(1-X)K_1] - [2,6\text{-ANS}]_0X\}}{[X/(1-X)K_1]\{[BP]_0 - [CD]_0 + [X/(1-X)K_1] + [2,6\text{-ANS}]_0X\}}, \quad (1)$$

where K_1 and K_{ass} are the association constant of β-CD with 2,6-ANS and that of β-CD with bisphenol, respectively. X is defined as $\Delta F'/\Delta F_\infty$, where ΔF_∞ is the ΔF value at $[CD]_0 = \infty$, and $\Delta F'$ is the ΔF value in the presence of both β-CD and bisphenol (total concentration of bisphenol, $[BP]_0$). Using a similar procedure, the K_{ass} values for the complexation of various bisphenols with various β-CD derivatives were determined.

Results and discussion

(a) Characterization of β-CD derivatives

The measurements by MALDI-TOF-Mass showed that the number-average molecular weight (M_n) and number-average degree of substitution (DS) of HE-β-CD and DM-β-CD molecules were 1409 ($M_w/M_n = 1.005$) and 1330 ($M_w/M_n = 1.122$), and 5.6 and 6.8, respectively. If anomeric hydroxyl groups are disregarded, a primary hydroxyl group at the 6-th position of the pyranose ring is the most reactive [9]. Therefore, HE-β-CD might have an additional 0.8 hydroxyethyl substituent on average at the C-6 position in each glucose unit. Similarly, in the case of DM-β-CD, methyl groups

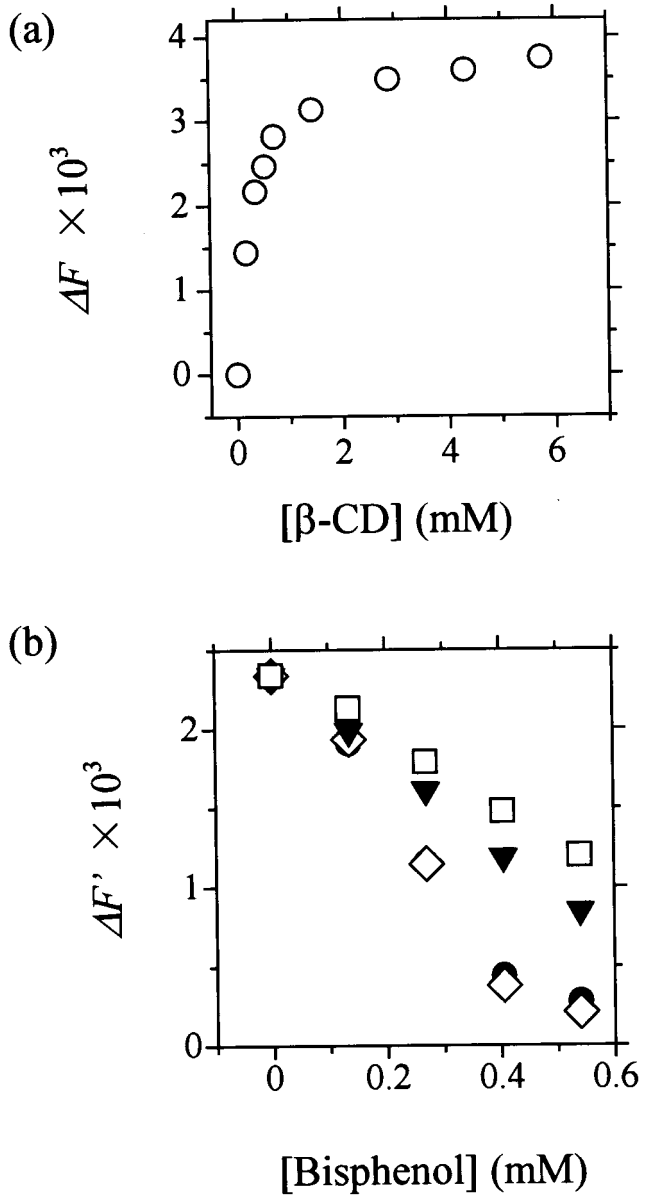
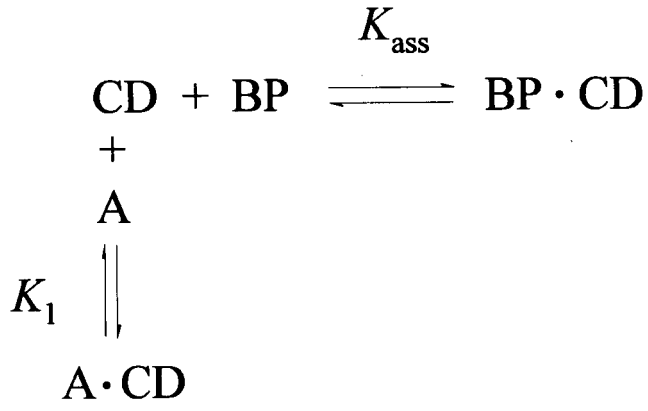


