

## Differential Effects of Substituent and Pressure on Induced Inclusion Complexation of 6-*O*- $\alpha$ -D-Glucosyl- $\beta$ -Cyclodextrin with 4-Substituted Phenols

YOSHIMI SUEISHI\*, NAOYA INAZUMI, TATSUHIRO IDE and TADASHI HANAYA  
*Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushima Naka, 700-8530, Okayama, Japan*

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### Abstract

The inclusion complexation of 6-*O*- $\alpha$ -D-glucosyl- $\beta$ -cyclodextrin (G- $\beta$ -CD), in which the glucosyl side chain is introduced to  $\beta$ -CD, with various kinds of phenols was studied spectrophotometrically at high pressures. The characteristic effects of substituent and pressure were found for the inclusion complexation of G- $\beta$ -CD. The association constants  $K$  for the G- $\beta$ -CD inclusion complexation increased with an increase in the bulkiness of the 4-substituent groups in phenols. As the external pressure increases, the inclusion constants for the G- $\beta$ -CD complexation increased and the reaction volumes were estimated to be  $-3.8$  to  $-19.4$  cm<sup>3</sup> mol<sup>-1</sup> from their pressure dependences. From analysis of the effect of pressure on the inclusion complexation with G- $\beta$ -CD, the number of water molecules included in the G- $\beta$ -CD cavity in water was estimated. The number of water molecules repelled from the CD cavity plays an important role in the change in volume upon inclusion. In addition, the structures of the inclusion complexes of G- $\beta$ -CD with phenols have been established by 1D and 2D NMR measurements. Based on the results, we suggested that the ability of the G- $\beta$ -CD inclusion complexation is enhanced by the interaction between guest molecules and the glucosyl side chain of G- $\beta$ -CD.

### Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that have hydrophobic cavities capable of forming guest–host or inclusion complexes with a variety of organic molecules in aqueous solution [1]. A number of branched CDs, in which mono- or di-saccharides are introduced on one or two primary hydroxyl groups of CDs, have been prepared for new applications different from those of the conventional nonbranched CDs [2–4]. Recently, branched CDs have attracted much attention because of their advantageous properties (high solubility in water, high biadaptability, apparent lack of toxicity, etc [5]) as drug carriers.

Previously, we demonstrated that the thermal isomerization (SP  $\rightarrow$  MC, Scheme 1) of 1',3', 3'-trimethylspiro[2*H*-1-benzopyran-2,2'-indoline]-6-sulfonate (6-SO<sub>3</sub>-SP) is used as a chemical indicator for estimating the unbound CD concentration and determine the equilibrium constants for the inclusion of  $\beta$ - and  $\gamma$ -CDs with phenols at high pressures without analyzing the absorption bands of the inclusion complexes [6]. Based on the results, we performed a detailed volumetric study to clarify the inclusion behavior of  $\beta$ - and  $\gamma$ -CDs.

The ability of inclusion complexation of branched CDs has not been established yet. In the inclusion

complexation of 6-*O*- $\alpha$ -D-glucosyl- $\beta$ -CD (G- $\beta$ -CD, Scheme 2) with phenols, we found differential inclusion behavior that stable inclusion complexes are formed with increasing the bulkiness of 4-substituents of phenols. In this study, we determined the association constants of G- $\beta$ -CD with various kinds of 4-substituted phenols for demonstration purposes and examined the pressure dependence of their inclusion equilibria by using the isomerization of 6-SO<sub>3</sub>-SP as a chemical indicator. Based on the results, we conducted a volumetric study concerning the inclusion behavior of G- $\beta$ -CD.

### Experimental

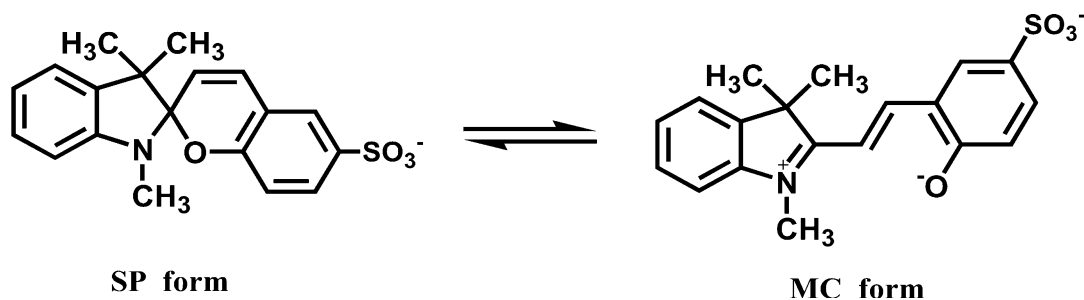
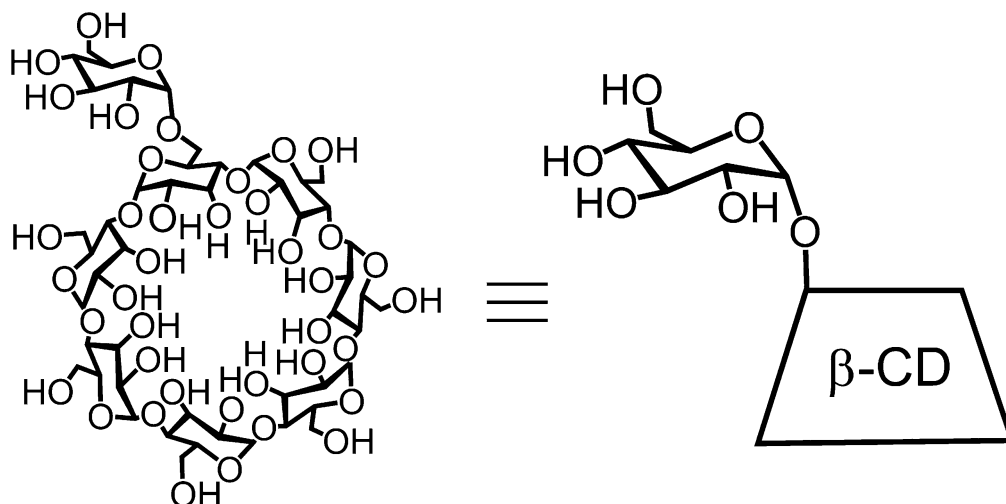
#### Materials

G- $\beta$ -CD was purchased from Tokyo Kasei Co. (Tokyo, Japan) and used without further purification. 4-Substituted phenols of reagent grade were obtained from Wako Pure Chemicals (Osaka, Japan) and used as received. Water was purified by distillation before use.

