Inclusion Complexes of Cyclodextrins with Methylene Blue and α-Naphthol Orange and Temperature Dependence of the Inclusion Complexation in Aqueous Solutions

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Methylene Blue (MB) has been found to form a complex with α-Naphthol Orange (αNO) in aqueous solution. From a simulation concerning the fluorescence quenching of MB by αNO, an equilibrium constant for the formation of the 1:1 MB–αNO complex has been estimated to be 79900 $\mathrm{mol^{-1}\,dm^{3}}$. Upon the addition of γ-CD to MB solution containing αNO, the absorption of the MB monomer band is enhanced in intensity without an appearance of the MB dimer band, indicating the formation of a ternary γ-CD–MB–αNO inclusion complex. To determine a stoichiometry of the ternary inclusion complex, a continuous variation method using the fluorescence intensities has been performed under the condition that the sum of the initial concentrations of γ-CD and αNO has been kept constant. The result of the continuous variation method indicates the formation of a 2:1:1 γ-CD–MB–αNO inclusion complex. The equilibrium constant for the formation of the γ-CD–MB–αNO inclusion complex from γ-CD and the 1:1 MB–αNO complex has been evaluated to be $1.86 \times 10^{9}\ \mathrm{mol^{-2}\,dm^{6}}$ from a simulation concerning the absorbance change. From the temperature dependence of the equilibrium constants, ΔH and ΔS values have been evaluated for the γ-CD–MB–αNO and γ-CD–MB–Acid Orange 7 systems. The ΔH and ΔS values for the formation of the γ-CD–MB–αNO inclusion complex have been positive in sign, in contrast to those for the formation of the MB–αNO and γ-CD–αNO complexes.

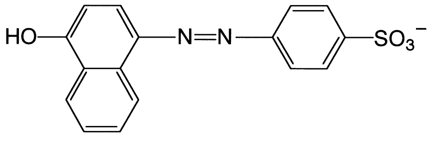
Cyclodextrins (CDs) are cyclic oligosaccharides made of D-glucopyranose residues. CDs having six, seven, and eight D-glucopyranose residues are called α-, β-, and γ-CD, respectively.[1] Because the cavity of torus-shaped CD is relatively hydrophobic, it has an ability of accommodating various kinds of organic compounds into its cavity to form inclusion complexes in aqueous solutions. The formation of the inclusion complexes of CDs with guest molecules affects the physicochemical properties to varying extents. In spectra of electronic absorption, fluorescence, phosphorescence, and so on, therefore, one can often observe characteristic spectral changes of a guest molecule upon the addition of CD.[2–10]

The interactions of an organic cation–organic anion complex with CD have firstly been investigated by Tan et al.[11] They 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. 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.

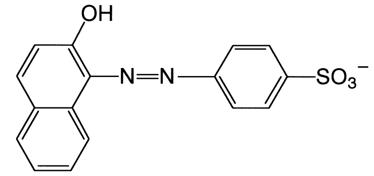
Previously, we have reported that Methylene Blue (MB) forms complexes with organic anions such as 1- and 2-naphthalenesulfonates.[12] The red shifts of absorption and fluorescence spectra of MB caused by the addition of the organic monoanion are attributed to the 1:1 complex formation between MB and the organic monoanion. Recently, we have investigated the interactions of thionine (organic cation) with 2-naphthalenesulfonate (organic anion), in the absence and presence of CDs.[13] 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 results in the formation of an inclusion complex of β-CD with thionine. Consequently, the thionine–2-naphthalenesulfonate complex is dissociated into the individual components upon the addition of β-CD. On the other hand, the 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.

We were interested in the size effects of an organic cation–organic anion complex on the complexation with CDs. MB and Acid Orange 7 (AO7) are bulkier than thionine and 2-naphthalenesulfonate, respectively (Chart 1). Because we have found the formation of a complex of MB with AO7 in aqueous solution, the interactions between the MB–AO7 complex with CDs have been examined.[14] The MB–AO7 complex is incorporated into the γ-CD cavities to form a 2:1:1 γ-CD–MB–AO7 inclusion complex. The addition of β-CD results in the dissociation of the MB–AO7 complex to individual components which are bound to β-CD to form 1:1 inclusion complexes.

Furthermore, it has been found that MB forms a complex



α-Naphthol Orange (αNO)



Acid Orange 7 (AO7)

Chart 1.

with tetrakis(4-sulfonatophenyl)porphyrin in aqueous solution.[15] For tetrakis(4-sulfonatophenyl)porphyrin, CD accommodates a sulfonatophenyl moiety.[10g,16] Consequently, the binding site towards CD is rather less bulky, although tetrakis(4-sulfonatophenyl)porphyrin is a bulky molecule. However, *γ*-CD does not form a ternary inclusion complex with MB and tetrakis(4-sulfonatophenyl)porphyrin.[15]

We have found that MB forms a complex with *α*-Naphthol Orange (*α*NO) (Chart 1). *α*NO is a positional isomer of AO7, in which the position of a hydroxy group is different from that of *α*NO. Using *α*NO as an organic anion in an organic cation–organic anion complex, we have examined the effects of the molecular structure of an organic anion on the complexation reaction of CDs. *α*NO has been found to form a ternary inclusion complex with *γ*-CD and MB. Equilibrium constants for the formation of the binary and ternary complexes have been estimated. In addition, we have evaluated the thermodynamic quantities for the formation of the MB–*α*NO complex, the *γ*-CD–*α*NO inclusion complex, and the *γ*-CD–MB–*α*NO inclusion complex from the temperature dependence of the equilibrium constants. For comparison, we have also estimated the thermodynamic quantities for the formation of the MB–AO7 complex, the *γ*-CD–AO7 inclusion complex, and the *γ*-CD–MB–AO7 inclusion complex.