Figure 3. (a) Changes in fluorescence intensity of 2,6-ANS in the presence of β-CD. $[ANS]_0$ = 90 μM. In the 9 mM phosphate buffer containing 10 (v/v)% MeOH (pH 7.0) at 25 °C. (b) Relationship between the $\Delta F'$ value and the concentration of various bisphenols in the presence of β-CD. $[ANS]_0$ = 90 μM, $[\beta\text{-CD}]_0$ = 135 μM. ●: BPA, ◇: BPB, ▼: BPF, □: BPS.



Scheme 1. Competitive inhibition of inclusional complexation between β-CD and BP by molecule A (2,6-ANS).

might be introduced mostly into the C-6 position, and partly to the C-2 position in each glucose unit, because the hydroxyl groups at C-2 of glucopyranoside is the most reactive among the secondary hydroxyl groups [9].

The number of CD residues in a L-Poly-β-CD molecule was estimated using a fluorescence titration with 2,6-ANS which was reported to form a 1:1 inclusion complex with free β-CD [7]. The plot of fluorescence intensity vs. the concentration of 2,6-ANS at a constant CD concentration showed a saturation curve, and above 200 μM, gradually decreased due to the self-quenching phenomenon. The crossing point of the initial slope and the saturated value corresponds to the effective concentration of CD residues (cavities). From the plot, the number of *effective* CD residues for the inclusion of 2,6-ANS in the L-Poly-β-CD molecule was calculated to be only 1.4 on average.

(b) Geometry and stoichiometry in complexation of β-CD with BPs

At first, the inclusion of BPA in a cavity of β-CD was examined by ^{1}H-NMR method. The measurements of the rotating frame nuclear Overhauser effects (^{1}H-^{1}H ROESY) in the solution of BPA-β-CD mixture (solvent, D_2O:CD_3OD = 9:1) showed four kinds of ROE cross-peaks (Figure 4). Cross-peaks A and B showed that protons at the 3rd position of β-CD correlate with methyl protons and the phenyl ring protons at 2nd and 3rd (or 5th and 6th) position of BPA, respectively. Cross-peaks C and D showed that methylene protons at the 6th position of β-CD correlate with protons at 3rd and 5th position in the phenyl ring and methyl protons of BPA, respectively. These results indicated inclusion of BPA in the cavity of β-CD (Scheme 2).

Geometry of the complexation between β-CD and other BPs were also examined. The cross-peaks observed in β-CD–BPB and β-CD–BPF systems showed the correlation of methylene protons at the 6th position of β-CD with the ethyl protons of BPB and phenyl ring protons of BPF, respectively (spectra not shown). In β-CD–BPS system, the cross-peak between phenyl ring protons of BPS and protons at the 3rd position of β-CD was observed (spectra not shown). Taking account of both the results of ROESY in the β-CD–BPB and β-CD–BPF systems and the spatially possible arrangement examined by the Corey–Pauling–Koltun (CPK) model, the correlation of the protons in the β-CD cavity with the phenyl ring protons of BPB and methylene protons of BPF is expect to be observed. However, these correlations expected were not observed, probably because those correlations were relatively very weak.

