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Complex Formation of Hexakis(2,3,6-tri-*O*-methyl)-α-Cyclodextrin with Substituted Benzenes in Aqueous Solution

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Abstract. The complex formation of hexakis (2,3,6-tri-*O*-methyl)-α-cyclodextrin with substituted benzenes has been investigated by circular dichroism (CD) spectroscopy. The sign and shape of the CD spectra markedly differ from the spectra of corresponding α-cyclodextrin complexes because of the distorted conformation of the host molecule and/or the difference in the geometry of the host-guest interaction. Enthalpy and entropy changes of the complex formation are determined by using the CD band intensities measured at various temperatures and host concentrations. Negative values of ΔH and ΔS indicate that the hydrophobic interaction is not the major driving force for the complex formation. The guest molecule is suggested to be tightly bound within the host cavity through the induced-fit conformational change of the host molecule.

Key words: Permethylated α-cyclodextrin, induced circular dichroism, inclusion complex, host-guest interaction.

1. Introduction

Cyclodextrins form inclusion complexes with a variety of guest molecules [1, 2]. Since the cavity of cyclodextrins, which can accommodate guest molecules, is limited in its size and shape, cyclodextrins show high selectivity in complex formation [3]. The chemical modification of cyclodextrins has been extensively investigated in order to improve the abilities of selective inclusion and enzyme-like catalysis, etc. [4]. One simple modification is permethylation, that is, all the hydroxyl groups are methylated. Permethylated cyclodextrins also form inclusion complexes in solution and in the solid state. X-ray analyses of several permethylated cyclodextrin complexes have shown remarkable differences in the macrocyclic conformation and the host-guest interaction, compared with the corresponding cyclodextrin complexes [5–7]. On the other hand, only a few investigations have been reported on complex formation in solution [8, 9]. We here report the circular dichroism (CD) spectra of hexakis (2,3,6-tri-*O*-methyl)-α-cyclodextrin (TM-α-CDx) complexes with substituted benzenes

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in aqueous solution, and will discuss the host-guest interaction in comparison with the α-cyclodextrin complexes.

2. Experimental

TM-α-CDx was prepared according to Casu *et al.* [10], and purified by successive recrystallization from hot water. Substituted benzenes were purchased from Nakarai Chemicals Ltd., and used without further purification. Solutions were prepared with deionized and distilled water. CD spectra were recorded on a JASCO J-40A Spectropolarimeter with a J-DPZ data-processor. The temperature was regulated by a Tokyo Rico TC-100 thermocontroller, and a water-jacketed cylindrical cell was used.

Thermodynamic parameters of the complex formation were determined on the assumption of a 1 : 1 complex formation by the least-squares curve-fitting method from the equations [11],

$$\ln K_d = \Delta H/RT_i - \Delta S/R \tag{1}$$

$$K_d = ab_j\theta_m/\theta_{ij} - a - b_j + \theta_{ij}/\theta_m \tag{2}$$

where a and b_j are concentrations of the guest and TM-α-CDx, respectively, θ_{ij} is the

