Effects of Substituent and Solvent on Inclusion Complexation of β-Cyclodextrins with Azobenzene Derivatives

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Abstract

The equilibrium constants (K) for the inclusion complex formation of β-cyclodextrin (β-CD) with Methyl Orange (MO) and substituted azoanilinium chlorides were determined spectrophotometrically. Based on the results, the substituent effect on the inclusion complexation of β-CD with azoanilinium chlorides was discussed in detail. Further, the solvent effects on the inclusion complexation of MO with β-CD and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) were examined in aqueous organic mixtures with water-miscible organic compounds (dimethylsulfoxide, acetonitrile, N, N-dimethylformamide, and acetone). It was found that the K value for the inclusion complex formation with β-CD and DM-β-CD decreases remarkably with increasing ratio of organic solvents, dependent of the surface tension of solvent mixtures.

Introduction

Cyclodextrins(CDs) are water-soluble oligosaccharides which form inclusion complexes with a large number of organic and inorganic molecules [1]. The ability of CDs to accommodate guest molecules of the appropriate size in their cavities has been utilized by many investigators to control the photochemical properties and chemical reactions of organic molecules [2].

Azo dyes have attracted much attention because of their photo- and thermochromic behaviors [3], and there are many reports on the inclusion complexations of CDs with azo dyes [4, 5]. The driving force for inclusion into the CD cavity has been attributed to the hydrophobic interaction between the internal wall of CD and guest molecule, and thus the inclusion with CD occurs effectively in aqueous solution. In the present study, we have examined the substituent effects on the inclusion complexations of β-CD with water-soluble azo dyes (Methyl Orange (MO) and azoanilinium chlorides). Based on the results, the inclusion behavior of azoanilinium chlorides is discussed. On one hand, since many organic compounds of guest molecules are insoluble in water, the measurements of inclusion complexes in aqueous-organic mixture of solvents are required. We have systematically examined the solvent effects on inclusion complexation of MO with β-CD and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) in solvent mixtures, and discussed the efficiency of the inclusion complexation with β-CDs.

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Experimental

Materials

β-CD, DM-β-CD, and MO were purchased from Aldrich Chemical Co. and used as received. Substituted azoanilinium chlorides (Scheme 1), prepared by azo coupling method and purified by column chromatography as described elsewhere [6, 7], were the gift from Prof. Dr. S. Yamamoto.
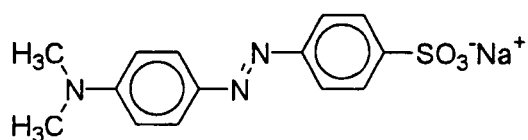
Measurements

The aqueous solution of β-CD or azo dyes is prepared by using distilled water. The representative concentrations used are 3×10^{-5} mol dm^{-3} for MO, 1.0×10^{-4} mol dm^{-3} for azoanilinium chlorides, and $0.2 \sim 10 \times 10^{-3}$ mol dm^{-3} for β-CD. Association constants of the inclusion complexes were determined spectrophotometrically using a Hitachi U-3200 spectrophotometer. The association constants obtained agreed within 5% error. The solution temperature was maintained to within ±0.1 K at 298 K by means of an external circulating water bath.

Results and discussion

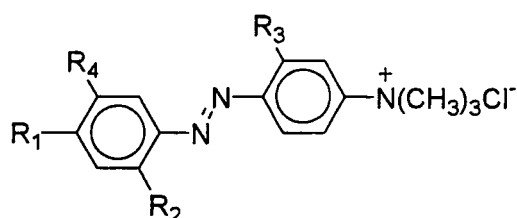### Association constants of β-CD with azobenzene derivatives

Figure 1 shows the UV-Vis absorption spectra of MO upon consecutive additions of excess β-CD. On adding β-CD, the spectrum of MO shows the blue shift (hypsochromic

H₃C
H₃C

Methyl Orange (MO)

Table 1. The association constants and free energy changes for the inclusion complexation of β-CD in aqueous solution

Compound	K (mol^{-1} dm^3)	$\Delta G°$ (kJ mol^{-1})
MO	3560	−20.3
1	860	−16.7
2	1300	−17.8
3	550	−15.6
4	460	−15.2
5	290	−14.0