Mention is made here of the stoichiometry for β-CD–BPs complexation. The stoichiometry couldn't be estimated by a direct method using the UV-vis spectrophotometer. However, it is thought that the complexations of β-CD–BPs are 1:1 from the spatially possible arrangement examined by the CPK model as mentioned above. Because of the strongly bent structure of BP (nearly at right angles), it seems to be quite difficult for BP to form a complex with another association ratio in β-CD-BPs systems. 2,6-ANS was reported to form a 1:1 inclusion complex with β-CD [7]. Therefore, al-

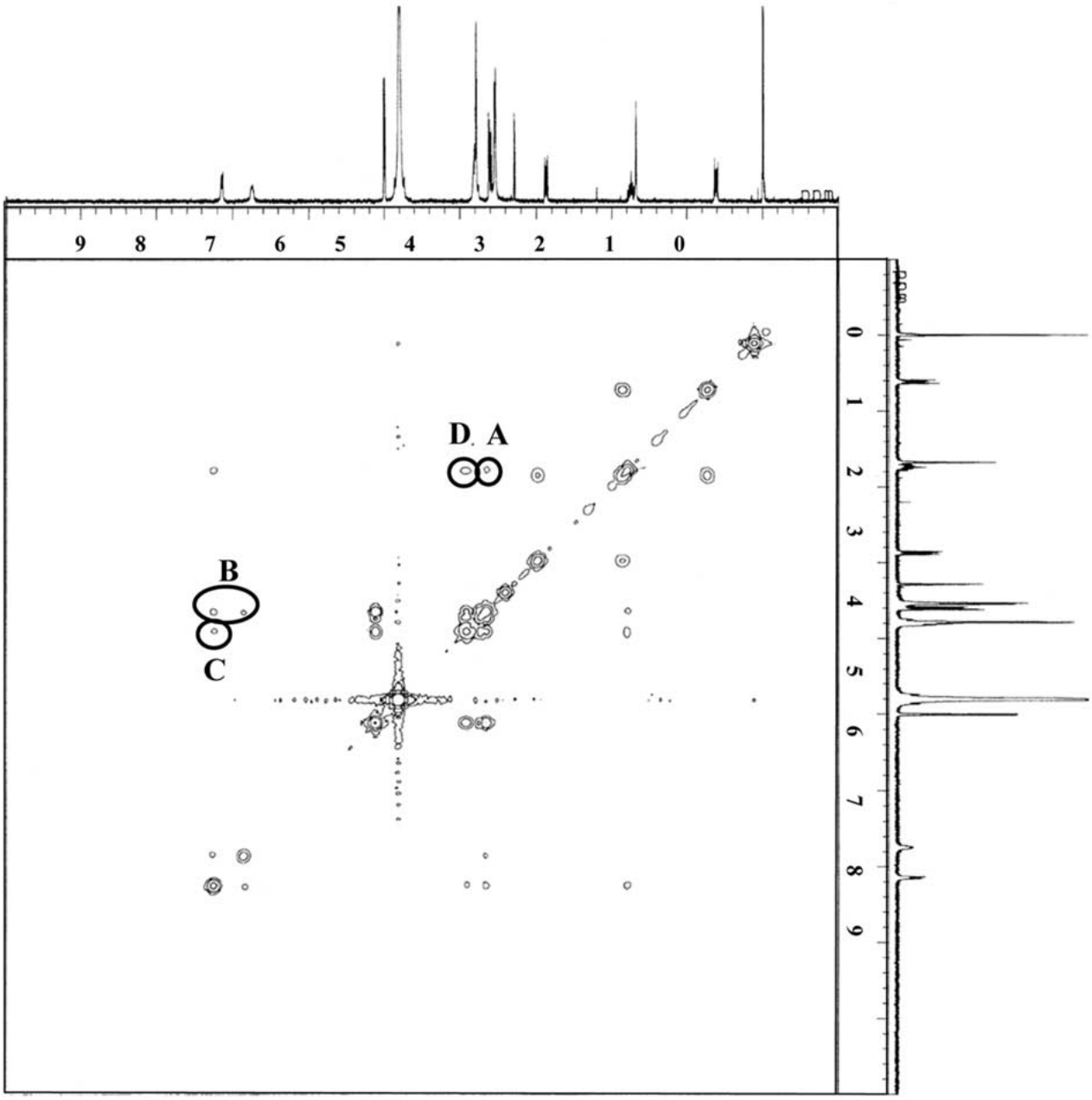


Figure 4. ^{1}H-^{1}H ROESY spectrum (400 MHz) for the mixture of β-CD and BPA in D_2O-CD_3OD (9:1) at 25 °C. [β-CD] = 0.8 mM. [BPA] = 0.8 mM.