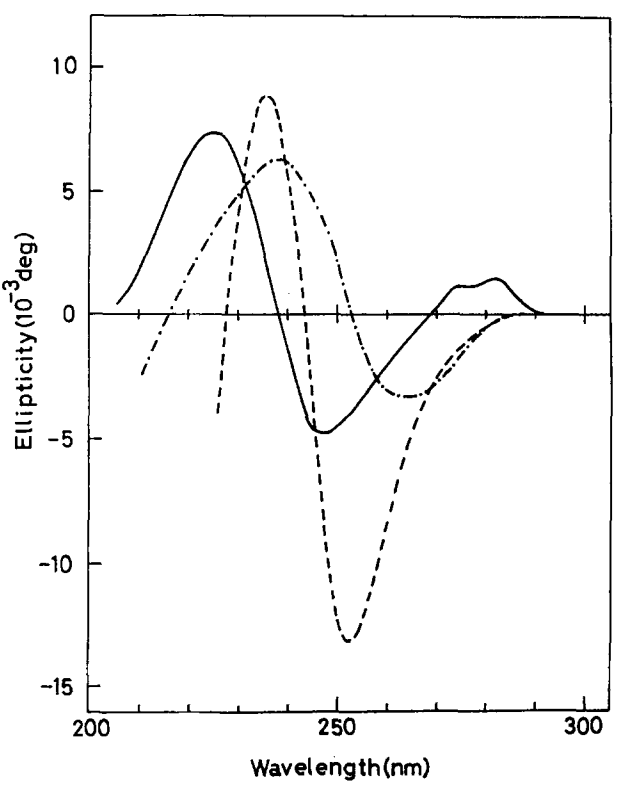

Fig. 1. CD spectra of TM-α-CDx complexes with benzoic acid (———), *m*-hydroxybenzoic acid (– – – –), and *p*-hydroxybenzoic acid (– · – · –) at 10 °C, with the concentrations of 7.98×10^{-3} M (TM-α-CDx), 1.95×10^{-3} M (benzoic acid), 1.23×10^{-3} M (*m*-hydroxybenzoic acid), and 1.95×10^{-3} M (*p*-hydroxybenzoic acid). A 0.5 mm cell was used.

observed CD intensity at the temperature T_i, and θ_m is the molecular ellipticity of the complex.

3. Results and Discussion

CD spectra of TM-α-CDx complexes with substituted benzenes are shown in Figures 1 and 2. In the benzoic acid complex, a weak positive CD is observed at 280 nm, and negative and positive CDs are observed at 250 nm and 225 nm, respectively. In the TM-α-CDx complexes with other benzene derivatives, a negative CD peak is found in the longer wavelength region, followed by a positive CD peak. These CD spectra are markedly different from the CD spectra of corresponding α-cyclodextrin complexes [12]. For example, the α-cyclodextrin complex with benzoic acid shows a weak negative CD peak at 280 nm, a weak positive shoulder near 250 nm, and a strong positive CD peak at 230 nm [12].

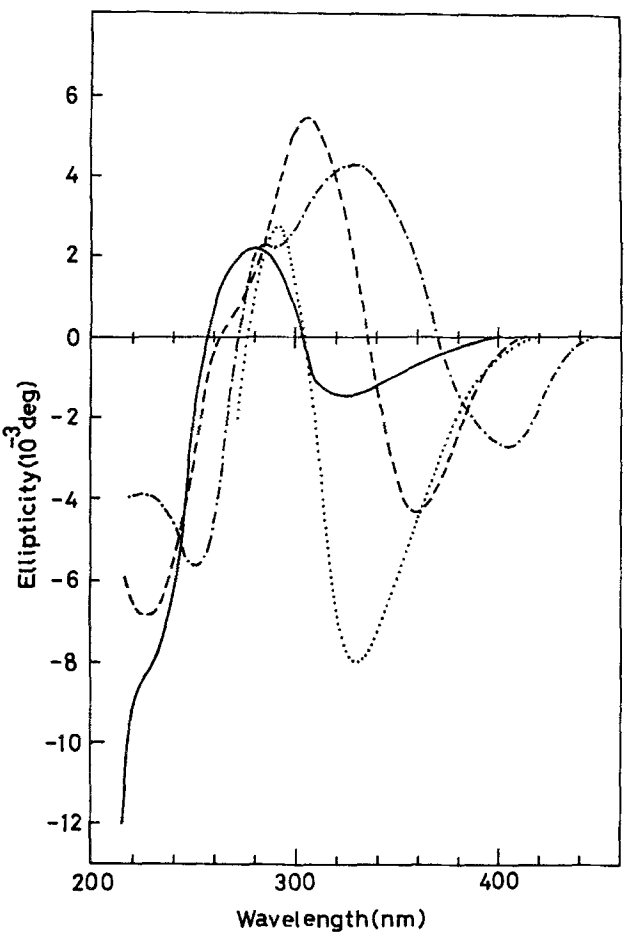

Fig. 2. CD spectra of TM-α-CDx complexes with *m*-nitrophenol (———), *p*-nitrophenol (– – – –), *m*-nitroaniline (· · · · ·), and *p*-nitroaniline (– · – · –) at 10 °C, with the concentrations of 7.98×10^{-3} M (TM-α-CDx), 2.08×10^{-3} M (*m*-nitrophenol), 2.20×10^{-3} M (*p*-nitrophenol), 2.27×10^{-3} M (*m*-nitroaniline), and 2.08×10^{-3} M (*p*-nitroaniline). A 0.5 mm cell was used.