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 Kogyou Co., Ltd., respectively, were used as received. *α*-Naphthol Orange (*α*NO) and Acid Orange 7 (AO7) from Tokyo Kasei Kogyou Co., Ltd. were recrystallized from ethanol. Concentrations of MB were 5.0×10^{-6} mol dm^{-3}, except for measurements of induced circular dichroism spectra, in which the MB concentration was 1.0×10^{-5} mol dm^{-3}. Samples were buffered at pH 4.9 using 0.01 mol dm^{-3} acetic acid and 0.01 mol dm^{-3} sodium acetate.

Absorption spectra were recorded on 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 taken with 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, and the evaluation of the thermodynamic quantities, for which measurements were performed at 4, 12, 25, and 35 °C.

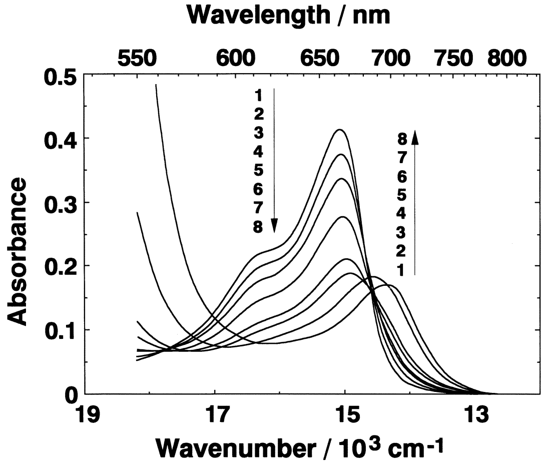


Fig. 1. Absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 4.9) containing various concentrations of *α*NO. Concentration of *α*NO: (1) 0, (2) 3.0×10^{-6}, (3) 7.0×10^{-6}, (4) 1.0×10^{-5}, (5) 2.0×10^{-5}, (6) 3.0×10^{-5}, (7) 1.0×10^{-4}, and (8) 3.0×10^{-4} mol dm^{-3}.

Results and Discussion

Complex Formation of MB with *α*NO in Aqueous Solution. Figure 1 shows absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing various concentrations of *α*NO. At low concentrations of *α*NO below around 7×10^{-6} mol dm^{-3}, the addition of *α*NO 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 *α*NO. When the *α*NO concentration is further raised, the red shift of the absorption peak becomes more prominent, with disappearance of the isosbestic point. These findings suggest that at low *α*NO concentrations a 1:1 MB–*α*NO complex (MB·*α*NO) is formed and that at high *α*NO concentrations the 1:1 MB–*α*NO complex associates with an additional *α*NO molecule to form a 1:2 MB–*α*NO complex (MB·(*α*NO)$_2$).

$$\mathrm{MB} + \alpha\mathrm{NO} \overset{K_1}{\rightleftarrows} \mathrm{MB}\cdot\alpha\mathrm{NO}, \tag{1}$$

$$\mathrm{MB}\cdot\alpha\mathrm{NO} + \alpha\mathrm{NO} \rightleftarrows \mathrm{MB}\cdot(\alpha\mathrm{NO})_2, \tag{2}$$

where K_1 is the equilibrium constant for the formation of the 1:1 MB–*α*NO complex. At low *α*NO concentrations where the isosbestic point is observed, the initial concentration of *α*NO is comparable to that of MB. Consequently, the 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 *α*NO is significantly higher than that of MB. As in the case of AO7, we used a simulation method to evaluate the K_1 value. The absorbance, A, of MB is given by

$$A = (\varepsilon_0 + \varepsilon_1 K_1[\alpha\text{NO}])[\text{MB}]d, \tag{3}$$

where ε_0 and ε_1 are the molar absorption coefficients of free MB and the MB–αNO complex, respectively, [αNO] and [MB] are the concentrations of free αNO and free MB, respectively, and d is the path length (1.0 cm) of a cell. The initial concentrations of MB and αNO, $[\text{MB}]_0$ and $[\alpha\text{NO}]_0$, are respectively represented by

$$[\text{MB}]_0 = (1 + K_1[\alpha\text{NO}])[\text{MB}], \tag{4}$$

$$[\alpha\text{NO}]_0 = (1 + K_1[\text{MB}])[\alpha\text{NO}]. \tag{5}$$

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

$$K_1[\text{MB}]^2 + (1 + K_1[\alpha\text{NO}]_0 - K_1[\text{MB}]_0)[\text{MB}] - [\text{MB}]_0 = 0. \tag{6}$$