Table 1. The association constants and thermodynamic parameters for the complexation of β-CD derivatives with 2,6-ANS[a]

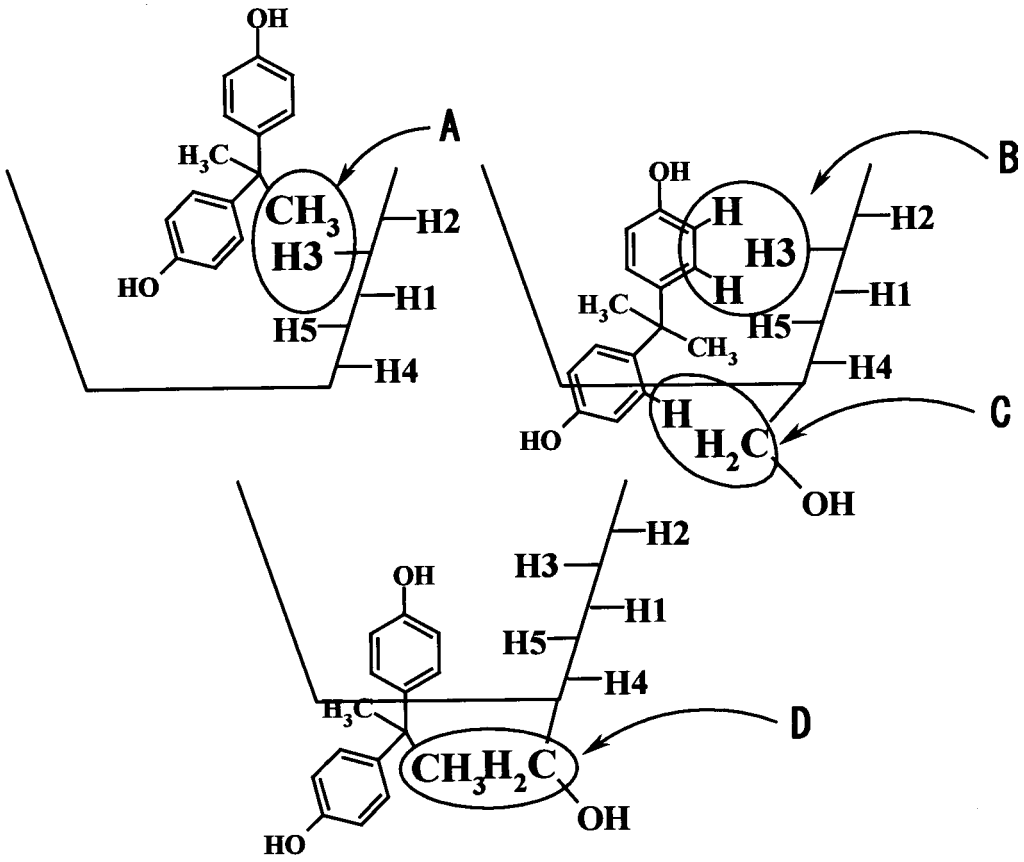
Host	K_1 ($\times 10^3$ M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol·K)
β-CD	0.88 ± 0.03	-16.8 ± 0.1	-27.5 ± 0.3	-36 ± 1
DM-β-CD	3.5 ± 0.3	-20.2 ± 0.2	-25.2 ± 0.2	-17 ± 1
HE-β-CD	1.3 ± 0.1	-17.8 ± 0.2	-23.3 ± 0.1	-18 ± 1
L-Poly-β-CD	3.7 ± 0.4	-20.4 ± 0.2	-33.1 ± 0.4	-43 ± 2

[a]In 10 (v/v)% MeOH-phosphate buffer (pH 7.0, 9 mM).

though definitive evidence couldn't be shown, stoichiometry for the competitive inhibition of inclusional complexation between β-CD and BPs by 2,6-ANS is supposed to be 1:1.