Since the benzene derivatives are optically inactive, the circular dichroism is induced by the complex formation with TM-α-CDx. In the earlier papers, we have shown that the sign and intensity of the induced CD of the cyclodextrin complexes are expressed by the equation [12, 13],

$$R(\lambda) = A(\lambda)(1 + 3\cos 2\theta)\mu(\lambda)^2 \tag{3}$$

where $A(\lambda)$ is a constant depending on the wavelength and the host structure, and θ is the angle made by the symmetry axis of the host molecule and the electric transition-dipole-moment, $\mu(\lambda)$, of the guest chromophore. When Equation (3) is applied to elucidate the geometry of the host-guest interaction in the TM-α-CDx complexes, a difficulty arises in the interpretation of the spectra. In the benzoic acid complex, two positive CD bands at 280 and 225 nm suggest that these two transition-moments are in the same direction. But, it has been experimentally established that the transition-dipole-moments of these absorption bands are nearly perpendicular to each other [14]. This suggests that Equation (3) is not strictly applicable to interpret the CD spectra of the TM-α-CDx complexes.

The CD spectrum of the TM-α-CDx-*p*-nitrophenol complex markedly differs from the CD spectra of the *p*-nitrophenol complexes with α-cyclodextrin and hexakis(2,6,-di-*O*-methyl)-α-cyclodextrin (DM-α-CDx) as shown in Figures 2 and 3. The comparison of the CD spectra between the complexes of α-cyclodextrin and DM-α-CDx suggests that the methylation at the O(2) and O(6) positions does not much affect the macrocyclic conformation and the host-guest interaction. Our recent X- ray analysis has also shown that the DM-α-CDx molecule has a round and symmetrical structure similar to that of α-cyclodextrin [15]. Therefore, the remarkable difference in the CD spectra of the TM-α-CDx complexes is considered to be brought about by the

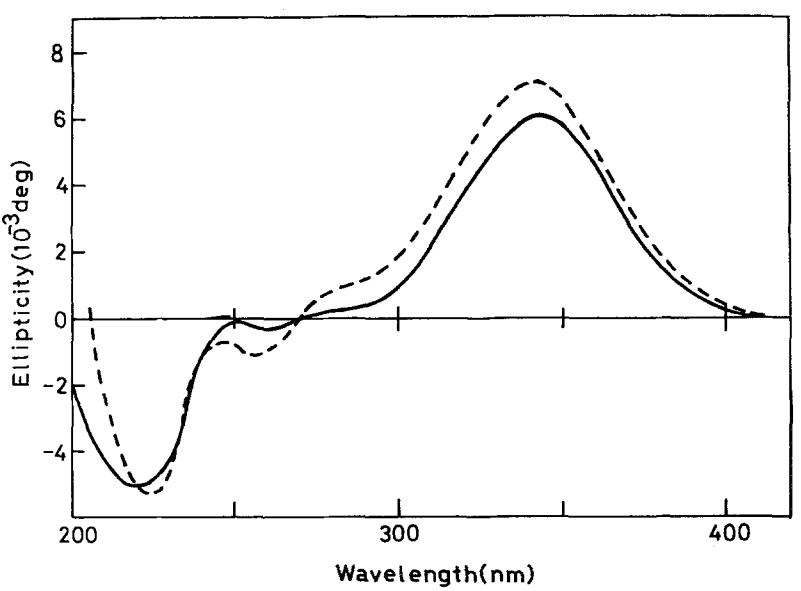

Fig. 3. CD spectra of the DM-α-CDx (9.0×10^{-3} M) complex (———) with *p*-nitrophenol (8.68×10^{-4} M) and α-cyclodextrin (18.5×10^{-3} M) complex (– – – –) with *p*-nitrophenol (1.12×10^{-3} M) measured at 20 °C by using 1mm cell.

methyl groups attached to the O(3) oxygen atoms. In the crystalline state, the TM-α-CDx molecule is distorted more than α-cyclodextrin or DM-α-CDx because of the steric hindrance involving methyl groups and the inability of forming intramolecular O(2)···O(3) hydrogen bonds to maintain the round structure [7]. The hydroxyphenyl group of *p*-nitrophenol is inserted into the TM-α-CDx cavity from the O(2), O(3) side with the upside-down orientation [16] compared with the corresponding α-cyclodextrin complex [17]. On the other hand, Inoue *et al.* have suggested on the basis of the NMR study in solution [9] that the nitrophenyl group is included within the TM-α-CDx cavity with the same orientation as that found in the α-cyclodextrin complex. The CD spectrum does not give sufficient information to elucidate whether the nitrophenyl group or hydroxyphenyl group is included, but strongly indicates that the macrocyclic conformation of TM-α-CDx and/or the geometry of the host-guest interaction differ from those of the α-cyclodextrin and DM-α-CDx complexes. The distortion of the host molecule or lateral shift of the guest molecule relative to the symmetry axis makes the elucidation of the geometry of the host-guest interaction difficult because Equation (3) is derived on the assumption that the host molecule has an *n*-fold symmetry axis, on which the guest chromophore is located. The TM-α-CDx molecule is highly flexible, therefore, it is plausible that the conformation of TM-α-CDx changes depending on the size and shape of the guest molecule through the induced-fit conformational change as observed in the crystalline state [18].