#### Measurements

A phosphate buffer (pH = 6.9 and ionic strength = 0.1), which was prepared from phosphate salts, was used as a solvent. A sample solution ([6-SO<sub>3</sub>-SP] = about

\* Author for correspondence. E-mail: ysueishi@cc.okayama-u.ac.jp

Scheme 1. Isomerization of 6-SO<sub>3</sub>-SP.

### Mono-glucosyl- $\beta$ -CD (G- $\beta$ -CD)

Scheme 2. Chemical structure of G- $\beta$ -CD.

$1.5 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $[\text{G-}\beta\text{-CD}] = 3 \times 10^{-4} \text{ mol dm}^{-3}$ , and  $[\text{phenols}] = 0.3\text{--}3 (\times 10^{-3}) \text{ mol dm}^{-3}$  deoxygenated by bubbling nitrogen was irradiated with a projection lamp (650 W) to produce the SP form. The photo and thermal isomerizations of 6-SO<sub>3</sub>-SP were reproducible.

The high-pressure inner cell and procedures for measurements at high pressures are described elsewhere [6]. The spectral changes of 6-SO<sub>3</sub>-SP at high pressures were monitored by using a Hitachi U-3200 spectrophotometer (Tokyo, Japan). The reaction temperature was maintained at 313 K. Thermal isomerization from the SP form to the MC form in the presence of G- $\beta$ -CD was followed at high pressures by monitoring the changes in the absorption spectra ( $\lambda_{\text{max}} = 508 \text{ nm}$ ) of the MC form. The thermal isomerization of 6-SO<sub>3</sub>-SP obeys first-order kinetics regardless of the concentrations of phenols and G- $\beta$ -CD. The first-order rate constants were obtained within a 5% error. The phenols used in this study (except 4-NO<sub>2</sub>-phenol) do not have absorption bands in the visible region. Using the isomerization rates of 6-SO<sub>3</sub>-SP as a chemical indicator enabled estimation of the concentration of the unbound G- $\beta$ -CD in solution and determination of the inclusion equilibrium constants with 4-substituted phenols.

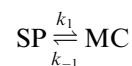
The <sup>1</sup>H-NMR spectra were measured in D<sub>2</sub>O with a Varian Inova AS600 instrument (600 MHz) at room

temperature. Chemical shifts were reported as  $\delta$  values relative to HOD ( $\delta$  4.79) as an internal standard [7]. The concentrations of phenols and G- $\beta$ -CD for the NMR measurements were  $0.941\text{--}3.59 (\times 10^{-2}) \text{ mol dm}^{-3}$  and  $1.08\text{--}5.64 (\times 10^{-3}) \text{ mol dm}^{-3}$ , respectively. 2D ROESY-NMR experiment was recorded at 600 MHz in D<sub>2</sub>O on a Varian Inova AS600 NMR spectrometer at room temperature. Mixing times for ROESY experiments were set at 300 ms.

## Results and discussion

### *Inclusion complexation of G- $\beta$ -CD with phenols*

When G- $\beta$ -CD is added to a solution of 6-SO<sub>3</sub>-SP, the peak in the vicinity of 220 nm increases and the peak at 508 nm decreases. This suggests that analogous to the 1:1 inclusion complexation of  $\beta$ -CD with 6-SO<sub>3</sub>-SP [6, 8], the SP form of 6-SO<sub>3</sub>-SP is preferentially included in the G- $\beta$ -CD cavity. As suggested previously [8], the reaction scheme of the thermal isomerization of 6-SO<sub>3</sub>-SP in the presence of G- $\beta$ -CD can be given as follows:





The isomerization rate (SP → MC) of 6-SO<sub>3</sub>-SP can be given by:

$$-\frac{d[\text{SP}_{\text{Total}}]}{dt} = -\left(\frac{d[\text{SP}]}{dt} + \frac{d[\text{SP-CD}]}{dt}\right) = k_1[\text{SP}] - k_{-1}[\text{MC}] \quad (1)$$

$$[\text{SP}_{\text{Total}}] = [\text{SP}] + [\text{SP-CD}] \quad (2)$$

$$[\text{SP}] + [\text{MC}] + [\text{SP-CD}] = [\text{SP}]_e + [\text{MC}]_e + [\text{SP-CD}]_e \quad (3)$$

where [SP]<sub>e</sub>, [MC]<sub>e</sub>, and [SP-CD]<sub>e</sub> denote the concentrations of SP, MC, and SP-CD in equilibrium, respectively. Under the condition [CD] ≫ [6-SO<sub>3</sub>-SP], the isomerization rate (SP → MC) can be expressed from Equations (1) to (3) as follows:

$$-\frac{d[\text{SP}_{\text{Total}}]}{dt} = \left(k_{-1} + \frac{k_1}{1 + K_{\text{CD}}[\text{G-}\beta\text{-CD}]}\right) \times ([\text{SP}_{\text{Total}}] - [\text{SP}_{\text{Total}}]_e) \quad (4)$$

The apparent rate constants ( $k_{\text{obs}}$ ) for the thermal isomerization (SP → MC) of 6-SO<sub>3</sub>-SP can be given by Equation (5), and are dependent on the concentration of the unbound G-β-CD [8].

$$k_{\text{obs}} = k_{-1} + \frac{k_1}{1 + K_{\text{CD}}[\text{G-}\beta\text{-CD}]} \quad (5)$$