Compd	R₁	R₂	R₃	R₄
1	H	H	H	H
2	CH₃	H	H	H
3	CH₃	CH₃	CH₃	H
4	Cl	CH₃	CH₃	H
5	H	CH₃	CH₃	NO₂

Scheme 1.

shift) and the peak in the vicinity of 480 nm due to MO decreases, as can be seen in Figure 1. The existence of several isosbestic points is related to the following simple 1 : 1 inclusion equilibrium.

$$MO + CD \rightleftharpoons MO - CD$$
$$K = \frac{[MO - CD]}{[MO][CD]}, \tag{1}$$
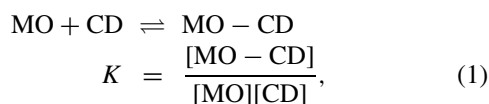
where MO − CD denotes the inclusion complex. Using the Benesi–Hildebrand's Equation (2), extensive studies for 1 : 1 complex formations have been made by many investigators [8].

$$\frac{[MO]_0[CD]_0}{\Delta E} = \frac{1}{K\Delta\epsilon} + \frac{[CD]_0}{\Delta\epsilon}, \tag{2}$$

where $[MO]_0$ and $[CD]_0$ are the total concentrations of Methyl Orange and β-CD, respectively. $\Delta\epsilon$ is the difference in the molar extinction coefficients for bound and unbound MO with β-CD, and ΔE is the change in the absorption intensity of MO solution on adding β-CD. The spectral data in Figure 1(a) can be analyzed according to the Benesi–Hildebrand's Equation (2). As shown in Figure 1(b), a linear relationship between $[MO]_0[CD]_0/\Delta E$ and $[CD]_0$ can be obtained, indicating the 1 : 1 inclusion complex formation under these conditions. The association constants K for the inclusion complexation of β-CD with MO can be obtained by dividing the slope by the intercept. From the similar

spectral analysis, the association constants of azoanilinium chlorides **1** $\sim$ **5** with β-CD in water were determined by Equation (2), and are given in Table 1.

When we inspect the data in Table 1, we notice some interesting points: (1) The K value for MO is much larger than those of azoanilinium chlorides **1** $\sim$ **5**. It is recognized that the SO_3^- and $N(CH_3)_3^+$ ions are located outside the CD cavity (in hydrophilic environments). The above result suggests that the fitness of the N, N-dimethylamino-phenyl ring of MO to the β-CD cavity is better than the others. (2) The K value for azoanilinium chlorides **2** is larger than that for **1**, which is attributed to the hydrophobic interaction between the phenyl ring having the methyl group R_1 and the internal wall of CD. (3) The K value for **3** is smaller than that for **2**, and comparable to that for **4**. The Corey–Pauling–Koltun (CPK) space-filling models showed that the fragment of phenyl group having the methyl substituents R_2 and R_3 is too large to pass through the β-CD cavity. The above K values suggest that the steric hindrance of the methyl group R_2 prevents a deep penetration from the methyl R_1 side of **3** into the β-CD cavity, as depicted in Scheme 2(a). Bertrand *et al.* [9] determined the association constants for the complexation of substituted phenols with β-CD: $K = 250$ mol^{-1} dm^3 for 4-methylphenol and $K = 410$ mol^{-1} dm^3 for 4-chlorophenol. From the NMR study, Inoue *et al.* [10] suggested that in inclusion complexation of substituted phenols, the OH group of phenols is located in hydrophilic environments (Scheme 2(b)). The above K values for **3** and **4**, compared with those of 4-methyl- and 4-chlorophenols, indicate that the molecular disposition of azobenzene derivatives in inclusion complexes is similar to those of 4-substituted phenols. (4) The K value for **5** is small. Previously, we determined the association constant for the inclusion complexation of 4-nitrophenol with β-CD as $K = 350$ mol^{-1} dm^3 [11]. This value is comparable to that of **5**, which suggests that the inclusion of **5** by β-CD occurs from the NO_2 side, as depicted in Scheme 2(c).

