Inclusion Complexes of Cyclodextrins with Methylene Blue and Acid Orange 7 in Aqueous Solutions

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Upon the addition of Acid Orange 7 (AO7) to aqueous Methylene Blue (MB) solution buffered at pH 5.0, the absorption spectrum of MB is reduced in intensity, accompanied by a red shift of the absorption peak, indicating the formation of a complex between MB and AO7. From a simulation concerning the fluorescence intensity change of MB, the equilibrium constant for the formation of the 1 : 1 MB–AO7 complex has been estimated to be 66300 mol^{-1} dm^3. γ-Cyclodextrin (γ-CD) forms a 1 : 2 and a 1 : 1 inclusion complex with MB and AO7, respectively. From the absorbance changes, equilibrium constants for the formation of the 1 : 2 γ-CD–MB and 1 : 1 γ-CD–AO7 inclusion complexes have been evaluated to be 2.95×10^7 mol^{-2} dm^6 and 11000 ± 300 mol^{-1} dm^3, respectively. When γ-CD is added to MB solution containing AO7, the absorption band of MB is enhanced in intensity without an appearance of the MB dimer band, in contrast to MB solution without AO7. This finding indicates the formation of a ternary γ-CD–MB–AO7 inclusion complex. To determine the stoichiometry of the ternary inclusion complex, a continuous variation method using the absorption and fluorescence intensities has been performed under the conditions that the sum of the initial concentrations of γ-CD and AO7 has been kept constant. Both the results indicate the formation of a 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex. The equilibrium constant for the formation of the ternary inclusion complex from γ-CD and the 1 : 1 MB–AO7 inclusion complex has been evaluated to be 3.40×10^{10} mol^{-2} dm^6 from the fluorescence intensity change.

Commercially available cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven, and eight D-glucopyranose residues, which are called α-, β-, and γ-CD, respectively.[1] CDs are shaped like a truncated cone with a relatively hydrophobic cavity. Consequently, they can accommodate many kinds of organic compounds into their cavities to form inclusion complexes in aqueous solutions. Upon the formation of the inclusion complexes of CDs with guest molecules, their physicochemical properties are varied to some extents. Consequently, the incorporation of a guest molecule by CD often induces characteristic spectral changes of a guest; such changes have been observed in spectroscopy of electronic absorption, fluorescence, phosphorescence, etc.[2—8]

Tan et al. have reported that the complexation of γ-CD with an organic anion (2,6-naphthalenedicarboxylate) is greatly enhanced by the addition of an organic cation (2,6-bis(1-pyridiniomethyl)naphthalene dibromide) as a space-regulator.[9] The formation of a ternary inclusion complex among γ-CD, the organic anion, and the organic cation has been confirmed by employing ^{1}H NMR, induced circular dichroism, and UV absorption spectroscopy. From a standpoint of host-guest chemistry, the organic cation-organic anion complex is incorporated into the γ-CD cavity as a guest.

In a previous paper, we have reported that MB forms complexes with organic anions such as 1- and 2-naphthalenesulfonates.[10] The red shifts of absorption and fluorescence spectra of MB caused by the addition of the organic monoanion are attributed to the 1 : 1 complex formation between MB and the organic monoanion. Recently, we have investigated the interactions of thionine with an organic monoanion, 2-naphthalenesulfonate, in the absence and presence of CDs.[11] Thionine has been found to form a 1 : 1 complex with 2-naphthalenesulfonate. β- and γ-CDs form a 1 : 1 and a 1 : 2 host–guest inclusion complex with thionine, respectively. Addition of β-CD to thionine solutions containing 2-naphthalenesulfonate, in which the thionine–2-naphthalenesulfonate complex exists, results in the formation of an inclusion complex of β-CD with thionine. The thionine–2-naphthalenesulfonate complex is dissociated to the individual components upon the addition of β-CD. On the other hand, addition of γ-CD results in the formation of a 1 : 1 : 1 γ-CD–thionine–2-naphthalenesulfonate inclusion complex. This implies that within the γ-CD cavity the interactions between thionine and 2-naphthalenesulfonate are stronger than those between two thionine molecules.

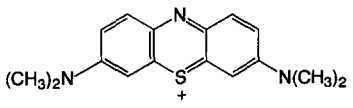
If two guest molecules in the ternary inclusion complex of γ-CD are bulkier than thionine and 2-naphthalenesulfonate, respectively, there is the possibility that the formation of a ternary inclusion complex is inhibited or that more than one γ-CD molecule participates in the formation of a ternary inclusion complex with the cationic and anionic guests. Thus, we investigated the interactions of β- and γ-CDs with two bulky guest molecules, Methylene Blue and Acid Orange 7, which are larger in size than thionine and 2-naphthalenesul-

fonate, respectively. In this paper, we report the formation of a ternary inclusion complex of γ-CD–Methylene Blue–Acid Orange 7, which includes two γ-CD molecules.

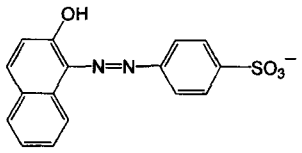
Experimental

β-Cyclodextrin (β-CD) obtained from Nakalai tesque, Inc., was recrystallized twice from water. γ-CD and Methylene Blue (MB), which were obtained from Wako Pure Chemical Industries, Ltd. and Tokyo Kasei Kogyo Co., Ltd., respectively, were used as received. Acid Orange 7 (AO7) from Tokyo Kasei Kogyo Co., Ltd. was recrystallized from ethanol (Chart 1.). Concentrations of MB were about 5.0×10^{-6} mol dm^{-3}. Samples were buffered at pH 5.0 using 0.01 mol dm^{-3} acetic acid and 0.01 mol dm^{-3} sodium acetate.