Equation (5) can be rewritten as:

$$\frac{1}{k_{\text{obs}} - k_{-1}} = \frac{1}{k_1} + \frac{K_{\text{CD}}}{k_1} [\text{G-}\beta\text{-CD}] \quad (6)$$

where [G-β-CD] denotes the concentration of unbound G-β-CD. In a previous paper, the 10<sup>3</sup>k<sub>-1</sub> (s<sup>-1</sup>) values for the isomerization (MC → SP) of 6-SO<sub>3</sub>-SP were estimated as 2.27 (at 1 bar), 1.93 (at 200 bar), 1.63 (at 500 bar), and 1.34 (at 800 bar) [8]. Figure 1 shows the plots of 1/(k<sub>obs</sub> - k<sub>-1</sub>) against the G-β-CD concentration. Their linear relationships indicate the 1:1 inclusion complex formation of 6-SO<sub>3</sub>-SP with G-β-CD: the values of the intercept (10<sup>-2</sup>(1/k<sub>1</sub>) (s)) and slope (10<sup>-6</sup>(K<sub>CD</sub>/k<sub>1</sub>) (mol<sup>-1</sup> dm<sup>3</sup> s)) were 3.17 ± 0.09 and 1.02 ± 0.03 (at 1 bar), 3.28 ± 0.05 and 1.08 ± 0.02 (at 200 bar), 3.73 ± 0.05 and 1.17 ± 0.02 (at 500 bar), and 4.24 ± 0.05 and 1.19 ± 0.02 (at 800 bar), respectively.

Upon the addition of phenols to a solution of 6-SO<sub>3</sub>-SP and G-β-CD ([G-β-CD] and [phenols] ≫ [6-

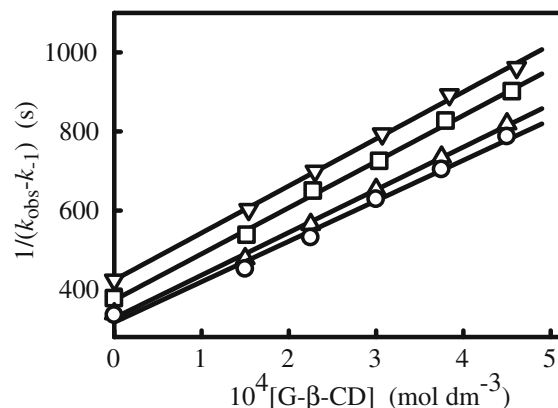


Figure 1. Plots of 1/(k<sub>obs</sub> - k<sub>-1</sub>) against G-β-CD concentration at high pressures: ○ at 1 bar, Δ at 200 bar, □ at 500 bar, and ▽ at 800 bar.

SO<sub>3</sub>-SP]), the concentration of unbound G-β-CD cannot be approximated to the initial concentration (total concentration) of G-β-CD, because analogous to the inclusion complexation of β-CD [6], G-β-CD forms 1:1 inclusion complexes with phenols. As suggested above, the apparent isomerization rate of 6-SO<sub>3</sub>-SP in the presence of G-β-CD is expressed as a function of the unbound G-β-CD concentration according to Equation (6). In the solution of 6-SO<sub>3</sub>-SP, G-β-CD, and phenols, the apparent isomerization rate of 6-SO<sub>3</sub>-SP from SP to MC was estimated and then the concentration of unbound G-β-CD in solution was determined from the isomerization rate. By using these linear relationships in Figure 1, estimating the thermal isomerization rate ( $k_{\text{obs}}$ ) of 6-SO<sub>3</sub>-SP allows us to determine the concentration of unbound CD in solution. In the presence of excess G-β-CD and phenols over 6-SO<sub>3</sub>-SP concentration, the concentration of inclusion complex between 6-SO<sub>3</sub>-SP and G-β-CD is negligible for phenols and G-β-CD. Therefore, the 1:1 association constants ( $K$ ) of phenols (P) with G-β-CD can be given by:

$$\begin{aligned} \text{P} + \text{G-}\beta\text{-CD} &\xrightleftharpoons{K} \text{P-CD} & K &= \frac{[\text{P-CD}]}{[\text{P}][\text{G-}\beta\text{-CD}]} \\ & & &= \frac{[\text{G-}\beta\text{-CD}]_0 - [\text{G-}\beta\text{-CD}]}{([\text{P}]_0 - [\text{G-}\beta\text{-CD}]_0 + [\text{G-}\beta\text{-CD}])[\text{G-}\beta\text{-CD}]} \end{aligned} \quad (7)$$

where [P]<sub>0</sub> and [G-β-CD]<sub>0</sub> denote the initial concentrations (total concentrations) of phenols and G-β-CD, respectively. [P-CD] is the concentration of the inclusion complex of G-β-CD with phenols: [P]<sub>0</sub> = [P] + [P-CD] and [G-β-CD]<sub>0</sub> = [G-β-CD] + [P-CD]. By using the concentration of unbound CD in solution estimated from the rate of the 6-SO<sub>3</sub>-SP isomerization, we determined the association constants of 4-substituted phenols with G-β-CD at high pressures according to Equation (7) (Table 1). There is a possibility that G-β-CD forms the 1:2 inclusion complex with phenols having the long alkyl chain such as 4-CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>-Phenol. The association values for the 1:1 equilibrium between G-β-CD and

Table 1. Association constants and reaction volumes for inclusion complex formation of G- $\beta$ -CD with phenols at 313 K