The free energy change for inclusion complex formation of azobenzene derivatives with β-CD can be given by

$$\Delta G° = -RT \ln K. \tag{3}$$

The $\Delta G°$ values, listed in Table 1, express the stability of inclusion complexes, which are compared with those of inclusion complexation of 4-substituted phenols [9]. The hy-
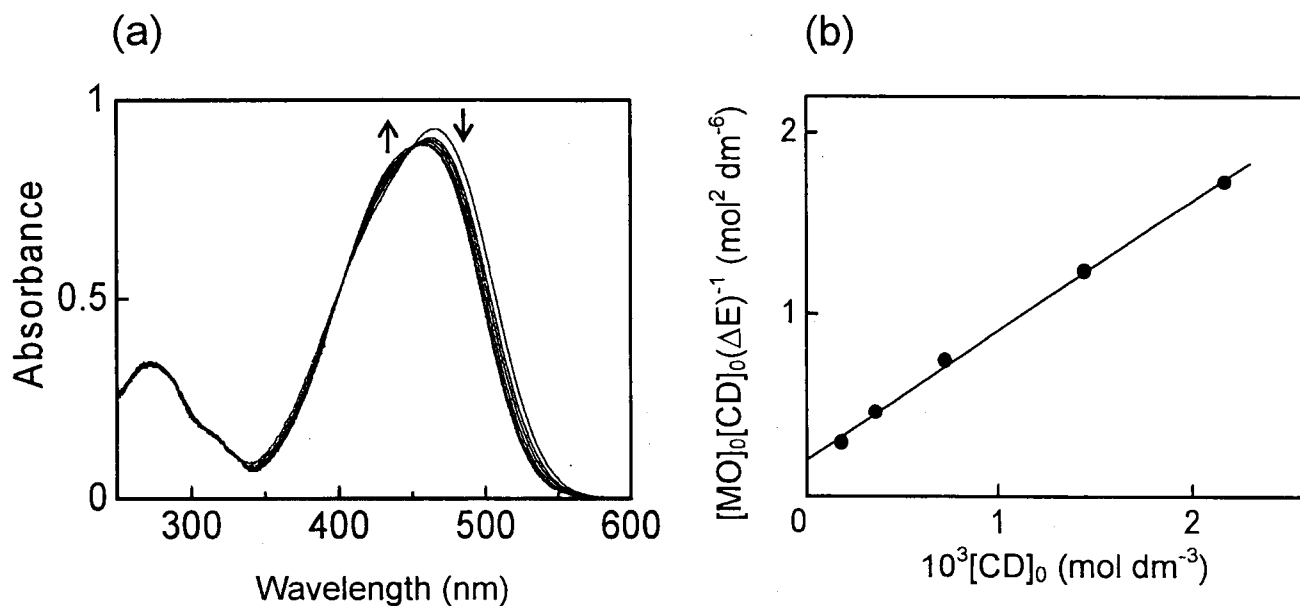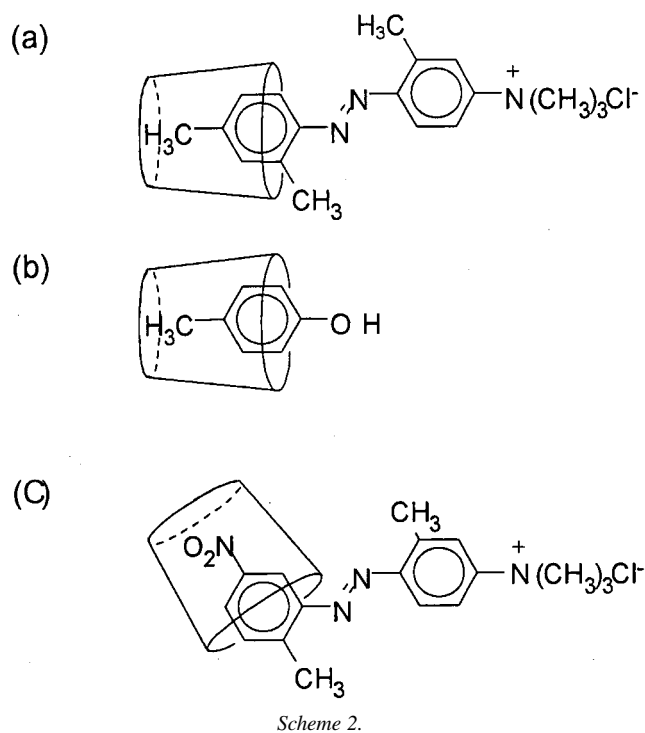
Figure 1. (a) Absorption spectra of MO (3×10^{-5} mol dm^{-3}) at various β-CD concentrations from 0 to (0.20 $\sim$ 2.17) $\times 10^{-3}$ mol dm^{-3}. (b) Determination of the inclusion equilibrium constant of MO with β-CD according to Equation (2).