Absorption spectra were taken with a Shimadzu UV-260 spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-501 spectrofluorometer equipped with a cooled Hamamatsu R-943 photomultiplier. Fluorescence spectra were corrected for the spectral response of the fluorometer. Induced circular dichroism spectra were recorded on a JASCO J-400X spectropolarimeter interfaced to a JASCO DP-500 data processor. Spectroscopic measurements were made at 25±0.1°C, except for the induced circular dichroism spectra, which were measured at 25±2°C.



Methylene Blue (MB)



Acid Orange 7 (AO7)

Chart 1.

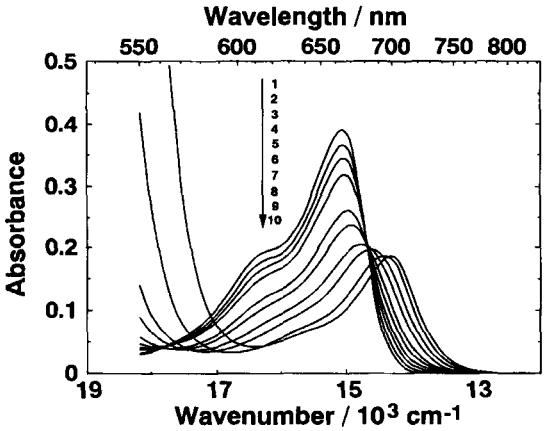


Fig. 1. Absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing various concentrations of AO7. Concentration of AO7: (1) 0, (2) 3.0×10^{-6}, (3) 6.0×10^{-6}, (4) 1.0×10^{-5}, (5) 2.0×10^{-5}, (6) 3.0×10^{-5}, (7) 6.0×10^{-5}, (8) 1.0×10^{-4}, (9) 3.0×10^{-4}, and (10) 1.0×10^{-3} mol dm^{-3}.

Results and Discussion

Complex Formation of MB with AO7 in Aqueous Solution. Figure 1 shows absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing various concentrations of AO7. In the AO7 concentration range below about 2×10^{-5} mol dm^{-3}, the addition of AO7 to MB solutions results in a slight red shift of the absorption peak of MB at 664 nm, accompanied by an isosbestic point at 680 nm and the reduction of the MB absorption intensity, indicating the formation of a complex between MB and AO7. As the AO7 concentration is further increased, the red shift of the absorption peak becomes more prominent. These findings suggest that at low AO7 concentrations a 1 : 1 MB–AO7 complex (MB·AO7) is formed and that at high AO7 concentrations the 1 : 1 MB–AO7 complex associates with an additional AO7 molecule to form a 1 : 2 MB–AO7 complex (MB·(AO7)$_2$).

$$\mathrm{MB} + \mathrm{AO7} \overset{K_1}{\rightleftharpoons} \mathrm{MB{\cdot}AO7}, \quad (1)$$

$$\mathrm{MB{\cdot}AO7} + \mathrm{AO7} \rightleftharpoons \mathrm{MB{\cdot}(AO7)_2}, \quad (2)$$

where K_1 is the equilibrium constant for the formation of the 1 : 1 MB–AO7 complex. At low AO7 concentrations where the isosbestic point is observed, the initial concentration of AO7 is comparable to that of MB. Consequently, a usual double reciprocal plot cannot be used to evaluate a reliable K_1 value, because such a plot is based on an equation derived under the condition that the concentration of AO7 is significantly higher than that of MB. Thus, we used a simulation method. The absorbance, A, of MB is given by

$$A = (\epsilon_0 + \epsilon_1 K_1[\mathrm{AO7}])[\mathrm{MB}]d, \quad (3)$$

where ϵ_0 and ϵ_1 are the molar absorption coefficients of free MB and MB·AO7, respectively, [AO7] and [MB] are the concentrations of free AO7 and free MB, respectively, and d is the path length (1.0 cm) of a cell. The initial concentrations of MB and AO7, $[\mathrm{MB}]_0$ and $[\mathrm{AO7}]_0$, are respectively represented by

$$[\mathrm{MB}]_0 = (1 + K_1[\mathrm{AO7}])[\mathrm{MB}], \quad (4)$$

$$[\mathrm{AO7}]_0 = (1 + K_1[\mathrm{MB}])[\mathrm{AO7}]. \quad (5)$$

From Eqs. 4 and 5, a quadratic equation for [MB] is derived:

$$K_1[\mathrm{MB}]^2 + (1 + K_1[\mathrm{AO7}]_0 - K_1[\mathrm{MB}]_0)[\mathrm{MB}] - [\mathrm{MB}]_0 = 0. \quad (6)$$

Assuming a K_1 value, one can calculate [MB] from Eq. 6. Insertion of the calculated [MB] to Eq. 5 affords [AO7]. Consequently, the absorbance is estimated by using a known ϵ_0 value (82800 mol^{-1} dm^3 cm^{-1} at 665 nm) and assumed K_1 and ϵ_1 values. From this simulation (not shown), a K_1 value and an ϵ_1 value were evaluated to be 23500 mol^{-1} dm^3 and 69.6 mol^{-1} dm^3 cm^{-1}, respectively, although the absorbance change was not too large. This K_1 value for AO7 is significantly greater than the K_1 values (400 and 7400