(c) Estimation of K_{ass} values

The inclusion of BPA in a cavity of β-CD was examined fluorimetrically using 2,6-ANS as a probe. Fluorescence intensity of 2,6-ANS at 450 nm was increased by the addition of β-CD (Figure 3a) [8], and the association constant for β-CD-ANS complex (K_1) was determined to be 8.8×10^2 M^{-1} by the double reciprocal plot of the initial concentration of CD, $[CD]_0$, versus the increase in fluorescence intensity, ΔF, due to the presence of β-CD. This value is smaller than those reported previously (in water, 1.9×10^3 M^{-1} [7], and in a M/30 phosphate buffer (pH 7.2), 2.7×10^3 M^{-1} [8], due to the presence of 10 (v/v)% methanol which was added to dissolve the bisphenols. Table 1 shows the K_1 values of ANS with various β-CD derivatives.



Scheme 2. Geometry of the complexation between β-CD and bisphenol A based on the ROESY measurement.

The value of ΔF was decreased by the addition of BPA (Figure 3b), suggesting that the BPs, as well as ANS, were included in the cavity of CD. Actually, the association constant of β-CD with BP (K_{ass}) was unequivocally determined using Equation (1), which was derived by the assumption of competitive inhibition (Scheme 1). With the same procedure, the K_{ass} values for three kinds of β-CD derivatives with four kinds of bisphenols could be determined and are compiled in Table 2.

In the case of L-Poly-β-CD, which had 1.4 effective CD residues within the molecule, the addition of bisphenols somewhat acceleratingly decreased the fluorescence intensity corresponding to the ANS-CD complex (Figure 5), which is quite different from that of other β-CD derivatives. This is probably due to the increase in hydrophobicity in the vicinity of L-Poly-β-CD molecule upon the inclusion of bisphenols, which further promotes the inclusion of BP into the cavity. Therefore, the K_{ass} value for L-Poly-β-CD shown in the table corresponds to the value calculated at a very low concentration of bisphenols (135 μM) (Table 2). Such a unique behavior of L-Poly-β-CD would be advantageous to effectively remove bisphenols from wastewater. It was roughly estimated that 3.2 g/L of L-Poly-β-CD could capture 23% of BPA from its 135 μM solution (solvent, phosphate buffer (9 mM, pH 7.0)–10 (v/v)% MeOH).

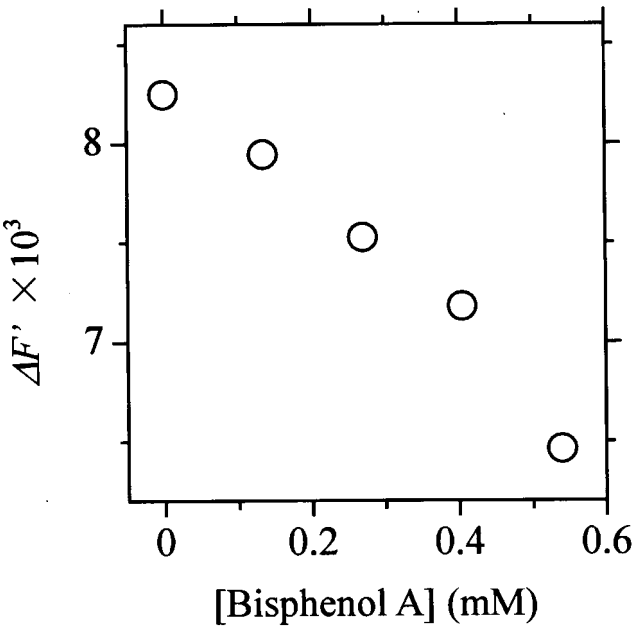


Figure 5. Relationship between the $\Delta F'$ value and the concentration of bisphenol A in L-Poly-β-CD solution. In the 9 mM phosphate buffer containing 10 (v/v)% MeOH (pH 7.0) at 25 °C.