| Guest   | $10^2 K$ (dm <sup>3</sup> mol <sup>-1</sup> ) |             |             |             | $\Delta V$ (cm <sup>3</sup> mol <sup>-1</sup> ) | $-\Delta V_{\text{inclu}}$ (cm <sup>3</sup> mol <sup>-1</sup> ) | $\Delta V_{\text{repet}}$ (cm <sup>3</sup> mol <sup>-1</sup> ) |
|---|---|-------------|-------------|-------------|---|---|--|
|   | 1 bar   | 200 bar     | 500 bar     | 800 bar     |   |   |  |
| Phenol  | 2.55 ± 0.02                                   | 2.60 ± 0.02 | 2.65 ± 0.02 | 2.80 ± 0.02 | -3.8 ± 0.4                                      | 87.2 <sup>a</sup>   | 83.4 ± 0.4   |
| 4-CH <sub>3</sub> -Phenol                                 | 4.52 ± 0.03                                   | 4.74 ± 0.03 | 4.80 ± 0.02 | 5.06 ± 0.02 | -4.5 ± 0.4                                      | 96.5 <sup>a</sup>   | 92.0 ± 0.4   |
| 4-NO <sub>2</sub> -Phenol                                 | 4.77 ± 0.05                                   | 4.82 ± 0.05 | 4.98 ± 0.05 | 5.17 ± 0.05 | -3.7 ± 0.2                                      | 109.8 <sup>a</sup>  | 106.1 ± 0.2  |
| 2-Naphthol  | 5.01 ± 0.05                                   | 5.30 ± 0.05 | 5.74 ± 0.06 | 6.26 ± 0.06 | -8.3 ± 0.1                                      | 112.4 <sup>a</sup>  | 104.1 ± 0.1  |
| 4-CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub> -Phenol | 66.6 ± 0.7                                    | 73.4 ± 0.7  | 86.4 ± 0.9  | 93.3 ± 0.9  | -12.4 ± 1.9                                     | 117.3   | 104.9 ± 1.9  |
| 4-CH <sub>3</sub> (CH <sub>2</sub> ) <sub>5</sub> -Phenol | 96.9 ± 0.9                                    | 116 ± 1     | 134 ± 1     | 174 ± 2     | -19.4 ± 2.1                                     | 117.3   | 97.9 ± 2.1   |

<sup>a</sup>Cited from Ref. [6].

4-CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>-Phenol, calculated from Equation (7), were constants, whereas the values tentatively calculated from the 1:2 equilibrium equation obviously changed with the concentrations of 4-CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>-Phenol. Judging from these estimated equilibrium constants [8], we found that 1:1 binding of G- $\beta$ -CD takes place with 4-CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>-Phenol. 6-SO<sub>3</sub>-SP serves as a chemical indicator, and the isomerization rate of 6-SO<sub>3</sub>-SP gives the concentration of unbound CD in solution. The equilibrium constant for the G- $\beta$ -CD inclusion can be estimated without analyzing the absorption bands due to the inclusion complex.

On the basis of the Benesi–Hildebrand treatment [9], extensive studies for 1:1 complex formations have been made by many investigators. 4-NO<sub>2</sub>-Phenol has an absorption band in the visible region ( $\lambda_{\text{max}} = 407$  nm), as can be seen in Figure 2a. On adding G- $\beta$ -CD to the 4-NO<sub>2</sub>-phenol solution ( $[\text{G-}\beta\text{-CD}]_0 \gg [4\text{-NO}_2\text{-phenol}]_0$ ), the absorption band of 4-NO<sub>2</sub>-phenol in the vicinity of 410 nm increases and that in the vicinity of 320 nm decreases. The existence of the isosbestic point is related to the 1:1 inclusion equilibrium. The spectral data were analyzed according to Benesi–Hildebrand Equation (8).

$$\frac{[\text{NP}]_0[\text{G-}\beta\text{-CD}]_0}{\Delta E} = \frac{1}{K\Delta\epsilon} + \frac{[\text{G-}\beta\text{-CD}]_0}{\Delta\epsilon} \quad (8)$$

where  $[\text{NP}]_0$  and  $[\text{G-}\beta\text{-CD}]_0$  are total concentrations of 4-NO<sub>2</sub>-phenol and G- $\beta$ -CD, respectively.  $\Delta\epsilon$  is the difference in the molar extinction coefficients for bound and unbound 4-NO<sub>2</sub>-phenol with G- $\beta$ -CD, and  $\Delta E$  is the change in the absorption intensity of the 4-NO<sub>2</sub>-phenol solution. As shown in Figure 2b, a good linear relationship between  $[\text{NP}]_0[\text{G-}\beta\text{-CD}]_0/\Delta E$  and  $[\text{G-}\beta\text{-CD}]_0$  can be obtained, indicating 1:1 inclusion complex formation. The association constant  $K$  for the inclusion complexation of G- $\beta$ -CD can be estimated according to Equation (8):  $K = (4.80 \pm 0.09) \times 10^2 \text{ mol}^{-1} \text{ dm}^3$ , which is in good agreement with the  $K$  value estimated from the measurements of the isomerization rate of 6-SO<sub>3</sub>-SP.

When we inspect the data in Table 1, we notice some interesting points. The  $K$  values for inclusion of phenols tend to increase with an increase in the bulkiness of the 4-substituent groups. The  $K$  values for 4-substituted phenols having long chain substituents are much larger

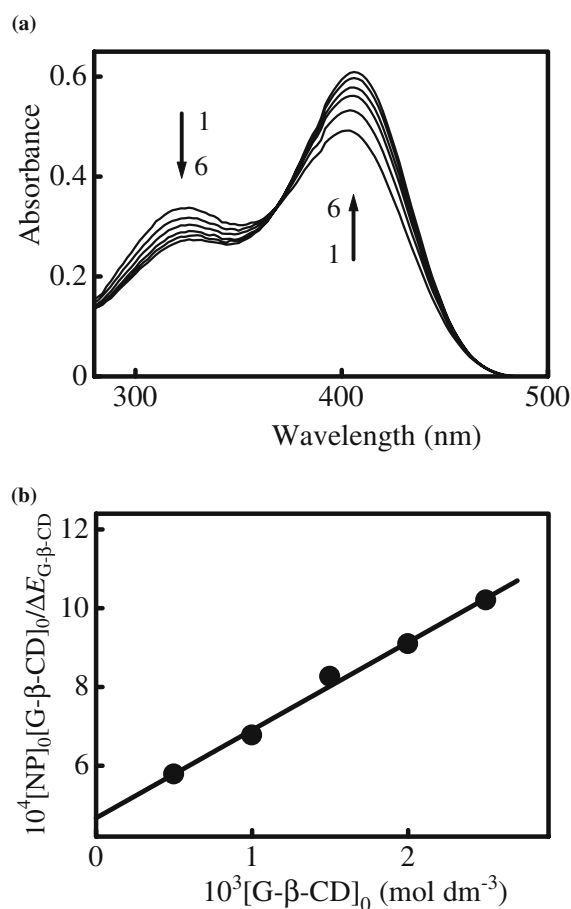


Figure 2. (a) Absorption spectra of 4-NO<sub>2</sub>-phenol ( $[\text{NP}]_0 = 4.94 \times 10^{-5} \text{ mol dm}^{-3}$ ) at various G- $\beta$ -CD concentrations at 313 K:  $[\text{G-}\beta\text{-CD}]_0 =$  (1) 0, (2)  $5.00 \times 10^{-4}$ , (3)  $1.00 \times 10^{-3}$ , (4)  $1.50 \times 10^{-3}$ , (5)  $2.00 \times 10^{-3}$ , and (6)  $2.50 \times 10^{-3} \text{ mol dm}^{-3}$ . (b) Determination of 1:1 inclusion equilibrium constant according to Equation (8).