Assuming a K_1 value, one can calculate [MB] from Eq. 6. Insertion of the calculated value of [MB] to Eq. 5 affords [αNO]. Thus, using a known ε_0 value (83800 mol^{-1} dm^3 cm^{-1} at 665 nm) and assumed K_1 and ε_1 values, the absorbance is estimated from Eq. 3. From this simulation (not shown), a K_1 value and an ε_1 value were evaluated to be 79700 mol^{-1} dm^3 and 29500 mol^{-1} dm^3 cm^{-1}, respectively, although the absorbance change was not too large. The error for the K_1 value was estimated to be less than 15%. This K_1 value for αNO is more than three times greater than that for AO7 (23500 mol^{-1} dm^3), indicating that the interactions between MB and αNO are stronger than those between MB and AO7.[14] The K_1 values for αNO and AO7 are remarkably greater than those (400 and 7400 mol^{-1} dm^3) for 2-naphthalenesulfonate and 2-anthracenesulfonate,[12] suggesting that the interactions between MB and the azo dyes (αNO 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 several concentrations of αNO in the low concentration range of αNO. When αNO is added to an MB solution, the fluorescence intensity of MB is reduced relative to that of free MB, without a shift of the fluorescence band. This finding also indicates the formation of the MB–αNO complex. Similar fluorescence quenching of MB has been observed for AO7.[14] The fluorescence quenching by the formation of the MB–αNO complex may be due to unshared electron pairs of an azo group in αNO, because the fluorescence of the MB–2-naphthalenesulfonate complex has been observed in aqueous solution.[12]

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

$$I_\text{f} = (a_0 + a_1 K_1[\alpha\text{NO}])[\text{MB}], \tag{7}$$

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–αNO complex, respectively.

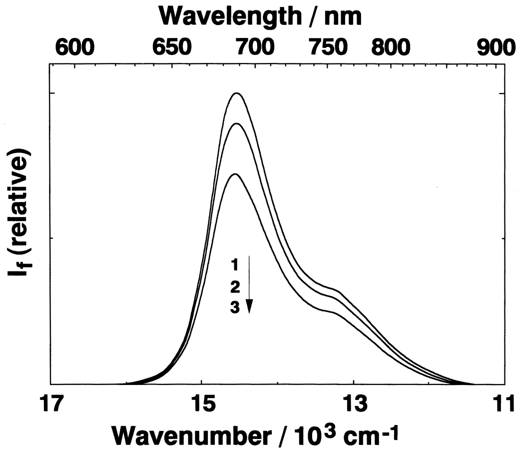


Fig. 2. Fluorescence spectra of MB (5.0 × 10^{-6} mol dm^{-3}) in aqueous solutions (pH 4.9) containing several concentrations of αNO. Concentration of αNO: (1) 0, (2) 3.0 × 10^{-6}, and (3) 7.0 × 10^{-6} mol dm^{-3}. λ_ex = 625 nm.

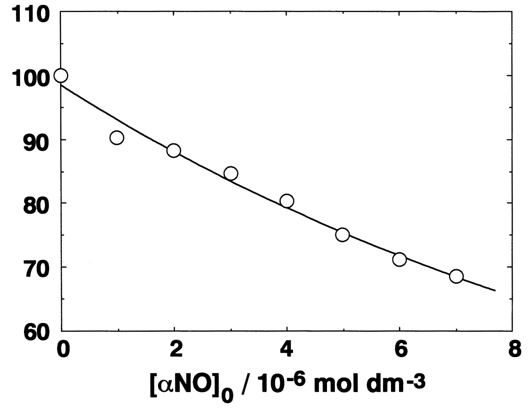


Fig. 3. Comparison of the best fit least-squares simulation curve for the fluorescence intensity with the observed fluorescence intensity data for MB (5.0 × 10^{-6} mol dm^{-3}) solution containing αNO. The simulation curve was calculated with an a_0 value of 1.97 × 10^7 mol^{-1} dm^3, an a_1 value of 2.54 × 10^3 mol^{-1} dm^3, and a K_1 value of 7.99 × 10^4 mol^{-1} dm^3. λ_ex = 555 nm. λ_obs = 685 nm.

From a simulation based on Eqs. 4–7, which is similar to that for the absorbance change, one can evaluate a K_1 value. In the simulation, the absorption of αNO at the excitation wavelength is negligible, because the αNO concentrations of less than 7 × 10^{-6} mol dm^{-3} are very low. Figure 3 illustrates the best-fit least-squares simulation curve, which has been calculated using a_0 = 1.97 × 10^7, a_1 = 2.54 × 10^3, and K_1 = 79900 mol^{-1} dm^3, together with the observed fluorescence intensity data. This K_1 value is in good agreement with the K_1 value estimated from the simulation based on the absorbance change. Although the evaluated a_1 value is not zero, it is four orders of magnitude less than the a_0 value, being consistent with the fluorescence quenching of MB in the presence of αNO.

Inclusion Complex Formation of αNO with γ-CD in Aqueous Solution. Figure 4 shows absorption spectra of αNO (3.0 × 10^{-5} mol dm^{-3}) in aqueous solutions containing various concentrations of γ-CD. As the γ-CD concentration is

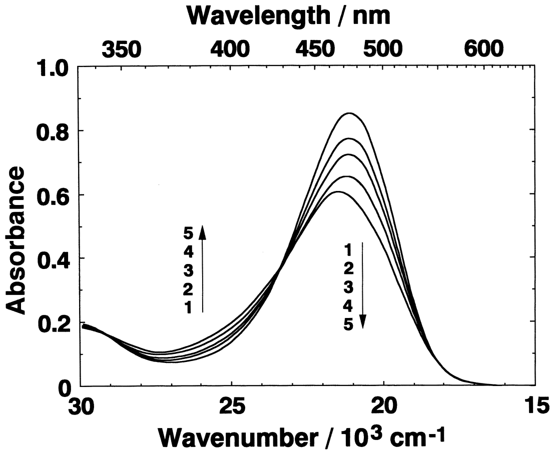


Fig. 4. Absorption spectra of αNO (3.0×10^{-5} mol dm^{-3}) in aqueous solutions (pH 4.9) containing various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 3.0×10^{-4}, (3) 7.0×10^{-4}, (4) 2.0×10^{-3}, and (5) 1.0×10^{-2} mol dm^{-3}.