The table indicates that the K_{ass} values obtained for BPB–β-CD derivatives except for L-Poly-β-CD were the largest among the bisphenols examined. This is mostly because of the hydrophobic interaction between the non-polar cavity of β-CD derivatives and the BPB molecule. Meanwhile, friction between the ethyl group in the BPB and the rim of the CD molecule might affect the forward and backward reaction rate constants for the complexation of

Table 2. The association constants and thermodynamic parameters for the complexation of β-CD derivatives with bisphenols[a]

Host	Guest (Bisphenol)	K_{ass} ($\times 10^3$ M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol·K)
β-CD	A	35 ± 1	-25.9 ± 0.1	-54.2 ± 1.7	-95 ± 10
	B	40 ± 4	-26.3 ± 0.2	-62.1 ± 5.0	-120 ± 17
	F	5.0 ± 0.6	-21.1 ± 0.3	-47.9 ± 2.1	-90 ± 8
	S	4.0 ± 0.2	-20.6 ± 0.1	-50.4 ± 5.1	-100 ± 13
DM-β-CD	A	100 ± 16	-28.5 ± 0.4	-37.1 ± 2.1	-29 ± 8
	B	140 ± 20	-29.4 ± 0.4	-37.4 ± 2.9	-27 ± 11
	F	13 ± 4	-23.5 ± 0.7	-30.7 ± 2.3	-24 ± 8
	S	4.0 ± 0.2	-20.6 ± 0.3	-42.7 ± 4.0	-74 ± 10
HE-β-CD	A	36 ± 16	-26.0 ± 0.9	-40.0 ± 4.0	-47 ± 13
	B	42 ± 10	-26.4 ± 0.5	-32.4 ± 2.4	-20 ± 8
	F	12 ± 4	-23.3 ± 0.7	-33.7 ± 2.3	-31 ± 8
	S	3.6 ± 0.3	-20.3 ± 0.2	-34.1 ± 0.8	-45 ± 3
L-Poly-β-CD[b]	A	3.3 ± 2.5	-20.1 ± 1.4	-66.9 ± 7.0	-157 ± 23
	B	2.8 ± 1.6	-19.7 ± 1.1	-69.8 ± 8.4	-168 ± 27
	F	3.1 ± 1.5	-19.9 ± 1.0	-66.7 ± 6.3	-157 ± 20
	S	3.3 ± 1.2	-20.1 ± 0.8	-65.1 ± 6.0	-151 ± 13

[a] In 10 (v/v)% MeOH-phosphate buffer (9 mM, pH 7.0).
[b] The K_{ass} values were determined only at $[BP]_0 = 135$ μM.

BPB with the CD molecule. The relatively smaller steric hindrance for BPA and BPF to enter and leave the CD cavity than that for BPB seemed to affect the rate constants, too. This point will be clarified by the estimation of forward and backward reaction rate constants using a stopped-flow or temperature jump method.

Furthermore, the K_{ass} values for the β-CD and HE-β-CD systems were quite similar, while those for DM-β-CD were the largest. This might be due to the extension of the non-polar cavity by the substituents at the rim of the cavity of the DM-β-CD molecule. The K_{ass} for L-Poly-β-CD systems was the smallest among the β-CD derivatives examined in this work, probably due to the steric hindrance of cross-links between the β-CD residues to the bisphenols entering the CD cavity.

Except for the L-Poly-β-CD system, furthermore, the K_{ass} values for BPS were the smallest among the bisphenols examined. The CPK model showed that a sulfonyl group at the center of the BPS molecule can attach to the OH groups at the rim of β-CD to form hydrogen bonding. Actually, the K_{ass} value for BPS with β-CD (1.1×10^5 M^{-1}) was the largest among four kinds of bisphenols (BPS > BPB > BPA > BPF) [10]. However, the K_{ass} value for the BPS-β-CD system was the smallest among the bisphenols examined, probably due to the too large diameter of the β-CD cavity for BPS to realize a tight contact of the sulfonyl group with the O–H groups at the rim of the cavity, which resulted in the domination of the largest polarity of BPS over the K_{ass} value. The K_{ass} values for β-CD derivatives were therefore in the order BPB > BPA > BPF > BPS.