Scheme 2.

drophobic interaction between the internal wall of CD and guest molecules is an important factor for the stability of inclusion complexes. In the case of **2** and **3**, it may safely be considered that the difference in the magnitude of the hydrophobic interaction is related to that of the contact area of the guest molecule for the internal wall of CD. The ratio ($=\Delta G_3^\circ / G_2^\circ$) of ΔG° for **2** and **3** is 0.88. The decrease in the contact area of the phenyl fragment of **3** in the hydrophobic CD cavity is ascribed to a shallow penetration caused by the steric hindrance of the methyl substituent R_2. This is supported from the CPK model studies.

Solvent effects on the association constants of MO

It is recognized that CDs in water form stable inclusion complexes with organic molecules owing to hydrophobic interactions in the CD cavity. In order to melt many organic compounds, however, an aqueous mixture with water-miscible organic compounds is often used for studying inclusion complexation of CD as a solvent. Thus, we have systematically examined solvent effects on the inclusion complexation in mixture of solvents. For demonstration purpose, we have determined the association constants of MO with β-CD and DM-β-CD in aqueous mixtures with water-miscible organic solvents such as dimethylsulfoxide (DMSO), acetonitrile, N,N-dimethylformamide (DMF), and acetone: dielectric constant $\epsilon = 48.9$ for DMSO, $\beta = 37.5$ for CH$_3$CN, $\epsilon = 36.7$ for DMF, and $\epsilon = 20.5$ for acetone.

In DM-β-CD, the methylation of O(2)H and O(6)H groups of the CD rim lengthens the CD cavity without any significant distortion of the ring, and enhances the inclusion ability [12]. In fact, the large association constant for the DM-β-CD-MO system in water was obtained ($K = 9540$ mol^{-1} dm^3), which is about 2.5 times larger than that of β-CD. The ratios of association constants of MO with β-CD and DM-β-CD in aqueous-organic solvents to that in water are listed in Table 2. As can be seen in Table 2, the addition of organic solvents causes the drastic decrease in the association constants of inclusion. The K values in water are about 10 times larger than those in mixtures containing 16% (V/V) organic solvents.

Connors *et al.* [5] interpreted the effects of organic cosolvents in α-CD complexes with MO in terms of competitive complexing of organic cosolvents. However, α-CD of which cavity is larger than that of α-CD does not form efficiently the inclusion complex with the relatively small organic compounds used as a solvent in this study [13]. In β-CD complexations, Örstan and Ross [14] suggested that

Table 2. The association constants for the inclusion complexation of MO in aqueous-organic mixture of solvents (organic solvents: $0 \sim 20\%$ (V/V))

CD	Solvent	$K/K(H_2O)$						
		0	4	8	12	16	20	$(V/V\%)$
β-CD	H_2O—DMSO	1	0.293	0.264	0.287	0.204	0.103	
		(72.0)[a]	(71.7)	(71.4)	(71.0)	(70.7)	(70.3)	
	H_2O—CH$_3$CN	1		0.226		0.086		
		(72.0)		(70.7)		(69.3)		
	H_2O—DMF	1		0.274		0.082		
		(72.0)		(71.3)		(70.4)		
DM-β-CD	H_2O—DMSO	1	0.444	0.387	0.315	0.196	0.099	
	H_2O—CH$_3$CN	1		0.320		0.098	0.077	
							(68.6)	
	H_2O-acetone	1	0.605	0.332		0.096	0.068	
		(72.0)	(71.5)	(71.0)		(69.8)	(69.2)	