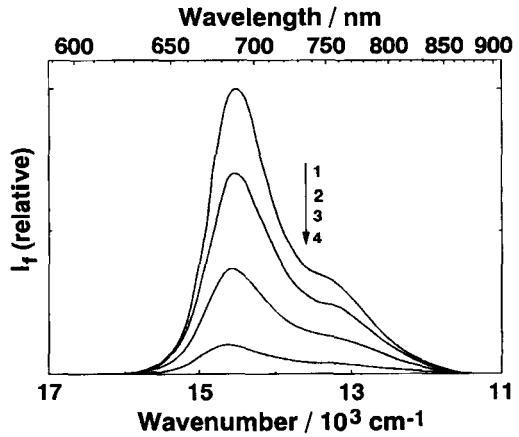


Fig. 2. Fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing various concentrations of AO7. Concentration of AO7: (1) 0, (2) 1.0×10^{-5}, (3) 3.0×10^{-5}, and (4) 1.0×10^{-4} mol dm^{-3}. λ_{ex} = 630 nm.

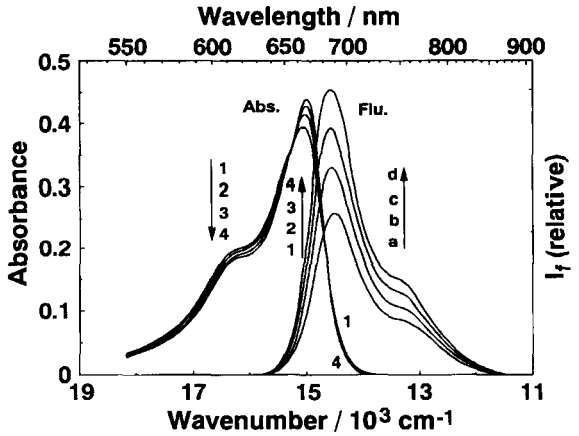


Fig. 3. Absorption and fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, (4) 1.0×10^{-2}, (a) 0, (b) 1.0×10^{-3}, (c) 3.0×10^{-3}, and (d) 1.0×10^{-2} mol dm^{-3}. λ_{ex} = 659 nm.

mol^{-1} dm^3) for 2-naphthalenesulfonate and 2-anthracenesulfonate,[10] suggesting that the interactions between MB and AO7 are stronger than those between MB and the aromatic sulfonates.

Figure 2 exhibits fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing various concentrations of AO7 in the low concentration range of AO7. When AO7 is added to an MB solution, the fluorescence intensity of MB is remarkably reduced relative to that of free MB, without a shift of the fluorescence peak. This finding also indicates the formation of the MB–AO7 complex. The fluorescence quenching of MB by the formation of the MB–AO7 complex may be due to the presence of an azo group in AO7, because the fluorescence of the MB–2-naphthalenesulfonate complex has been observed in aqueous solution.[10] Another azo dye, α-naphthol orange, was also found to quench the MB fluorescence in aqueous solution. This finding seems to support the quenching mechanism that an azo group is responsible for the fluorescence quenching in the MB–AO7 complex.

In the presence of AO7, the fluorescence intensity of MB is given as

$$I_f = (a_0 + a_1K_1[\text{AO7}])[\text{MB}], \quad (7)$$

where I_f, a_0, and a_1 are the fluorescence intensity of MB, an experimental constant including the fluorescence quantum yield of free MB, and that of the MB–AO7 complex, respectively. From a simulation (not shown) based on Eq. 7, which is similar to that for the absorbance change, the K_1 value was estimated to be 66300 mol^{-1} dm^3. This K_1 value is 2.8 times greater than that evaluated from the absorbance change. In this simulation, a_1, which is proportional to the fluorescence quantum yield of the MB–AO7 complex, has been estimated to be zero. This finding is consistent with the fact that the fluorescence spectra of MB has been strongly quenched by AO7. Since the ratio of the reduction in the fluorescence intensity upon the addition of AO7 is large compared to the corresponding absorbance change, the K_1 value evaluated from the fluorescence intensity change seems to be more reliable than that from the absorbance change.

Inclusion Complex Formation of MB with β- and γ-CD in Aqueous Solution. Figure 3 shows absorption and fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing various concentrations of β-CD. Upon the addition of β-CD, the intensity of the absorption maximum is enhanced with a sharpening of the absorption band. An isosbestic point is observed at 658 nm, indicating the formation of an inclusion complex of β-CD with MB. Taking into account the dimensions of the β-CD cavity and MB, the stoichiometry of the β-CD–MB complex is most likely 1 : 1.

$$\beta\text{-CD} + \text{MB} \overset{K_2}{\rightleftharpoons} \beta\text{-CD}\cdot\text{MB}. \quad (8)$$

Here, K_2 is the equilibrium constant for the formation of the 1 : 1 β-CD–MB inclusion complex.