Previously, Murai et al. evaluated the K_{ass} value for the complexation of β-CD with phthalic acid esters (PAEs) using 6-(*p*-toluidino)-2-naphthalenesulfonic acid (TNS) as a fluorescence probe [11]. The K_{ass} values for diheptyl phthalate and di-(2-ethylhexyl) phthalate were, for example, 2142 M^{-1} and 930 M^{-1}, respectively. These values are slightly smaller than those for the bisphenols examined here, due to the difference in the hydrophobicity and the molecular shape of bisphenols and PAEs for inclusion into the β-CD cavity. Therefore, the above results show that the steric factor is very important (sometimes dominant) for the stable inclusional complexation by CDs.

(d) Thermodynamics in the complexation of BP with various β-CD derivatives

From a comparison of the association constants at various temperatures, thermodynamic parameters for the complexation were evaluated for various β-CD derivative-ANS systems (K_1, Table 1) and β-CD derivative-BP systems (K_{ass}, Table 2).

Many previous studies showed that the process of inclusional complexation by CD is usually favorable for enthalpy ($\Delta H < 0$) and unfavorable or slightly favourable for entropy ($\Delta S < 0$ or $\Delta S \geq 0$) [12–17] which is not inconsistent with the results obtained in this work. The tables show that the enthalpy changes for β-CD–BP systems were always much larger than that for the β-CD–2,6-ANS system. Furthermore, the entropy changes for BPs were much more negative than those for ANS, due to the tight fixation of BPs in the CD cavity. Rekharsky et al. reported that the entropy changes for the inclusion of primary and secondary aliphatic alcohols by α-CD were more negative than by β-CD [12], suggesting that the tighter binding induces a more negative entropy change, which is consistent with the tendency observed here.

Largely negative (unfavorable) entropy changes were reported for the complexation of β-CD with 2,7-naphthalenedisulfonate (-48 J/K·mol) [12]. The inclusion of p-nitrophenol (-88 J/K·mol) [18], meclofenamic acid (-90 J/K·mol) [19], and phenolphthalein (-174 J/K·mol at

pH 10.0) [20] by β-CD also showed largely negative entropy changes.

In the inclusion into the CD cavity, the negative entropic contribution due to the reduction in the mobility of the guest is compensated by the interaction enthalpy. By this enthalpy–entropy compensation, the expected gain in entropy due to the dehydration of non-polar moieties of guest and host molecules might become masked [21].

The tendency observed here ($\Delta H < 0$ and $\Delta S < 0$) is ascribable to a "non-classical" hydrophobic interaction, which has often been reported for hydrophobic complexation of "*semi-polar*" compounds (CD, ANS and BPs can be included into this category) as Jencks described [22]. In that model, the driving force for the association may appear as either a favorable enthalpy or entropy change. Therefore, the factor of hydrophobic interaction in the inclusion phenomena cannot be excluded simply from the unfavorable entropy change observed here.

Meanwhile, it was pointed out that bulk and pairwise hydrophobic interactions should be definitely distinguished [21, 23]. The former denotes association of a large number of non-polar molecules or moieties, which occurs in protein folding and micelle formation, for example. In such a process, each non-polar molecule completely changes its aqueous surroundings to non-aqueous ones. This situation resembles the transfer of non-polar solute to organic solvent. Therefore, the transfer dynamics ($\Delta H \geq 0$, $\Delta S > 0$) is a good model for the "classical" hydrophobic interaction. On the other hand, the pairwise interaction between two single molecules can be considered to be a partial reverse of the transfer process, though this assumption has not been validated at this moment. Hydrophobic interactions in host-guest complexes might involve both pairwise and bulk interactions depending on geometry of the complex, which might result in thermodynamic parameters ($\Delta H < 0$, $\Delta S < 0$) different from those for the "classical" hydrophobic interaction.