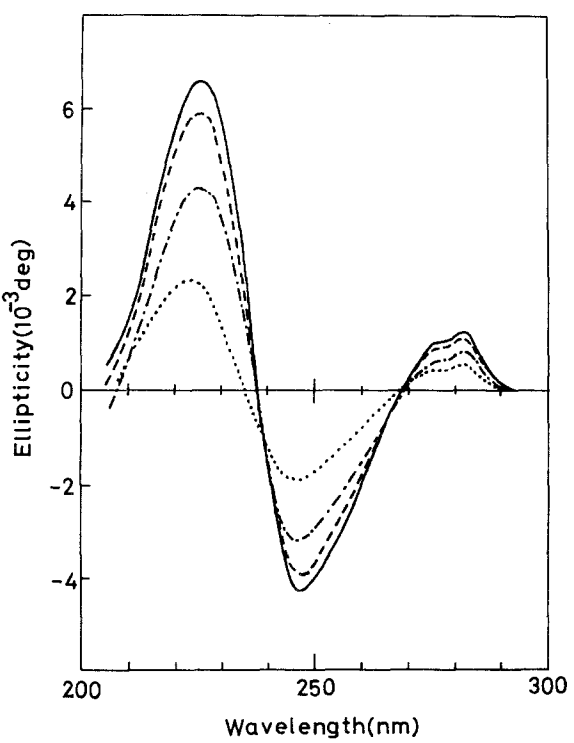

Fig. 4. CD spectra of the TM-α-CDx complexes with benzoic acid at various concentrations of the host with the guest concentrations of 1.95×10^{-3} M and the cell length of 0.5 mm. ———: 7.82×10^{-3} M, 10 °C; – – – –: 3.91×10^{-3} M, 10 °C; – · – · –: 1.95×10^{-3} M, 10 °C; · · · · ·: 7.82×10^{-3} M, 50 °C.

Figure 4 shows the CD spectra of the TM-α-CDx complex with benzoic acid at various conditions. The spectral shape is not affected by the change in the host-guest molar ratio or temperature change. The intensity change at 225 nm is shown in Figure 5. The CD intensity monotonously decreases with the decrease of the TM-α-CDx concentration and the increase of the temperature. Thermodynamic parameters, obtained by assuming a 1 : 1 complex formation, are given in Table I. The calculated CD intensity by using these parameters is in good agreement with the observed one as shown by the solid lines in Figure 5. The free energy of the complex formation at 298 K is in the range from –3.73 to –4.74 kcal/mol. These values are *ca.* 1 kcal/mol greater than the free energy change found in α-cyclodextrin complexation, which is in the range from –2.55 to –3.64 kcal/mol [12]. No significant difference in the thermodynamic parameters is observed between the complexes with *meta* isomers and *para* isomers. ΔH and ΔS values are greater than the corresponding values of α-cyclodextrin complexes. The large negative enthalpy change indicates a strong host-guest interaction, which may be brought about through the induced-fit conformational change of the TM-α-CDx ring. The negative entropy change may be ascribed to the strong restriction on the conformational flexibility of the host molecule and on the translational and rotational freedom of the guest molecule, which are imposed by the inclusion. ΔH and ΔS are highly compensated by each other as shown in Figure 6. As a result, in spite of the change in ΔS values among the complexes, ΔG values are found in a relatively narrow region. Such compensation