increased, the intensity of the absorption maximum is decreased with a slight blue shift of the absorption maximum. In addition, an isosbestic point is observed at 430 nm. These findings indicate the formation of an inclusion complex of γ-CD with αNO. When a 1:1 γ-CD–αNO inclusion complex is formed, a usual double reciprocal plot would show a straight line:

$$1/(A - A_0) = 1/b + 1/(bK_2[\gamma\text{-CD}]_0), \qquad (8)$$

where A, A_0, b, K_2, and $[\gamma\text{-CD}]_0$ are the absorbances in the presence and absence of γ-CD, a constant, the equilibrium constant for the formation of the 1:1 γ-CD–αNO inclusion complex, and the initial concentration of γ-CD, respectively. The double reciprocal plot for the γ-CD$-\alpha$NO system exhibits a straight line, with a K_2 value of 1380 ± 30 mol^{-1} dm^3 (not shown). Consequently, this suggests that the stoichiometry of the γ-CD–αNO inclusion complex is 1:1.

Effects of γ-CD on the Complexation between MB and αNO in Aqueous Solution. Figure 5 illustrates absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing αNO (2.0×10^{-5} mol dm^{-3}) and various concentrations of γ-CD. When the γ-CD concentration is increased, the MB monomer band is increased in intensity, accompanied by a slight red shift of the absorption maximum. At a γ-CD concentration of 2.0×10^{-4} mol dm^{-3}, the MB absorption band is sharpened compared to that at a low γ-CD concentration. It has been reported that γ-CD forms a 1:2 inclusion complex with MB, in which MB exists as a dimer.[14,17] Over the whole γ-CD concentration range examined, the MB dimer band at 609 nm does not appear, indicating that a 1:2 γ-CD–MB inclusion complex is not formed upon the addition of γ-CD. Therefore, the absorption spectral change by the addition of γ-CD suggests that an inclusion complex is formed among γ-CD, MB, and αNO, and that only one MB molecule is included into the γ-CD–MB–αNO inclusion complex. The sharpening of the MB absorption band at a γ-CD concentration of 2.0×10^{-4} mol dm^{-3} is due to the formation of the γ-CD–MB–αNO inclusion complex. The environment around an MB molecule located within the ternary inclusion complex is less polar than that located within bulk water. This may explain the sharpening of the MB absorption band.

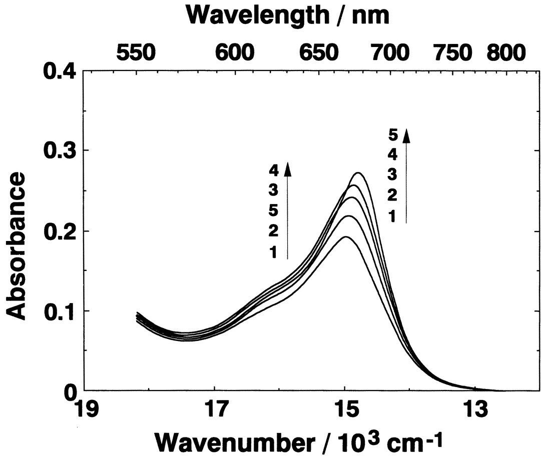


Fig. 5. Absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 4.9) containing αNO (2.0×10^{-5} mol dm^{-3}) and various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 2.0×10^{-5}, (3) 4.0×10^{-5}, (4) 6.0×10^{-5}, and (5) 2.0×10^{-4} mol dm^{-3}.

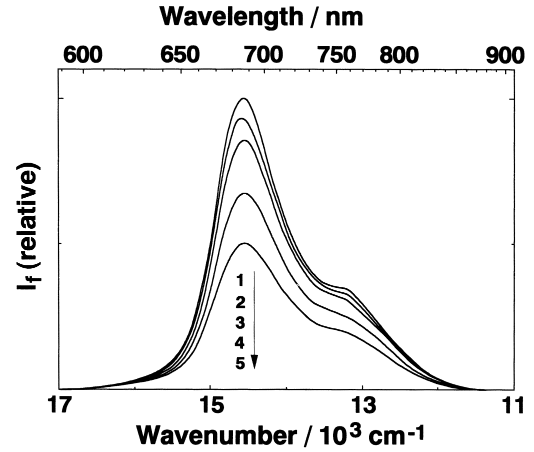


Fig. 6. Fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 4.9) containing αNO (2.0×10^{-5} mol dm^{-3}) and various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 2.0×10^{-5}, (3) 4.0×10^{-5}, (4) 1.0×10^{-4}, and (5) 2.0×10^{-4} mol dm^{-3}. $\lambda_{ex} = 580$ nm.