As the β-CD concentration is increased, the fluorescence intensity is increased with a slight blue shift of the fluorescence peak (Fig. 3), indicating the formation of the β-CD–MB inclusion complex. From the fluorescence intensity change, the K_2 value can be evaluated according to the equation:

$$1/(I_f - I_f^0) = 1/b + 1/(bK_2[\beta\text{-CD}]_0), \quad (9)$$

where I_f, I_f^0, b, and $[\beta\text{-CD}]_0$ are the fluorescence intensities in the presence and absence of β-CD, a constant, and the initial concentration of β-CD, respectively. From a double reciprocal plot (not shown) based on Eq. 9, the K_2 value was evaluated to be 420±10 mol^{-1} dm^3, which is nearly the same as reported values (400 and 350 mol^{-1} dm^3).[12]

Figure 4 shows absorption and fluorescence spectra of MB (4.8×10^{-6} mol dm^{-3}) in aqueous solutions containing various concentrations of γ-CD. When γ-CD is added, the absorption band of the MB dimer centered at 609 nm is enhanced at the expense of the intensity of the monomer band at 664 nm. This finding indicates that the dimerization

of MB is induced by the addition of γ-CD. In other words, γ-CD forms an inclusion complex with two MB molecules. Hirai et al. have reported the formation of a 1 : 2 γ-CD–MB inclusion complex.[13]

$$\gamma\text{-CD} + 2\text{MB} \overset{K_3}{\rightleftharpoons} \gamma\text{-CD}\cdot(\text{MB})_2. \quad (10)$$

Here, K_3 is the equilibrium constant for the formation of the 1 : 2 γ-CD–MB inclusion complex (γ-CD·(MB)$_2$). In the γ-CD–MB system, the absorbance of MB is given as

$$A = (\epsilon_0 + \epsilon_2 K_3[\gamma\text{-CD}]_0[\text{MB}])[\text{MB}]d, \quad (11)$$

where ϵ_2 is the molar absorption coefficient of the 1 : 2 inclusion complex. The initial concentration of MB is represented by

$$[\text{MB}]_0 = [\text{MB}] + 2[\gamma\text{-CD}\cdot(\text{MB})_2]. \quad (12)$$

Using K_3, one derives a quadratic equation concerning [MB] from Eq. 11:

$$2K_3[\gamma\text{-CD}]_0[\text{MB}]^2 + [\text{MB}] - [\text{MB}]_0 = 0. \quad (13)$$

Assuming a K_3 value, [MB] can be calculated from Eq. 13. Employing a known ϵ_0 value at 665 nm and values of ϵ_2 and K_3 as parameters, we performed a simulation concerning the MB absorbance. Figure 5 exhibits the least-squares best fit curve for the MB absorbance, which has been calculated with an ϵ_2 value of 5.91×10^4 mol^{-1} dm^3 cm^{-1} and a K_3 value of 2.95×10^7 mol^{-2} dm^6. This K_3 value is about 6 times greater than the one reported (5.3×10^6 mol^{-2} dm^6).[13] It has been reported that γ-CD induces the dimerization of Acridine Orange, roccellin, Pyronine Y, etc.[13–15]

As shown in Fig. 4, the fluorescence intensity is reduced upon the addition of γ-CD, suggesting that the MB dimer within the γ-CD cavity is non-fluorescent. As in the case of the absorbance change, we evaluated a K_3 value to be 3.21×10^7 mol^{-2} dm^6 from a simulation using the fluorescence intensity change. This K_3 value is nearly the same as that estimated from the absorbance change.

Effects of β-CD on the Complexation between MB and AO7 in Aqueous Solution. Figure 6 shows absorption and fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing AO7 (1.0×10^{-4} mol dm^{-3}) and various concentrations of β-CD. When the β-CD concentration is increased, the absorption band is intensified, accompanied by a blue shift and a sharpening of the band. The fluorescence intensity is remarkably enhanced with the increase in the β-CD concentration. These findings suggest that the MB–AO7 complex is dissociated into the individual components, followed by the formation of an inclusion complex of β-CD with MB. At a β-CD concentration of 1.0×10^{-2} mol dm^{-3}, the absorbance of the peak is less than 0.4, whereas the absorbance at the same β-CD concentration in Fig. 3, where the absorption spectra in the absence of AO7

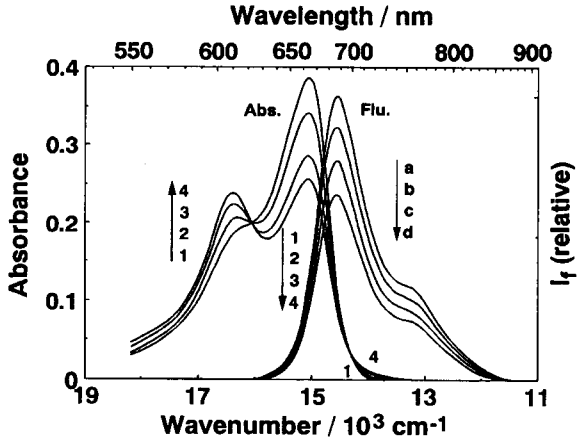


Fig. 4. Absorption and fluorescence spectra of MB (4.8×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 1.0×10^{-3}, (3) 4.0×10^{-3}, (4) 1.0×10^{-2}, (a) 0, (b) 1.0×10^{-3}, (c) 3.0×10^{-3}, and (d) 1.0×10^{-2} mol dm^{-3}. λ_{ex} = 622 nm.