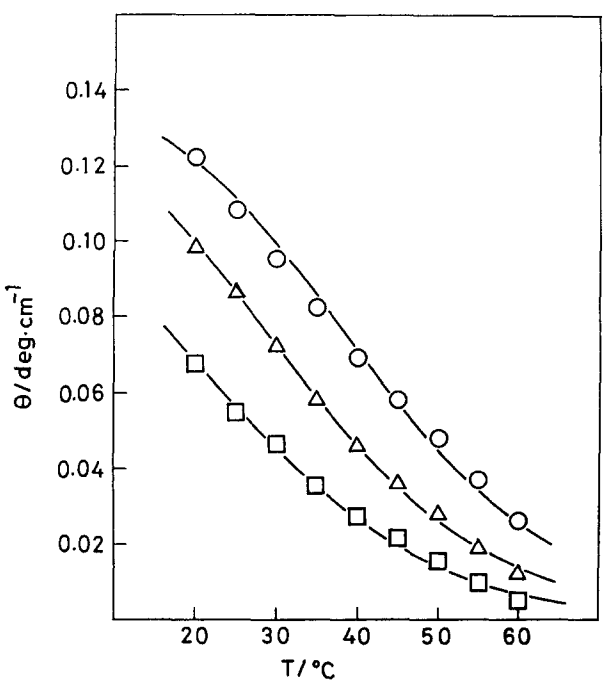

Fig. 5. The temperature dependence of the CD intensity of the TM-α-CDx complex with benzoic acid at 225 nm with TM-α-CDx concentrations of 7.82×10^{-3} M (○), 3.91×10^{-3} M (△), and 1.95×10^{-3} M (□). The calculated intensity change on the basis of the thermodynamic parameters obtained by the curve-fitting method is shown by solid lines.

Table I. Thermodynamic parameters of TM-α-CDx complexes with substituted benzenes[a]

Guest	ΔG(kcal/mol)[b]	ΔH(kcal/mol)	ΔS(cal/K·mol)
Benzoic acid	−3.73	−16.4(0.4)	−43(2)
m-Hydroxybenzoic acid	−4.17	−17.3(0.5)	−44(2)
p-Hydroxybenzoic acid	−4.07	−14.4(0.5)	−34(2)
m-Nitrophenol	−3.85	−10.2(0.3)	−21(1)
p-Nitrophenol	−3.91	−14.9(0.4)	−37(1)
m-Nitroaniline	−4.16	−11.5(0.2)	−27(1)
p-Nitroaniline	−4.74	−14.4(0.2)	−33(1)

[a] Standard deviations are given in parentheses.
[b] At 298 K.

phenomena between ΔH and ΔS are widely observed in the complex formation of cyclodextrins [11, 12, 19, 20].

Permethylation generally extends the host cavity and may make the inside of the cavity more hydrophobic. The thermodynamic parameters in Table I, however, give no clear indication of the importance of the hydrophobic interaction in the complex formation of TM-α-CDx, since the hydrophobic interaction is characterized by the positive values of ΔH and ΔS. The strong host-guest interaction, such as van der Waals interaction, may overwhelm the effect of hydrophobic interaction. The conformation of TM-α-CDx is considerably flexible and TM-α-CDx changes its conformation so that the shape of the TM-α-CDx cavity becomes more suitable to include the guest molecule tightly [18]. Such host-guest interaction also imposes a strong restriction on the conformational flexibility and the freedom of the guest molecule, and therefore, causes the unfavorable negative entropy change. The greater

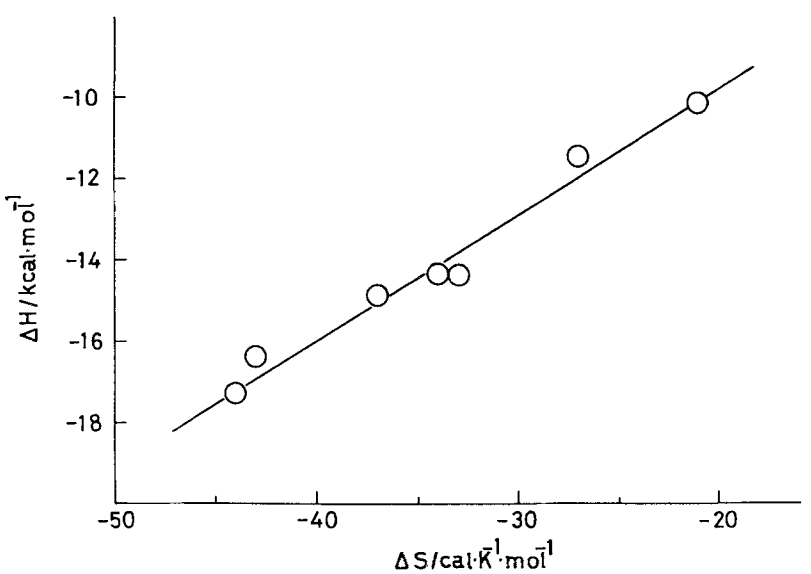

Fig. 6. Plot of ΔH against ΔS for the guests listed in Table I.

negative-signed enthalpy change, however, not only compensates the effect of such a negative entropy change, but also makes the complex more stable than α-cyclodextrin complexes.

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