Figure 6 depicts fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing αNO (2.0×10^{-5} mol dm^{-3}) and various concentrations of γ-CD. With an increase in the γ-CD concentration, the MB fluorescence intensity is reduced without a shift of the fluorescence peak wavelength. The dimerization of MB within the γ-CD cavity, i.e., the formation of a 1:2 γ-CD–MB inclusion complex, leads to the fluorescence quenching of MB.[14] In an MB solution containing αNO, the MB fluorescence is reduced by the addition of γ-CD, although the formation of the MB dimer is not observed (Fig. 5). Consequently, the reduction in the fluorescence intensity of MB indicates the formation of the ternary in-

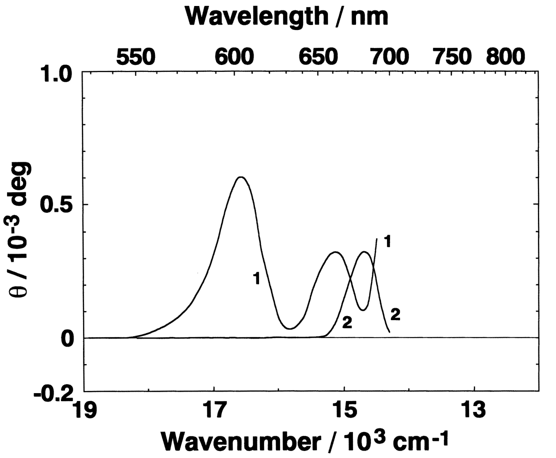


Fig. 7. Induced circular dichroism spectra of MB (1.0×10^{-5} mol dm^{-3}). Curve 1: in aqueous solution (pH 4.9) containing γ-CD (1.0×10^{-2} mol dm^{-3}). Curve 2: in aqueous solution (pH 4.9) containing both γ-CD (1.0×10^{-4} mol dm^{-3}) and αNO (3.0×10^{-5} mol dm^{-3}).

clusion complex among γ-CD, MB, and αNO. The fluorescence reduction in the γ-CD–MB–αNO inclusion complex is in marked contrast to the enhancement of the MB fluorescence in the γ-CD–MB–AO7 inclusion complex and that of the thionine fluorescence in the γ-CD–thionine–2-naphthalenesulfonate inclusion complex.[13,14] In the ternary inclusion complexes of γ-CD–MB–αNO and γ-CD–MB–AO7, the relative orientation of MB and αNO within the γ-CD cavity may be different from that of MB and AO7, due to the different substitution positions of hydroxy groups of αNO and AO7. This might be responsible for the difference in the fluorescence intensity change upon the formation of the ternary inclusion complexes.

Figure 7 exhibits induced circular dichroism (icd) spectra of MB (1.0×10^{-5} mol dm^{-3}) in aqueous solutions containing γ-CD (1.0×10^{-2} mol dm^{-3}) in the absence and presence of αNO (3.0×10^{-5} mol dm^{-3}). The icd spectrum of MB in γ-CD solution containing αNO shows a positive signal peak at around 680 nm, whereas the icd spectrum of MB in γ-CD solution without αNO exhibits positive signal peaks at 603 and 660 nm, which are due likely to the MB dimer and monomer bands, respectively. The icd spectral change in the absence and presence of αNO confirms the formation of the γ-CD–MB–αNO inclusion complex. In MB solution containing both γ-CD and αNO, no appearance of the icd band corresponding to the 603-nm band is consistent with the absence of the MB dimer.

From the absorption spectral change (Fig. 5), one may conclude that a single MB molecule is involved in the γ-CD–MB–αNO inclusion complex. To determine the stoichiometry of the inclusion complex, we applied a continuous variation method regarding the fluorescence intensity under the conditions that the sum of the initial concentrations of γ-CD and αNO was fixed at 3.0×10^{-5} mol dm^{-3}. Figure 8 shows raw data (open circles) of the fluorescence intensity observed at 685 nm and the corrected fluorescence intensities (closed circles) subtracted from values on a straight line. Because the γ-CD concentration is very low, the fluorescence intensity in the absence of αNO is linearly dependent on the γ-CD concentration. Consequently, the corrected fluorescence intensities (ΔI_f) are proportional to the concentration of the γ-CD–MB–αNO inclusion complex. The ΔI_f value goes through a maximum at a $[\gamma\text{-CD}]_0/([\gamma\text{-CD}]_0 + [\alpha\text{NO}]_0)$ value of 0.67, indicating that the ratio of the numbers of γ-CD and αNO molecules in the ternary inclusion complex is 2 : 1. A single MB molecule is included within the ternary inclusion complex, because a MB dimer does not exist in the ternary inclusion complex. Taking into account the existence of the 1 : 1 MB–αNO complex, we conclude that the stoichiometry of the ternary inclusion complex is most likely 2 : 1 : 1 for γ-CD, MB, and αNO.

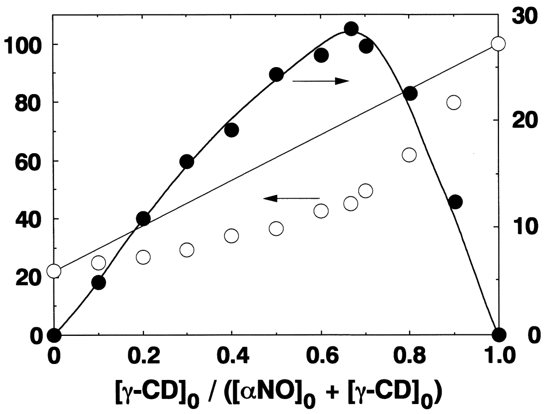


Fig. 8. Continuous variation plot for the fluorescence intensity of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 4.9) containing αNO and γ-CD. The sum of the initial concentrations of αNO and γ-CD is kept at 3.0×10^{-5} mol dm^{-3}. Open circles are raw data, and closed circles are corrected data that the value of the raw data is subtracted from a value on the straight line. λ_{ex} = 570 nm. λ_{obs} = 685 nm.

$$2\gamma\text{-CD} + \text{MB}\cdot\alpha\text{NO} \overset{K_3}{\rightleftarrows} (\gamma\text{-CD})_2\cdot\text{MB}\cdot\alpha\text{NO}. \quad (9)$$

Here, K_3 is the equilibrium constant for the formation of the 2 : 1 : 1 γ-CD–MB–αNO inclusion complex ($(\gamma\text{-CD})_2\cdot\text{MB}\cdot\alpha\text{NO}$).