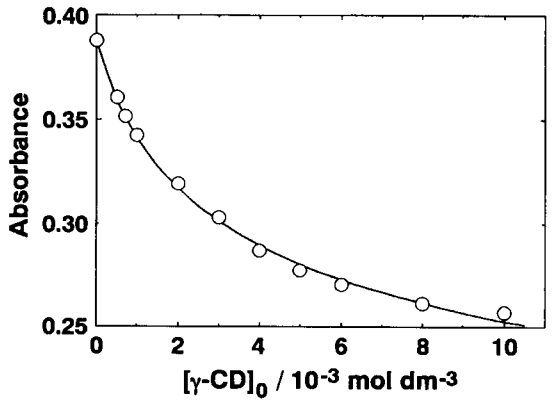


Fig. 5. Comparison of the best fit least-squares curve for the absorbance at 665 nm with the observed absorbance data for MB (4.8×10^{-6} mol dm^{-3}) solution containing γ-CD. The simulation curve was calculated with an ϵ_0 value of 82800 mol^{-1} dm^3 cm^{-1}, an ϵ value of 59100 mol^{-1} dm^3 cm^{-1}, and a K_3 value of 2.95×10^7 mol^{-2} dm^6.

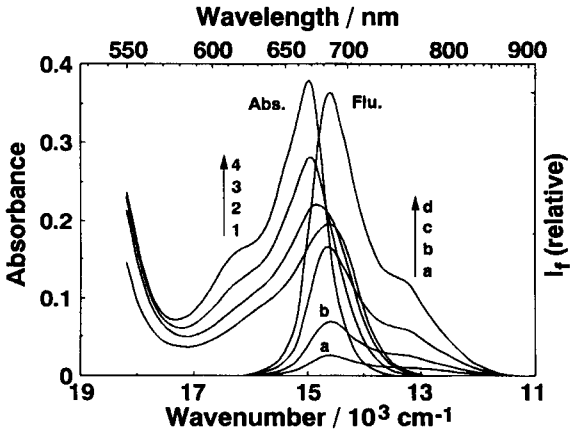


Fig. 6. Absorption and fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing AO7 (1.0×10^{-4} mol dm^{-3}) and various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, (4) 1.0×10^{-2}, (a) 0, (b) 1.0×10^{-3}, (c) 3.0×10^{-3}, and (d) 1.0×10^{-2} mol dm^{-3}. λ_{ex} = 620 nm.

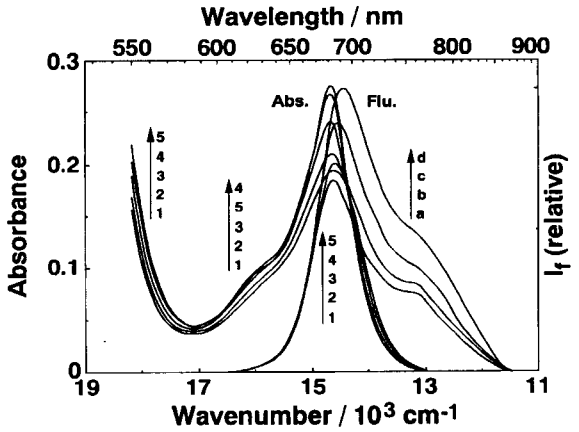


Fig. 7. Absorption and fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing AO7 (1.0×10^{-4} mol dm^{-3}) and various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 1.0×10^{-5}, (3) 3.0×10^{-5}, (4) 1.0×10^{-4}, (5) 3.0×10^{-4}, (a) 0, (b) 1.0×10^{-5}, (c) 3.0×10^{-5}, and (d) 1.0×10^{-4} mol dm^{-3}. λ_{ex} = 590 nm.

are shown, is greater than 0.4. The formation of an inclusion complex between β-CD and AO7 has been confirmed from the absorbance change upon the addition of β-CD to AO7 solution. Consequently, the smaller absorbance increase in the MB solution containing AO7 compared to the MB solution without AO7 is due to the consumption of β-CD for the formation of the inclusion complex between β-CD and AO7.

Effects of γ-CD on the Complexation between MB and AO7 in Aqueous Solution. Figure 7 illustrates absorption and fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing AO7 (1.0×10^{-4} mol dm^{-3}) and various concentrations of γ-CD. As the γ-CD concentration is increased below around 1×10^{-4} mol dm^{-3} of γ-CD, the absorption maximum is increased in intensity, accompanied by an isosbestic point at 696 nm. In spite of the addition of γ-CD, the MB dimer band at 609 nm does not appear. Consequently, the absorption spectral change upon the addition of γ-CD suggests the formation of an inclusion complex including a single MB molecule. There are two species which could be responsible for the spectral change; one species is a 1 : 1 : 1 γ-CD–MB–AO7 inclusion complex, and the other a 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex. To identify which species is responsible for the absorption spectral change shown in Fig. 7, we have applied a continuous variation method concerning the absorbance under the conditions that the sum of the initial concentrations of AO7 and γ-CD has been kept at 5.0×10^{-5} mol dm^{-3}. Figure 8 exhibits both the raw data of the absorbance at 680 nm and the corrected data that have been subtracted from values on a straight line. The corrected data are proportional to the amount of the γ-CD–MB–AO7 inclusion complex. Although there is some scatter of the data, the value of the corrected data goes through a maximum at a $[\gamma\text{-CD}]_0/([\gamma\text{-CD}]_0+[\text{AO7}]_0)$ value of about 0.67, indicating that the absorption spectral change shown in Fig. 7 is due to the formation of the 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex ($(\gamma\text{-CD})_2$·MB·AO7).