than those of phenol and 4-CH<sub>3</sub>-phenol. The stability of an inclusion complex is determined by how well a guest fits in the CD cavity and the hydrophobic interaction between the internal wall of the CD and the guest molecules is an important factor. The magnitude of the hydrophobic interaction is related to the contact area of a guest molecule for the internal wall of the CD. From the NMR study, Inoue *et al.* [10] suggested that the OH group of phenols in inclusion complexes is located in the hydrophilic environment and the hydrophobic parts such as a benzene ring are encapsulated in the

$\beta$ -CD cavity. 2-Naphthol, 4-CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>-phenol, and 4-CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>-phenol are too long to encapsulate into the  $\beta$ -CD cavity, which is supported by the Corey–Pauling–Koltun (CPK) space-filling model studies. Judging from the large  $K$  values for phenols having the bulky 4-substituents, we consider that the interaction between guest molecules and the glucosyl side chain introduced on the primary hydroxyl group of  $\beta$ -CD is operative.

#### NMR study of inclusion complex

<sup>1</sup>H NMR measurements give useful information on the complexation mode of the guest with the host. Figure 3

shows the 600 MHz <sup>1</sup>H-NMR spectra of G- $\beta$ -CD and its inclusion complexes in D<sub>2</sub>O. The assignments of the chemical shifts for the G- $\beta$ -CD protons were carried out with reference to the NMR spectral data of  $\alpha$ - and  $\beta$ -CDs reported by Inoue *et al.* [10]. The changes in the chemical shifts for the H-3 and H-5 protons of  $\beta$ -CD unit and the H-3' proton of 6-*O*- $\alpha$ -D-glucosyl moiety were observed for the inclusion complexes with three kinds of phenols, and were listed in Table 2. The chemical shift changes for the H-1 and H-2 protons located outside the CD cavity and the H-4' proton were not observed in the inclusion complexes of G- $\beta$ -CD with phenols. However, although the kind of interaction that

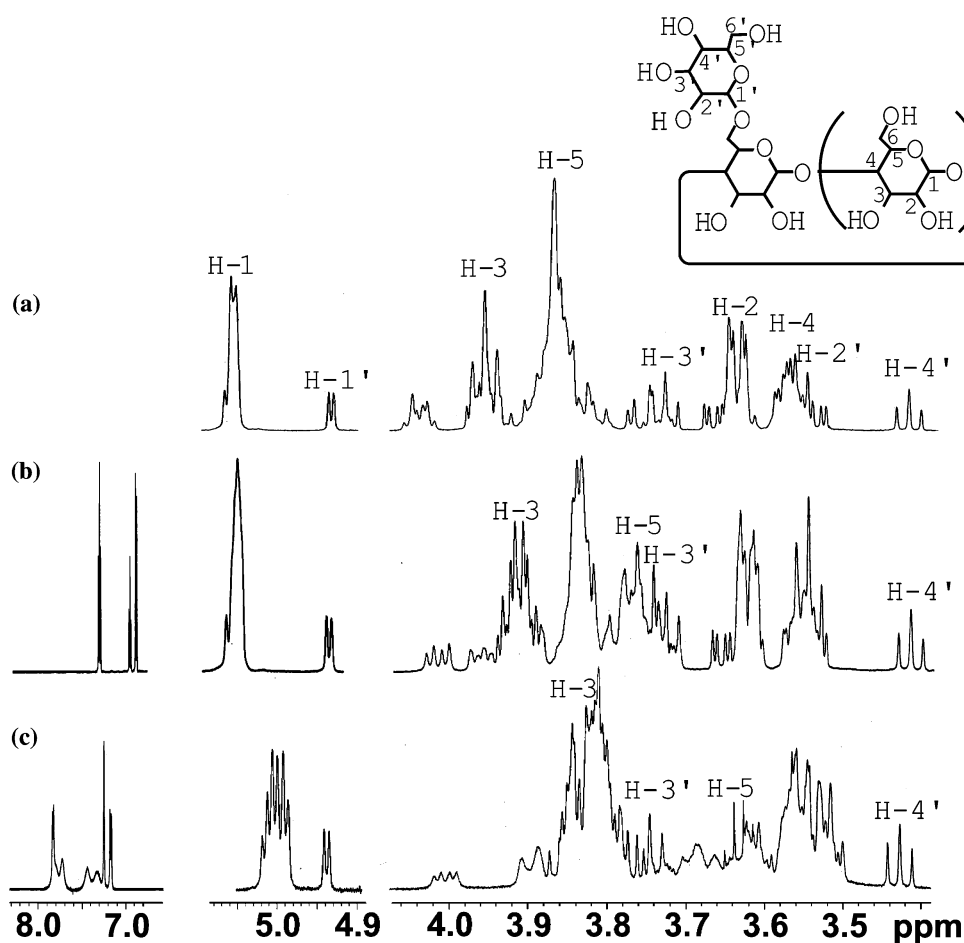


Figure 3. <sup>1</sup>H-NMR spectra of G- $\beta$ -CD and its inclusion complex: (a) G- $\beta$ -CD ( $3.32 \times 10^{-3} \text{ mol dm}^{-3}$ ), (b) G- $\beta$ -CD ( $5.15 \times 10^{-3} \text{ mol dm}^{-3}$ )-Phenol ( $9.41 \times 10^{-3} \text{ mol dm}^{-3}$ ), and (c) G- $\beta$ -CD ( $1.08 \times 10^{-3} \text{ mol dm}^{-3}$ )-2-Naphthol ( $1.12 \times 10^{-2} \text{ mol dm}^{-3}$ ).