(e) Pressure effect on the inclusion phenomena

From the measurements at various pressures (Figure 6), the volume changes (ΔV) for the inclusion of ANS, BPA and BPB into β-CD cavity were estimated to be $+13 \pm 1$ mL·mol^{-1}, -12 ± 2 mL·mol^{-1} and -10 ± 1 mL·mol^{-1}, respectively, using the van't Hoff equation (2). There have been some reports concerning the volume change at the inclusion by β-CD. However, no clear interpretation of the ΔV values has been given.

$$\frac{d \ln K}{dP} = -\frac{\Delta V}{RT}. \qquad (2)$$

Based on the ordinary interpretation for the volume changes, the positive ΔV value for the inclusion of ANS can be ascribed to the release of water molecules around the polar sulfonate group and secondary amino group in the ANS molecule, and those in the CD cavity. It was suggested that strongly hydrated *charged* groups do not allow the complete penetration of the guest into the macrocyclic cavity [24]. Only after the dehydration, which demands a large energy, would the penetration be completed, resulting in an increase in the total volume of the system ($\Delta V > 0$).

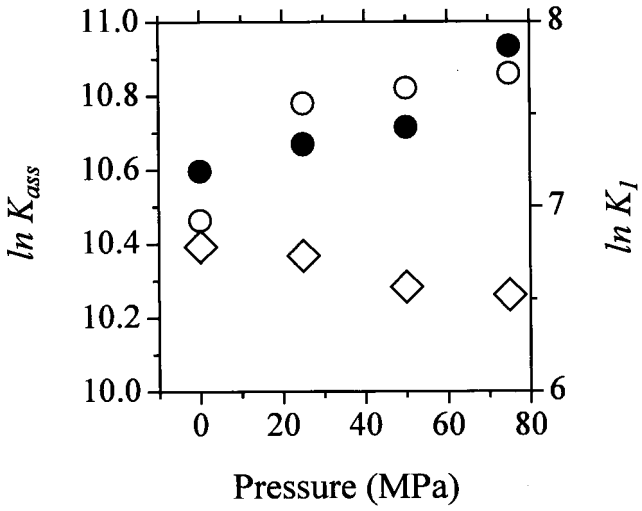


Figure 6. Effect of pressure on the association constant for the complexation of β-CD and bisphenols or 2,6-ANS in the 9 mM HEPES buffer containing 10 (v/v)% MeOH (pH 7.0) at 25 °C. K_{ass}: BPA (○), BPB (●). K_1: 2,6-ANS (◇).

On the other hand, non-polar moieties of guests with neutral polar groups in general can penetrate into the macrocyclic cavity without considerable dehydration. Therefore, the negative ΔV values for the inclusion of neutral BPA and BPB in the β-CD cavity seemed to be somewhat troublesome to explain. The formation of hydrogen bonding between CD and BP might partly contribute to the negative entropy change [25], though the hydrogen bonding could not be confirmed by the ROESY measurements. It has been pointed out that a simple hydrophobic association is accompanied by a *positive* ΔV value [26]. On the other hand, the hydrophobic association between protein (usually semi-polar) and small molecular weight ligand has been observed to show a negative ΔV value [27]. Such a discrepancy reminds us of the difference in the tendency of the ΔH and ΔS values between the "classical" and "non-classical" hydrophobic interactions discussed in the previous section. Furthermore, it should be emphasized here that the negative ΔV values for BPA-β-CD and BPB-β-CD systems were consistent with the negative ΔS values for these systems.

Previously, the ΔV value for the complexation of β-CD with *p*-nitrophenyl ferrocenylacrylate was reported to be -15 mL·mol^{-1} [28] and that for *m*-nitrophenylacetate -16 mL·mol^{-1} [29]. The negative ΔV values for these electrically neutral guests were in agreement with the tendency observed in this work. Therefore, the thermodynamic parameters including ΔH, ΔS and ΔV values for the BP-β-CD systems examined here indicate that the inclusion phenomena of BPs by β-cyclodextrin and its derivatives can be considered to be a model of a protein-ligand association system driven by the non-classical hydrophobic interaction.

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