To evaluate the K_3 value, we employed a simulation method similar to that used for the γ-CD–MB–AO7 system.[14] As a function of γ-CD concentration, the simulation regarding the absorbance was made for MB solutions containing 2.0×10^{-5} mol dm^{-3} of αNO. The absorbance of MB is given as

$$A = (\varepsilon_0 + \varepsilon_1 K_1[\alpha\text{NO}] + \varepsilon_2 K_1 K_3[\gamma\text{-CD}]^2[\alpha\text{NO}])[\text{MB}]d, \quad (10)$$

where ε_2 is the molar absorption coefficient of the 2 : 1 : 1 γ-CD–MB–αNO inclusion complex. The initial concentrations of MB, αNO, and γ-CD are respectively represented by

$$[\text{MB}]_0 = (1 + K_1[\alpha\text{NO}] + K_1 K_3[\gamma\text{-CD}]^2[\alpha\text{NO}])[\text{MB}], \quad (11)$$

$$\begin{aligned}[\alpha\text{NO}]_0 = (1 &+ K_1[\text{MB}] + K_2[\gamma\text{-CD}] \\ &+ K_1 K_3[\gamma\text{-CD}]^2[\text{MB}])[\alpha\text{NO}],\end{aligned} \quad (12)$$

$$[\gamma\text{-CD}]_0 = (1 + K_2[\alpha\text{NO}])[\gamma\text{-CD}] + K_1K_3[\text{MB}][\alpha\text{NO}][\gamma\text{-CD}]^2. \qquad (13)$$

Combining Eqs. 12 and 13, one may derive a cubic equation for $[\gamma\text{-CD}]$:

$$K_1K_3[\text{MB}][\gamma\text{-CD}]^3 + (K_2 + K_1K_3[\alpha\text{NO}]_0[\text{MB}] - K_1K_3[\gamma\text{-CD}]_0[\text{MB}])[\gamma\text{-CD}]^2 + (1 + K_1[\text{MB}] + K_2[\alpha\text{NO}]_0 - K_2[\gamma\text{-CD}]_0)[\gamma\text{-CD}] - (1 + K_1[\text{MB}])[\gamma\text{-CD}]_0 = 0. \qquad (14)$$

Because the K_1 and K_2 values are already evaluated, one can calculate $[\gamma\text{-CD}]$ from Eq. 14, assuming a K_3 value and [MB] to be $[\text{MB}]_0$. By inserting the calculated value of $[\gamma\text{-CD}]$ to Eq. 12, one may estimate $[\alpha\text{NO}]$. By use of the evaluated values of $[\gamma\text{-CD}]$ and $[\alpha\text{NO}]$, a new value of [MB] is estimated from Eq. 11. When we use the value of [MB] thus obtained and the assumed K_3 value, a new value of $[\gamma\text{-CD}]$ is calculated from Eq. 14. This procedure has been repeated until the values of $[\gamma\text{-CD}]$, $[\alpha\text{NO}]$, and [MB] have converged. Because these concentrations are estimated for the assumed K_3 value, the absorbance corresponding to the K_3 value can be evaluated from Eq. 10. Figure 9 depicts the best fit least-squares curve for the absorbance observed at 677 nm, with the known values of K_1 (79900 mol^{-1} dm^3) and ε_0 (60800 mol^{-1} dm^3 cm^{-1}), and variables of $K_3 = 1.86 \times 10^9$ mol^{-2} dm^6, $\varepsilon_1 = 19900$ mol^{-1} dm^3 cm^{-1}, and $\varepsilon_2 = 56200$ mol^{-1} dm^3 cm^{-1}. This K_3 value is an order of magnitude less than that (3.40×10^{10} mol^{-2} dm^6) for AO7.[14]

For the fluorescence intensity change, a similar simulation was performed (not shown). From the simulation, a K_3 value was estimated to be 2.94×10^8 mol^{-2} dm^6, which is about one sixth of the K_3 value obtained from the simulation concerning the absorbance change. In high concentrations of γ-CD in MB solutions containing αNO, the fluorescence intensity of MB was slightly decreased during the measurements of the MB fluorescence intensity. Therefore, the K_3 value obtained from the fluorescence intensity change seems to be less reliable than that obtained from the absorbance change.