Table 2. Changes ( $\Delta\delta$ ) in chemical shifts of G- $\beta$ -CD in the presence of phenols

| Guest                                  | $\Delta\delta^a$ (ppm) |       |        |        |
|--|------------------------|-------|--------|--------|
|  | H-3                    | H-3'  | H-4'   | H-5    |
| Phenol <sup>b</sup>                    | -0.048                 | 0.000 | -0.003 | -0.100 |
| 4-NO <sub>2</sub> -Phenol <sup>c</sup> | -0.128                 | 0.005 | 0.008  | -0.160 |
| 2-Naphthol <sup>d</sup>                | -0.122                 | 0.022 | 0.006  | -0.220 |

<sup>a</sup> $\Delta\delta = \delta(\text{G-}\beta\text{-CD bound with phenols}) - \delta(\text{G-}\beta\text{-CD unbound with phenols})$  in D<sub>2</sub>O. Negative values indicate upfield shifts.

<sup>b</sup> $[\text{G-}\beta\text{-CD}]_0 = 5.15 \times 10^{-3} \text{ mol dm}^{-3}$  and  $[\text{Phenol}]_0 = 9.41 \times 10^{-3} \text{ mol dm}^{-3}$ .

<sup>c</sup> $[\text{G-}\beta\text{-CD}]_0 = 5.64 \times 10^{-3} \text{ mol dm}^{-3}$  and  $[\text{4-NO}_2\text{-Phenol}]_0 = 3.59 \times 10^{-2} \text{ mol dm}^{-3}$ .

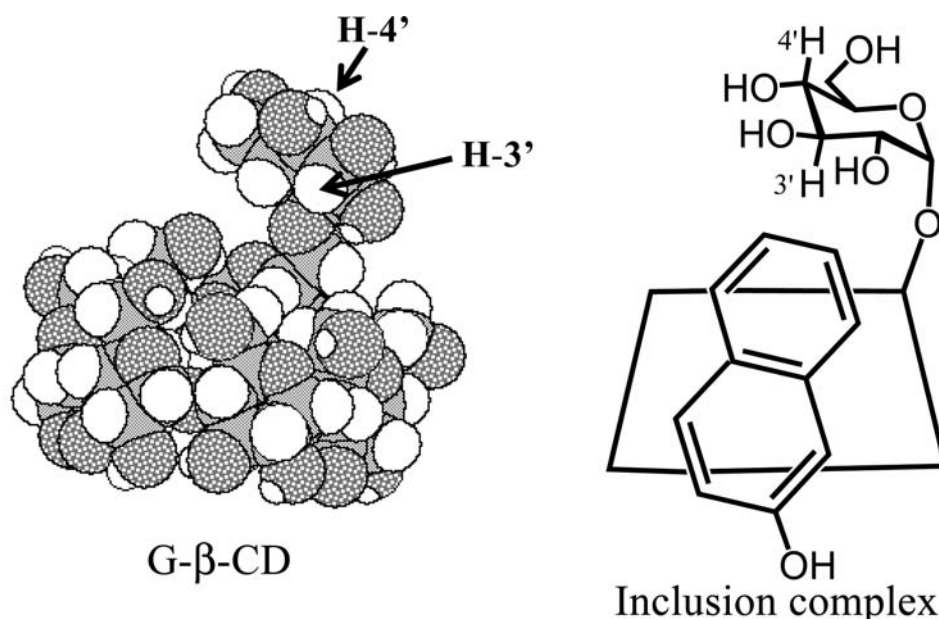
<sup>d</sup> $[\text{G-}\beta\text{-CD}]_0 = 1.08 \times 10^{-3} \text{ mol dm}^{-3}$  and  $[\text{2-Naphthol}]_0 = 1.12 \times 10^{-2} \text{ mol dm}^{-3}$ .

is operative has not been clarified, in the inclusion complex between G- $\beta$ -CD and 2-naphthol, the H-1 and H-2 protons showed upfield-induced chemical shifts ( $\Delta\delta$  = about 0.05 and 0.08 ppm, respectively), which is smaller than those for the H-3 and H-5 protons. The unambiguous assignments for the H-6 and H-6' protons were difficult because of the similarity of the proton structures and signal overlapping. It is instructive to obtain information on the induced chemical shifts of guest molecules for the discussion on the molecular disposition of inclusion complexes. We examined the NMR spectra for the inclusion complexes in excess of G- $\beta$ -CD (host), and the changes ( $\Delta\delta$ ) in chemical shifts of guests were obtained as follows:  $\Delta\delta$  = -0.017, -0.004, and 0.001 ppm for C<sup>2,6</sup>-H, C<sup>4</sup>-H, and C<sup>3,5</sup>-H of phenol, respectively ( $[\text{G-}\beta\text{-CD}]_0/[\text{phenol}]_0 = 4.4$ );  $\Delta\delta$  = 0.038 and -0.093 ppm for C<sup>2,6</sup>-H and C<sup>3,5</sup>-H of 4-NO<sub>2</sub>-phenol ( $[\text{G-}\beta\text{-CD}]_0/[\text{4-NO}_2\text{-phenol}]_0 = 2.3$ );  $\Delta\delta$  = -0.199, -0.166, -0.262, -0.659, -0.790, -0.678, and -0.643 ppm for C<sup>1,3,4,5,6,7,8</sup>-H of 2-naphthol ( $[\text{G-}\beta\text{-CD}]_0/[\text{2-naphthol}]_0 = 3.7$ ). The large induced chemical shifts for the protons of phenol molecules indicate an inclusion of the phenyl moiety into the CD cavity.