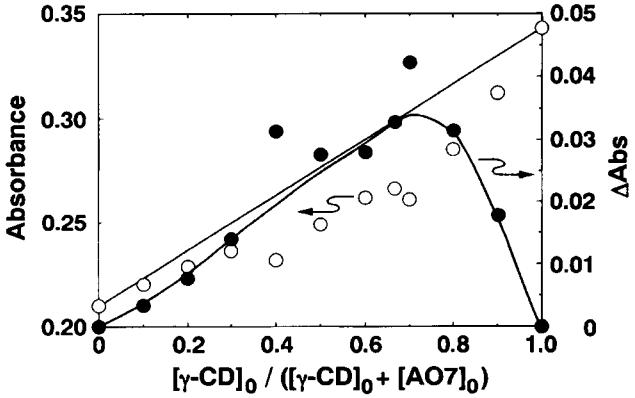


Fig. 8. Continuous variation plot for the absorbance of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing AO7 and γ-CD. The sum of the initial concentrations of γ-CD and AO7 is kept at 5.0×10^{-5} mol dm^{-3}. Open circles are raw data, and closed circles are corrected data that the value of the raw data is subtracted from a value on the straight line. λ_{obs} = 670 nm.

$$2\gamma\text{-CD} + \text{MB·AO7} \overset{K_4}{\rightleftharpoons} (\gamma\text{-CD})_2\text{·MB·AO7}. \quad (14)$$

A similar 2 : 1 : 1 inclusion complex including two CD molecules has been reported for the β-CD–1-cyanonaphthalene–anisole system.[16]

Figure 7 shows fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing AO7 (1.0×10^{-4} mol dm^{-3}) and various concentrations of γ-CD. When γ-CD is added, the fluorescence intensity is enhanced, accompanied by a red shift of the fluorescence peak. This is due to the formation of the inclusion complex of γ-CD, MB, and AO7. The enhancement also indicates no formation of the MB dimer within the γ-CD cavity, since the dimer is nonfluorescent as shown in Fig. 4. The fluorescence of MB, which forms a complex with AO7 in bulk water, is quenched as shown in Fig. 3. In contrast, the fluorescence of MB, which forms a complex with AO7 located within the γ-CD cavities, is enhanced (Fig. 7). The different behavior in the fluorescence intensity of the MB–AO7 complex bound to the γ-CD cavities is due likely to the constrained environment around the complex. The fluorescence quenching of MB by the azo group in AO7 is restricted within the narrow γ-CD cavities. The MB–AO7 complex most likely has a character of the charge transfer. The charge transfer interaction in the MB–AO7 complex seems to be responsible for the red-shift of the MB fluorescence in the AO7 solution containing γ-CD.

Using the fluorescence intensity, a continuous variation method has been performed under the conditions of $[\text{AO7}]_0+[\gamma\text{-CD}]_0 = 5.0\times10^{-5}$ mol dm^{-3} to further confirm the existence of the 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex. Figure 9 exhibits the raw data for the fluorescence intensity and the corrected data that have been subtracted from values on a

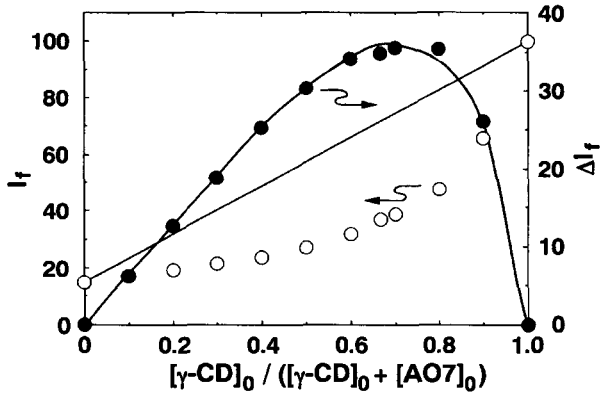


Fig. 9. Continuous variation plot for the fluorescence intensity of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 5.0) containing AO7 and γ-CD. The sum of the initial concentrations of γ-CD and AO7 is kept at 5.0×10^{-5} mol dm^{-3}. Open circles are raw data, and closed circles are corrected data that the value of the raw data is subtracted from a value on the straight line. λ_{ex} = 590 nm. λ_{obs} = 684 nm.

straight line. The curve for the corrected data reaches a maximum value at a $[\gamma\text{-CD}]_0/([\gamma\text{-CD}]_0+[AO7]_0)$ value of about 0.7. This finding provides additional evidence for the formation of the 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex. In the γ-CD–thionine–2-naphthalenesulfonate system, a 1 : 1 : 1 γ-CD–thionine–2-naphthalenesulfonate inclusion complex is formed.[11] The molecular size of MB is larger than that of thionine. In addition, AO7 is considerably larger in size than 2-naphthalenesulfonate. Consequently, the greater molecular dimensions of MB and AO7 cause the involvement of the second γ-CD molecule in the ternary γ-CD–MB–AO7 inclusion complex.