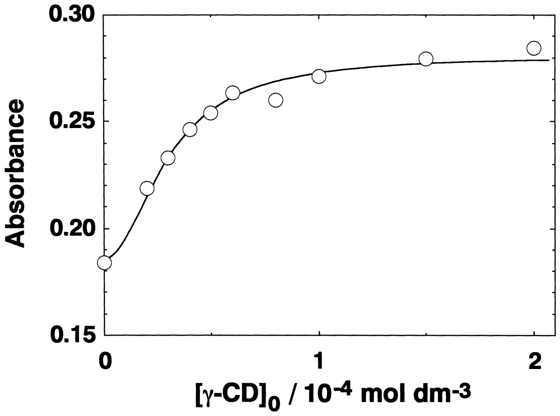


Fig. 9. Comparison of the best fit least-squares curve for the absorbance at 677 nm with the observed absorbance data. The simulation curve was calculated with an ε_0 value of 60800 mol^{-1} dm^3 cm^{-1}, an ε_1 value of 19900 mol^{-1} dm^3 cm^{-1}, an ε_2 value of 56200 mol^{-1} dm^3 cm^{-1}, and a K_3 value of 1.86×10^9 mol^{-2} dm^6. $[\text{MB}]_0 = 5.0 \times 10^{-6}$ mol dm^{-3}. $[\alpha\text{NO}]_0 = 2.0 \times 10^{-5}$ mol dm^{-3}.

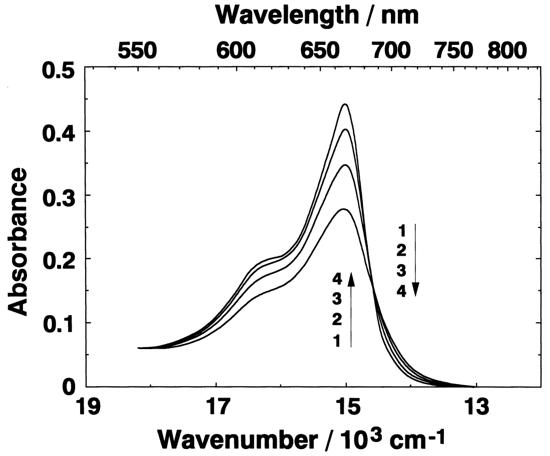


Fig. 10. Absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 4.9) containing αNO (8.0×10^{-6} mol dm^{-3}) and various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, and (4) 1.0×10^{-2} mol dm^{-3}.

Effects of β-CD on the Complexation between MB and αNO in Aqueous Solution. Figure 10 illustrates absorption spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing αNO (8.0×10^{-6} mol dm^{-3}) and various concentrations of β-CD. Upon the addition of β-CD, the intensity at the absorption peak is increased, accompanied by a red-shift and a sharpening of the absorption band. This finding indicates that the MB–αNO complex is dissociated in the presence of β-CD, followed by the complexation between β-CD and MB. An inclusion complex is also formed between β-CD and αNO, because the formation of a 1 : 1 β-CD–αNO inclusion complex has been confirmed in a binary system of β-CD and αNO. From a plot based on Eq. 8, the equilibrium constant for the formation of the 1 : 1 β-CD–αNO inclusion complex has been estimated to be 300 ± 10 mol^{-1} dm^3, which is slightly less than that (420 ± 10 mol^{-1} dm^3) for AO7.[14]

Figure 11 shows fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions containing αNO (8.0×10^{-6} mol dm^{-3}) and various concentrations of β-CD. As the increase in the β-CD concentration, the MB fluorescence is significantly enhanced. This finding indicates the dissociation of the MB–αNO complex, because the MB fluorescence is quenched by the formation of the MB–αNO complex. The result is consistent with the result obtained from the absorption spectral change upon the addition of β-CD. Because the cavity size of β-CD is smaller than that of γ-CD, it is unlikely that a ternary inclusion complex is formed among β-CD, MB, and αNO.

Thermodynamic Quantities for the Equilibria in the Systems of γ-CD–MB–αNO and γ-CD–MB–AO7. To further examine the complexation reactions in the γ-CD–MB–αNO system, we tried to estimate the thermodynamic quantities from the temperature dependence of the equilibrium constants. Figure 12 exhibits the temperature dependence of K_1 for αNO. From this plot, ΔH and ΔS values for the formation of the MB–αNO complex are evaluated to be −43 ± 4 kJ

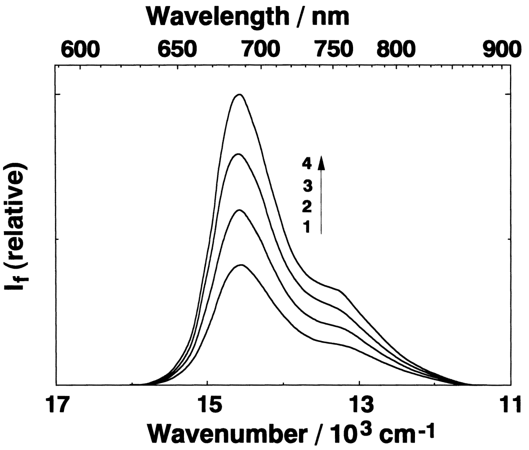


Fig. 11. Fluorescence spectra of MB (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 4.9) containing αNO (8.0×10^{-6} mol dm^{-3}) and various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, and (4) 1.0×10^{-2} mol dm^{-3}. λ_{ex} = 630 nm.