From the changes in chemical shifts for the protons of G- $\beta$ -CD we can make a few interesting observations. (1) On adding phenols, we can see that the peaks of H-3 and H-5 of G- $\beta$ -CD shift to a higher magnetic field. These are in good agreement with the results reported by Inoue *et al.* [10], who suggested that the chemical shift changes are ascribed to the ring-current effects and the phenol molecule is encapsulated into the CD cavity. (2) In the 6-*O*- $\alpha$ -D-glucosyl protons of G- $\beta$ -CD, the change in the chemical shifts of H-4' for the inclusion complexes are small on adding phenols; however a moderate lower field shift of H-3' is obtained for the inclusion complex with 2-naphthol. Such shift changes appear to be caused by enlargement of the region where the larger guest

molecule brings about the ring-current effect, which are similar to those observed for H-5 protons of the  $\alpha$ -CD inclusion complex [10]. The change  $|\Delta\delta|$  in the induced chemical shifts for H-3' and H-5 increases with an increase in the bulkiness of guest molecules. These findings suggest that the interaction between 2-naphthol and the 6-*O*- $\alpha$ -D-glucosyl moiety of G- $\beta$ -CD is operative in the inclusion complex, which is responsible for the large *K* value for the inclusion complexation of G- $\beta$ -CD with bulky guest molecules. Ueno *et al.* synthesized various kinds of branched CDs, and those branched CDs are recognized to be induced-fit types of guest binding [11, 12]. In the CD inclusion complexes, capping of the cavity by hydrophobic moiety is effective for enlarging the hydrophobic environment around the cavity. In Scheme 3, the plausible structure of G- $\beta$ -CD is depicted by using a simple molecular calculation, and its inclusion complex with 2-naphthol is shown based on the changes in the <sup>1</sup>H-NMR chemical shifts of the inclusion complexes. The CPK model study shows that these structures are most plausible.

The 2D ROESY-NMR experiments are instructive for the discussion on the position of the phenyl moiety towards the CD cavity. Figure 4 shows the ROESY-NMR spectrum of 2-naphthol-G- $\beta$ -CD in D<sub>2</sub>O solution. The intense cross peaks were detected between the inner protons (H-3 and H-5) of the G- $\beta$ -CD cavity and the phenyl moiety protons (C<sup>1,3,4,5,8</sup>-H) of 2-naphthol, which confirmed the inclusion between the 2-naphthol molecule and G- $\beta$ -CD. It is noteworthy that the cross peaks between the C<sup>6,7</sup>-H protons of 2-naphthol and the H-3,5 protons of G- $\beta$ -CD are not observed, while those between the C<sup>6,7</sup>-H protons and the H-3' proton of G- $\beta$ -CD appears, as enclosed in the rectangle in Figure 4. These ROESY-NMR signals arise from dipolar interactions between the protons of 2-naphthol and the protons in the glucosyl side chain of G- $\beta$ -CD. These



Scheme 3. Space-filling model of G- $\beta$ -CD and structure of its inclusion complex with 2-naphthol.

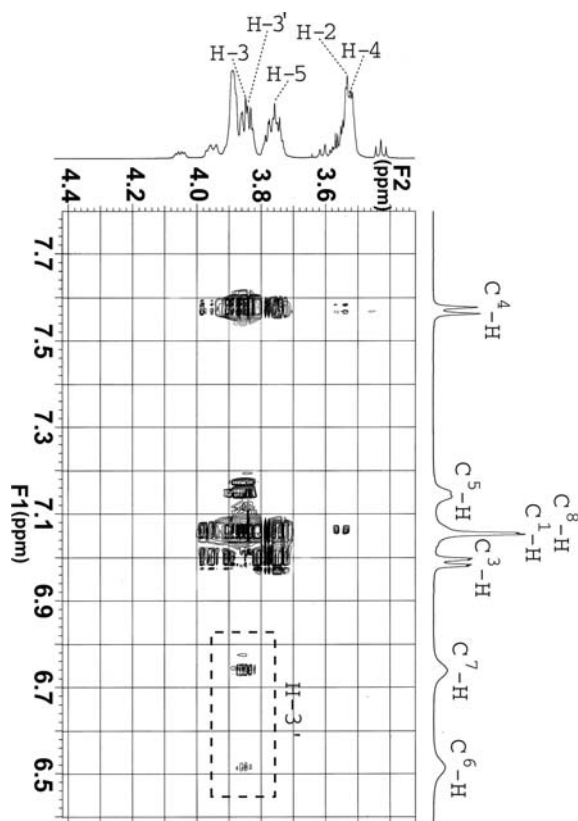


Figure 4. 2D ROESY-NMR spectrum (300 ms mixing time) at 298 K of a  $D_2O$  solution in which  $G\text{-}\beta\text{-CD}$  ( $5.02 \times 10^{-2} \text{ mol dm}^{-3}$ ) and 2-naphthol ( $4.95 \times 10^{-2} \text{ mol dm}^{-3}$ ).

results are in accord with the above structure of the inclusion complex of  $G\text{-}\beta\text{-CD}$  (Scheme 3), and support the induced inclusion complexation of branched CDs.

#### Volumetric study of inclusion complexation

The  $K$  values for inclusion complexation of  $G\text{-}\beta\text{-CD}$  become larger with increased external pressure. Using  $K$ -values shown in Table 1, the reaction volume  $\Delta V$  for the inclusion complexation of  $G\text{-}\beta\text{-CD}$  with 4-substituted phenols at 1 bar can be evaluated according to the following thermodynamic equations, and the results are also listed in Table 1.

$$\ln K = aP + b \quad (9)$$

$$\Delta V = -RT \left( \frac{\partial \ln K}{\partial P} \right)_T - \kappa_T RT \quad (10)$$

where  $\kappa_T$  is the isothermal compressibility of water and was calculated from available data [13], to be  $\kappa_T = 4.425 \times 10^{-5} \text{ bar}^{-1}$ . The reaction volumes estimated for the  $G\text{-}\beta\text{-CD}$  complexation are unexpectedly negative ( $\Delta V = -3.8$  to  $-19.4 \text{ cm}^3 \text{ mol}^{-1}$ ), and are obviously smaller than those for  $\beta\text{-CD}$  ( $\Delta V = 14.0$ – $2.5 \text{ cm}^3 \text{ mol}^{-1}$  [6]). The absolute value of  $\Delta V$  for  $G\text{-}\beta\text{-CD}$  increases with an increase in the bulkiness of

the 4-substituent group. Previously, using di-*tert*-butyl nitroxide [14] and spiropyran [8] as guest molecules, we demonstrated that CD inclusion equilibrium was pressure dependent. We also showed that the reaction volume for the CD inclusion is not solely due to the change in total (the guest plus CD) volume but consists of three discrete volume changes [8, 12, 15]:

$$\Delta V = \Delta V_{\text{inclu}} + \Delta V_{\text{desolv}} + \Delta V_{\text{repe}} \quad (11)$$

where  $\Delta V_{\text{inclu}}$  is the volume change related to the inclusion of the guest molecules in the CD cavity,  $\Delta V_{\text{desolv}}$  denotes the volume change accompanying desolvation around the guest molecule, and  $\Delta V_{\text{repe}}$  denotes the volume change caused by water molecules repelled out from the CD cavity [16]. Usually  $\Delta V_{\text{inclu}}$  is negative value, and  $\Delta V_{\text{desolv}}$  and  $\Delta V_{\text{repe}}$  are positive. The  $\Delta V_{\text{desolv}}$  value for the CD inclusion process of phenols can be assumed small ( $\Delta V_{\text{desolv}} \sim 0 \text{ cm}^3 \text{ mol}^{-1}$ ) because in the inclusion complex of phenols the OH group solvated by water is located outside the CD cavity [10]. Therefore,  $\Delta V$  for the CD inclusion of phenols can be expressed as follows:

$$\Delta V = \Delta V_{\text{inclu}} + \Delta V_{\text{repe}} \quad (12)$$

Taking into account of the molecular disposition of the 4-substituted phenols in the CD cavity in the CD inclusion complexes [10], we estimated the  $\Delta V_{\text{inclu}}$  values for inclusion of 4- $\text{CH}_3(\text{CH}_2)_4$ - and 4- $\text{CH}_3(\text{CH}_2)_5$ -phenols with the aid of the CPK models, as suggested previously [6]. The  $\Delta V_{\text{repe}}$  values caused by the repelling of water molecules from the CD cavity were calculated from the values of  $\Delta V$  and  $\Delta V_{\text{inclu}}$ , and are given in the right-hand side column in Table 1.

In the study on the inclusion complexation of phenyl acetates with  $\beta\text{-CD}$ , VanEtten *et al.* [17] indicated that exclusion of water molecules from the CD cavity is an important factor in forming the stable inclusion complexes. In the  $G\text{-}\beta\text{-CD}$  inclusion complexation, the values of  $\Delta V_{\text{repe}}$  for the inclusion of phenol and 4- $\text{CH}_3$ -phenol are small compared with the others. Similar trends were observed for the 1:1 inclusion complexation of  $\beta\text{-CD}$  with 4-substituted phenols [6]. Figure 5 shows plots of  $\Delta V_{\text{repe}}$  against  $-\Delta V_{\text{inclu}}$  for the inclusion complexation of 4-substituted phenols with  $G\text{-}\beta\text{-CD}$  together with those of  $\beta\text{-CD}$  estimated previously [6]. For the  $G\text{-}\beta\text{-CD}$  complexation, the saturation curve was obtained, analogous with the  $\beta\text{-CD}$  complexation: The saturated values were estimated to be 105 and  $115 \text{ cm}^3 \text{ mol}^{-1}$  for  $G\text{-}\beta\text{-CD}$  and  $\beta\text{-CD}$ , respectively. An X-ray diffraction study demonstrated that an average of 6.5 water molecules are situated in the  $\beta\text{-CD}$  cavity [16]. All or part of the water molecules in the CD cavity are repelled according to the size of the guest molecule upon the inclusion complexation. The saturated value of the  $\beta\text{-CD}$  complexation in Figure 5 corresponds to the

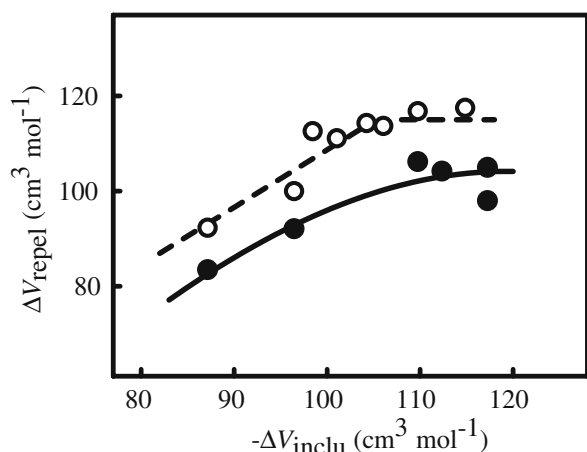


Figure 5. Relationship between  $\Delta V_{\text{repe}}l$  and  $-\Delta V_{\text{inclu}}$  for inclusion complexation of phenols with  $\beta$ -CD (○) and G- $\beta$ -CD (●).

volume of 6.5 water molecules included in  $\beta$ -CD cavity. This finding suggests that evaluations of components in reaction volume are reasonable. In the G- $\beta$ -CD complexation, the saturated value ( $\Delta V_{\text{repe}}l \sim 105 \text{ cm}^3 \text{ mol}^{-1}$ ) plotted in Figure 5 shows the average number of water molecules included in the G- $\beta$ -CD cavity in water. The average number of water molecules situated in the G- $\beta$ -CD cavity in water is evaluated to be 5.8, which is responsible for the downward shift of the plots for the G- $\beta$ -CD complexation in Figure 5. Most of the water molecules in the  $\beta$ -CD cavity are situated through the hydrogen bond of the OH group of the rim [16]. The decrease in the number of the water molecules included in the G- $\beta$ -CD cavity is attributed to the introduction of the glucosyl side chain into the OH group of the CD rim.

In summary, the interaction between the 6-*O*- $\alpha$ -D-glucosyl side chain of G- $\beta$ -CD and bulky guest molecules is operative, resulting in the enhanced stability of the G- $\beta$ -CD inclusion complex. The glucose unit in the side chain of G- $\beta$ -CD is flexible in motion, and its

orientation may be different between the free and complexed state. Although the present analysis of high-pressure results used a simplified model, the above results are reasonable and show that the number of water molecules repelled from the CD cavity upon the inclusion complexation may be an important aspect of CD inclusion complex formation dynamics. High-pressure studies on inclusion complexes may be able to provide useful insight into the CD inclusion behavior.

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