In addition to the two equilibria which are represented by Eqs. 1 and 14, in the γ-CD–MB–AO7 system, we have to take into account an equilibrium concerning the formation of an inclusion complex between γ-CD and AO7. It has been reported that, at high concentrations of AO7, γ-CD forms a 1 : 2 and a 2 : 2 γ-CD–AO7 inclusion complex.[17,18] At low concentrations below about 1×10^{-3} mol dm^{-3} of AO7, however, an isosbestic point was found to be at 535 nm. A usual double reciprocal plot for the absorbance, which is analogous to a plot based on Eq. 9, exhibited a straight line, indicating the formation of a 1 : 1 γ-CD–AO7 inclusion complex. This finding is consistent with the results of relaxation times obtained from kinetic studies.[17]

$$\gamma\text{-CD} + \text{AO7} \overset{K_5}{\rightleftharpoons} \gamma\text{-CD}\cdot\text{AO7}. \quad (15)$$

Here, K_5 is the equilibrium constant for the formation of the 1 : 1 γ-CD–AO7 inclusion complex (γ-CD·AO7). From the absorbance change by the addition of AO7, 11000±300 mol^{-1} dm^3 was evaluated as the K_5 value (not shown). To evaluate the K_4 value, we simulated the absorbance change upon the addition of γ-CD to MB solutions containing 1.0×10^{-4} mol dm^{-3} of AO7. The absorbance of MB is given as

$$A = (\epsilon_0 + \epsilon_1 K_1[AO7] + \epsilon_3 K_1 K_4[\gamma\text{-CD}]^2[AO7])[MB]d, \quad (16)$$

where ϵ_3 is the molar absorption coefficient of the 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex. The initial concentrations of MB, AO7, and γ-CD are respectively represented by

$$[MB]_0 = (1 + K_1[AO7] + K_1K_4[\gamma\text{-CD}]^2[AO7])[MB], \quad (17)$$

$$[AO7]_0 = (1 + K_1[MB] + K_5[\gamma\text{-CD}] + K_1K_4[\gamma\text{-CD}]^2[MB])[AO7], \quad (18)$$

$$[\gamma\text{-CD}]_0 = (1 + K_5[AO7])[\gamma\text{-CD}] + K_1K_4[MB][AO7][\gamma\text{-CD}]^2. \quad (19)$$

Combining Eqs. 17 and 18, the following cubic equation for $[\gamma$-CD] is derived:

$$K_1K_4[MB][\gamma\text{-CD}]^3 + (K_5 + K_1K_4[MB] - K_1K_4[\gamma\text{-CD}]_0[MB]) \times[\gamma\text{-CD}]^2 + (1 + K_1[MB] + K_5[AO7]_0 - K_5[\gamma\text{-CD}]_0)[\gamma\text{-CD}] -(1 + K_1[MB])[\gamma\text{-CD}]_0 = 0. \quad (20)$$

Assuming a K_4 value and [MB] to be $[MB]_0$, one can calculate $[\gamma$-CD] from Eq. 20. By inserting the calculated $[\gamma$-CD] to Eq. 18, one may estimate [AO7]. By use of the evaluated values of $[\gamma$-CD] and [AO7], [MB] is estimated from Eq. 17. When we use the value of [MB] thus obtained and the assumed K_4 value, a new value of $[\gamma$-CD] is calculated from Eq. 20. This procedure has been repeated until the values of $[\gamma$-CD], [AO7], and [MB] have been converged. Because these concentrations are estimated for the assumed K_4 value, the absorbance corresponding to the K_4 value can be evaluated from Eq. 16. Figure 10 depicts the best fit least-squares curve for the absorbance observed at 682 nm, with the known K_1 (66300 mol^{-1} dm^3), ϵ_0 (36000 mol^{-1} dm^3 cm^{-1}), variables of $K_4 = 7.61\times10^9$ mol^{-2} dm^6, ϵ_1 = 39900 mol^{-1} dm^3 cm^{-1}, and ϵ_3 = 53300 mol^{-1} dm^3 cm^{-1}. For the fluorescence intensity change, a similar simulation (not shown) was performed. From the simulation, a K_4 value was estimated to be 3.40×10^{10}

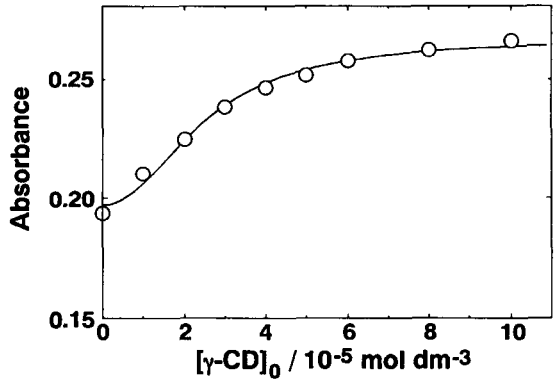


Fig. 10. Comparison of the best fit least-squares curve for the absorbance at 682 nm with the observed absorbance data. The simulation curve was calculated with an ϵ_0 value of 36000 mol^{-1} dm^3 cm^{-1}, an ϵ_1 value of 39900 mol^{-1} dm^3 cm^{-1}, an ϵ_3 value of 53300 mol^{-1} dm^3 cm^{-1}, and a K_4 value of 9.39×10^9 mol^{-1} dm^3. $[MB]_0 = 5.0\times10^{-6}$ mol dm^{-3}. $[AO7]_0 = 1.0\times10^{-4}$ mo dm^{-3}.