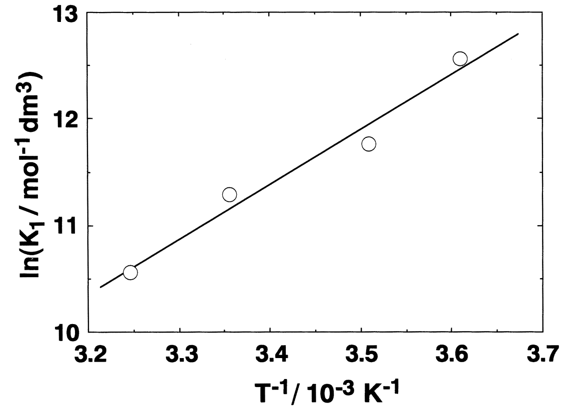


Fig. 12. Temperature dependence of K_1 for αNO in aqueous solution (pH 4.9).

mol^{-1} and -61 ± 12 J K^{-1} mol^{-1}, respectively. These thermodynamic quantities are summarized in Table 1. Table 1 also tabulates ΔH and ΔS values for the formation of the γ-CD–αNO inclusion complex, together with those for the formation of MB–1-naphthalenesulfonate, MB–2-naphthalenesulfonate, and MB–2-anthracenesulfonate complexes.[12] Figure 13 depicts the temperature dependence of K_3 for the γ-CD–MB–αNO system. From this plot, ΔH and ΔS values for K_3 are estimated to be 18 ± 2 kJ mol^{-1} and 230 ± 5 J K^{-1} mol^{-1}, respectively. In contrast to the ΔH and ΔS values for K_1 and K_2, the ΔH and ΔS values for K_3 are positive in sign, suggesting that the hydrophobic interaction exerts on the formation of the γ-CD–MB–αNO ternary inclusion complex.[18] The complexation between MB and αNO cancels the electric charges on MB and αNO, leading to an increase in the hydrophobicity of the MB–αNO complex. Consequently, the hydrophobic interactions may play a critical role in the formation of the 2:1:1 γ-CD–MB–αNO inclusion complex from γ-CD and the MB–αNO complex. In the formation of the γ-CD–MB–αNO inclusion complex, the large positive ΔS value for K_3 indicates an entropy-driven reaction.

Table 1. The ΔH and ΔS Values for K_1, K_2, and K_3 in the Systems of γ-CD–MB–NO and γ-CD–MB–AO7 and Those for K_1 of MB–1-Naphthalenesulfonate (1NS), MB–2-Naphthalenesulfonate (2NS), and MB–2-Anthracenesulfonate (2AS) Complexes

		ΔH/kJ mol^{-1}	ΔS/J K^{-1} mol^{-1}
NO	K_1	-43 ± 4	-51 ± 12
	K_2	-35 ± 2	-55 ± 7
	K_3	18 ± 2	230 ± 5
AO7	K_1	-33 ± 1	-20 ± 2
	K_2	-30 ± 1	-24 ± 5
	K_3	-36 ± 8	61 ± 28
MB–1NS	K_1	-30[a]	-53[a]
MB–2NS	K_1	-28[a]	-46[a]
MB–3NS	K_1	-49[a]	-92[a]

a) Ref.12.

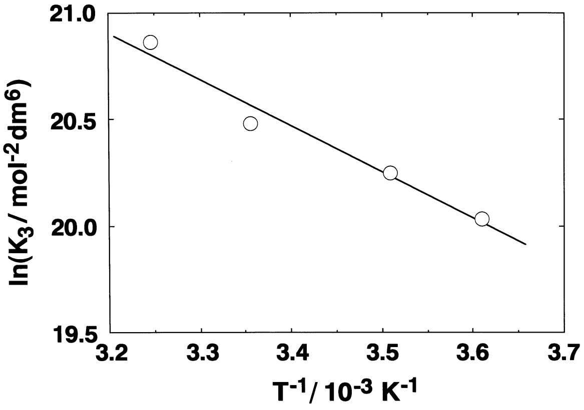


Fig. 13. Temperature dependence of K_3 for the γ-CD–MB–αNO inclusion complex in aqueous solution (pH 4.9).

For comparison, we have also evaluated the thermodynamic quantities for K_1, K_2, and K_3 of the γ-CD–MB–AO7 system; they are summarized in Table 1. The absolute values of ΔH and ΔS for K_1 and K_2 of AO7 are less than those of αNO. Especially, the ΔS values for AO7 are about half of those for αNO. Consequently, the MB–AO7 and γ-CD–AO7 complexes are likely more loose than the MB–αNO and γ-CD–αNO complexes, respectively. Although the negative ΔH value is evaluated for K_3 of AO7, the ΔS value for K_3 of AO7 is positive in sign. As in the case of αNO, the hydrophobic interaction may contribute to the formation of the γ-CD–MB–AO7 inclusion complex.

Conclusions

A cationic dye, MB, forms a complex with an anionic dye, αNO, in aqueous solution (pH 4.9). From a simulation concerning the fluorescence intensity of MB, the equilibrium constant for the formation of the 1:1 MB−αNO complex is evaluated to be 79900 mol^{-1} dm^3. The MB–αNO complex associates with γ-CD to form a 2:1:1 γ-CD–MB–αNO inclusion complex, with the equilibrium constant of 1.86×10^9 mol^{-2} dm^6. From the temperature dependence of the equilibrium constants, ΔH and ΔS values have been evaluated for the formation of the MB–αNO complex, the γ-CD–αNO inclusion complex, and the γ-CD–MB–αNO inclusion complex. These values have also been evaluated for the corresponding complexes of AO7. For the equilibria in the formation of the MB–αNO complex and the γ-CD–αNO inclusion complex, negative values of ΔH and ΔS have been obtained. In contrast, the ΔH and ΔS values for K_3 (formation of the γ-CD–MB–αNO inclusion complex) are positive, suggesting that the hydrophobic interaction works as a driving force of the formation of the ternary inclusion complex. For AO7, the positive ΔS value has been evaluated for K_3, although the ΔH for K_3 is negative in sign.

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