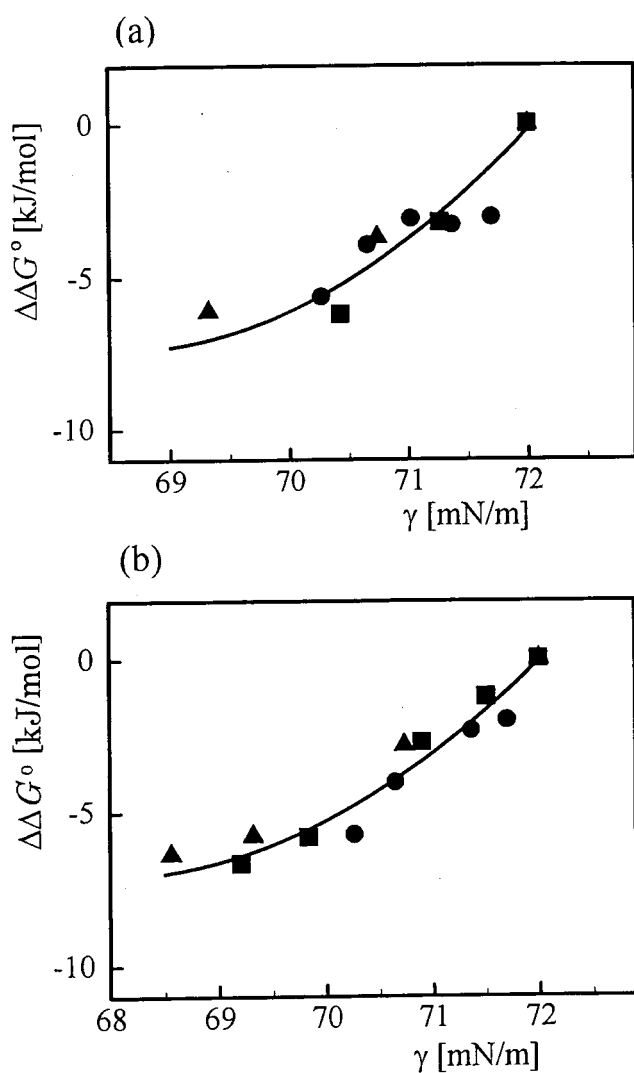[a] The calculated surface tension of solvent mixtures.

Figure 2. The relationship between $\Delta\Delta G°$ and surface tension values of solvent mixtures: (a) β-CD complexations in H_2O—DMSO (●), in H_2O—CH$_3$CN (▲), and in H_2O—DMF (■). (b) DM-β-CD complexations in H_2O—DMSO (●), in H_2O—CH$_3$CN (▲), and in H_2O-acetone (■).

surface tension is the major controlling factor, or more explicitly, that the change in molecular surface area exposed to solvent is a critical factor in determining complex stability. Similarly, Connors explained the medium effect on inclusion complex formation by using the solvent surface tension [15]. According to their suggestions, the free energy change for the complexation between CD and guest molecules is related to the surface area change during the reaction and to the macroscopic surface tension of solvent. It is instructive to examine the effect of surface tension on inclusion complexations. The difference in the stabilities of inclusion complexes in water and in solvent mixtures can be expressed by the difference in the free energy changes for the inclusion complex formation ($\Delta\Delta G° = RT \ln(K/K(H_2O)) = \Delta G°(H_2O) - \Delta G°$ (solvent mixtures)). Surface tension values of acetone, CH$_3$CN, DMF, DMSO, and water are 23.5, 28.7, 35.2, 42.9, and 72.0 mN/m (at 298 K), respectively [16]. Addition of acetonitrile and DMF, which have smaller surface tensions than water, to water decreases largely the surface tension. In fact, as shown in Table 2, the inclusion complex of MO with β-CD is more instable in H_2O—CH$_3$CN (and DMF) mixtures than in H_2O—DMSO mixtures containing a similar concentration of cosolvent. Since the surface tension values of solvent mixtures were not available, we tentatively estimated the number average surface tension values (γ) of solvent mixtures (Table 2). The $\Delta\Delta G°$ values for the inclusion complexation in solvent mixtures are plotted as a function of the surface tension of solvent mixtures in Figure 2, where two remarkable features are seen. One is that the stability of inclusion complex in solvent mixtures decreases as the surface tension values decrease. Another is that the plots of $\Delta\Delta G°$ against the γ values fall roughly on a same curve irrespective of the kinds of solvent mixtures. These findings strongly indicate that the CD inclusion complex formation is controlled by macroscopic surface tension of solvents. We expected a larger solvent effect for the inclusion complex formation with DM-β-CD having the enlarged hydrophobic cavity, while the significant difference in the solvent effects on the β-CD and DM-β-CD complex formations is not obtained (Figure 2(a) and (b)). The small

K values in aqueous-organic mixture of solvents are attributed to the similarity between the environment in solvent mixtures and microenvironment in the CD cavity, which is responsible for the macroscopic surface tension of solvent mixtures.

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