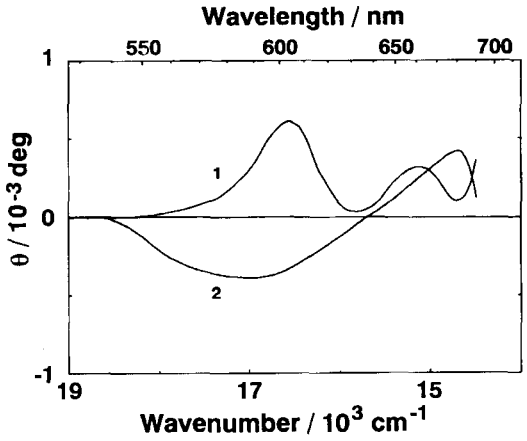


Fig. 11. Induced circular dichroism spectra of MB (1.0×10^{-5} mol dm^{-3}). Curve 1: in aqueous solution (pH 5.0) containing γ-CD (1.0×10^{-2} mol dm^{-3}). Curve 2: in aqueous solution (pH 5.0) containing both γ-CD (3.0×10^{-4} mol dm^{-3}) and AO7 (1.0×10^{-4} mol dm^{-3}).

mol^{-2} dm^6, which is 4.5 times greater than the K_4 value obtained from the simulation concerning the absorbance change. The K_4 value obtained from the fluorescence intensity change seems to be more reliable than that obtained from the absorbance change, because the absorbance change is relatively small.

Induced Circular Dichroism Spectra of the MB Inclusion Complexes. Figure 11 illustrates an induced circular dichroism (icd) spectrum of MB (1.0×10^{-5} mol dm^{-3}) in γ-CD (1.0×10^{-2} mol dm^{-3}) solution and that in γ-CD (3.0×10^{-4} mol dm^{-3}) solution containing AO7 (1.0×10^{-2} mol dm^{-3}). The icd spectrum of MB in γ-CD solution exhibits a positive signal in the wavelength range from 550 to 690 nm. This icd spectrum is similar to that observed by Hirai et al.[13] On the other hand, the icd spectrum for solutions containing both γ-CD and AO7 exhibits the negative and positive signals in the wavelength ranges of 540—640 and 640—690 nm, respectively. In γ-CD solutions without AO7, the 1 : 2 γ-CD–MB inclusion complex is formed, while the 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex is formed in γ-CD solution with AO7. Therefore, the orientation of an MB molecule in the ternary inclusion complex relative to the γ-CD molecules is definitely different from that in the 1 : 2 γ-CD–MB inclusion complex. The orientation of MB relative to γ-CD in the 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex is likely tilted compared to that in the 1 : 2 γ-CD–MB inclusion complex.

Conclusions

MB forms a complex with AO7 in aqueous solution (pH 5.0). In aqueous solutions, β- and γ-CDs form a 1 : 1 and a 1 : 2 CD–MB inclusion complex, respectively. This implies that γ-CD induces the dimerization of MB. When β-CD is added to an MB solution containing AO7, the MB–AO7 complex is dissociated to the individual components, MB and AO7, which form inclusion complexes with β-CD. This suggests that MB and AO7 cannot enter the β-CD cavity (cavities) as the MB–AO7 complex due to the small β-CD cavity size. On the other hand, the addition of γ-CD results in the formation of the 2 : 1 : 1 γ-CD–MB–AO7 inclusion complex, indicating that two γ-CD molecules encapsulate the MB–AO7 complex. Although the γ-CD cavity is wider than the β-CD cavity, a single γ-CD molecule is not large enough to incorporate the MB–AO7 complex. In the case of a thionine–2-naphthalenesulfonate complex, a single γ-CD molecule accommodates the thionine–2-naphthalenesulfonate complex.[11] The difference in the number of γ-CD molecules involved in the ternary inclusion complexes is ascribed to the bulkiness of the complex located within the γ-CD cavity (cavities).

The equilibrium constants for the formation of the relevant complexes have been evaluated from the absorbance and/or fluorescence intensity changes. The K_1 value for the MB–AO7 complex is significantly greater than that for the MB–2-naphthalenesulfonate complex,[10] indicating stronger interactions between MB and AO7. In bulk water, the MB fluorescence is quenched in the MB–AO7 complex. An azo group in AO7 may be responsible for the fluorescence quenching. The MB fluorescence in the MB–AO7 complex is enhanced by the formation of the γ-CD–MB–AO7 inclusion complex. This difference in the fluorescence behavior of MB is due likely to the degree of freedom around the MB–AO7 complex. In the γ-CD–MB–AO7 complex, the quenching of the MB fluorescence by the azo group in AO7 is obstructed by the cavity wall of γ-CD, resulting in the fluorescence enhancement compared to bulk water containing AO7.

The charge transfer interaction most likely contributes to the formation of the MB–AO7 complex. The interactions between the guests and CDs seem to involve the hydrophobic interactions. To further understand the interactions between these component molecules and those between CD and the MB–AO7 complex, it is necessary to estimate thermodynamic quantities concerning the relevant equilibria. Because the inclusion phenomenon of CDs is generally related to the dimensions of the CD cavity and guest molecules, it is also important to examine the behavior of organic cation–organic anion complexes different in size from the MB–AO7 complex.

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