

Size-selective Inclusion Complexes of Phenothiazine Dye Monomers and Dimers with Cyclodextrins

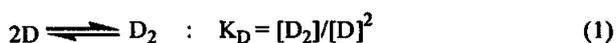
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The effects of cyclodextrins (α -, β -, and γ -CDs) on the monomer-dimer equilibria of phenothiazine dyes (methylene blue (MB), thionine (TH), and 1,9-dimethyl-methylene blue (DMMB)) have been studied using absorption spectroscopy. The positions of the monomer-dimer equilibria do not change by the presence of α -CD, while the addition of β -CD suppresses the dimerization. This is attributed to that the cavity of β -CD is large enough to stabilize dye monomers by the formation of inclusion complexes, while that of α -CD is too narrow to include the dye molecules. The apparent dimerization is enhanced upon addition of γ -CD for MB and TH due to inclusion complexation of the dimers of dyes with γ -CD. However, the suppression of the apparent dimerization by addition of γ -CD is observed for DMMB. It can be explained in terms of size-selective formation of inclusion complexes of phenothiazine dye monomers and dimers with γ -CD: the cavity size of γ -CD is not large enough to accommodate dimers of DMMB though it accommodate the dimers of MB or TH. The dimerization constants (K_D) were determined as $9.6 \times 10^3 \text{ M}^{-1}$ for MB, $3.2 \times 10^3 \text{ M}^{-1}$ for TH, $1.6 \times 10^5 \text{ M}^{-1}$ for DMMB. The association constants of dye monomers (K_{C1}) and dimers (K_{C2}) with CDs are determined from the dependence of the apparent dimerization constants (K_D') on concentrations of CDs. The K_{C2} values obtained for MB and TH with γ -CD are larger than the corresponding K_{C1} values by two orders of magnitude. An opposite trend is found for binding with β -CD.

Keywords Cyclodextrins, phenothiazine, dimerization, inclusion complexes, association constants

The phenothiazine dyes have been widely-used materials, for examples, as sensitizers in solar energy conversion,¹ redox mediators in catalytic oxidation reactions,² and active species in electrochromism.³ All of these applications stem from the redox properties of the phenothiazine dyes. However, the redox chemistry of the phenothiazine dyes (D) are often complicated by the dimerization of dye molecules⁴:

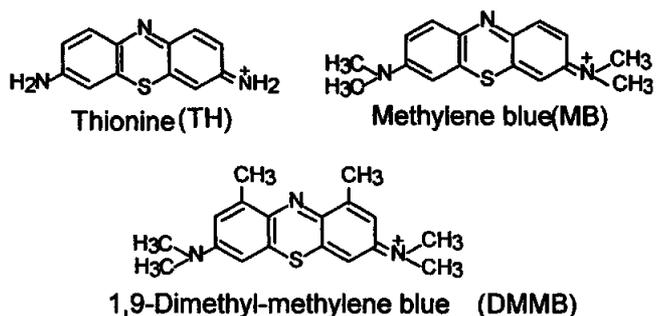


where K_D is the equilibrium constant for the monomer-dimer equilibrium.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of 6, 7 or 8 glucose units and called α , β or γ -CD, respectively.⁵ CDs have hydrophobic cavities and are capable to form inclusion complexes by admitting the guest molecules into their cavities. This property enables to shift the position of

monomer-dimer equilibria of the phenothiazine dyes depending on the dye species and the cavity size of CDs. It was found that β -CD suppresses the formation of dimer whereas γ -CD enhances the dimerization due to the difference in cavity sizes. However, there is a paucity of reports on the quantitative analysis of the association constants between the phenothiazine dye species and CD,⁶ where the determination of K_D value itself was also difficult presumably due to the ambiguity of the UV-vis spectroscopic parameters.^{7,8}

We have great interest in monomer-dimer equilibria of organic molecules, e.g., various viologen species, with CDs.⁹⁻¹³ In this study, we investigated the effects of α , β and γ -CDs on the monomer-dimer equilibria of three phenothiazine dyes, methylene blue (MB), thionine (TH), and 1,9-dimethyl-methylene blue (DMMB), using absorption spectroscopy. These dyes were selected because they have gradual changes in their sizes and symmetric



structures as shown in the above. The spectroscopic parameters of the monomers and dimers of the dyes were deduced. Also, the association constants of the monomer and dimer with β and γ -CDs were obtained by fitting the dependence of the apparent dimerization constants (K_D') on the concentration of CDs to a multistep equilibrium scheme.

Experimental

TH, MB and DMMB were obtained from Aldrich and recrystallized from CH_2Cl_2 - CH_3OH before use. α and β -CDs were obtained from Aldrich and γ -CD were from Tokyo Kasei and used as received. Solutions were prepared from reverse osmosed water which was further purified by passage through a Millipore purification train. All solutions contained 0.1 M KCl and 0.01 M HCl through the experiments.⁸ The concentrations of CD solutions were calculated from the reported optical rotation data.⁵

Absorption spectra were measured using a Hewlett-Packard Model 8452A or GBC Model 920 spectrophotometer equipped with thermostated cell holders. The temperature was maintained at 25 °C except when the effects of temperature were investigated. Three quartz cells with light pathlength (b) of 0.2, 1 and 10 cm were used to maintain the absorbances below 1.

Results and Discussion

Effects of CDs on UV-vis Absorption Spectral Behavior of Phenothiazine Dyes

The shifts in the position of the monomer-dimer equilibria of the phenothiazine dyes by the addition of CDs cause changes in the visible absorption spectrum because of distinct absorption bands of the monomer and dimer forms of TH, MB and DMMB. For an example, MB shows absorption maximum

Table 1 Effects of CDs on the monomer-dimer equilibria of phenothiazine dyes^a

	α -CD	β -CD	γ -CD
TH	N.E.	--	++
MB	N.E.	--	++
DMMB	N.E.	-	--

a. The symbols of 'N.E.', '-', '--', and '++' denote negligible effect, moderate suppression of dimerization, strong suppression of dimerization, and strong enhancement of dimerization, respectively.

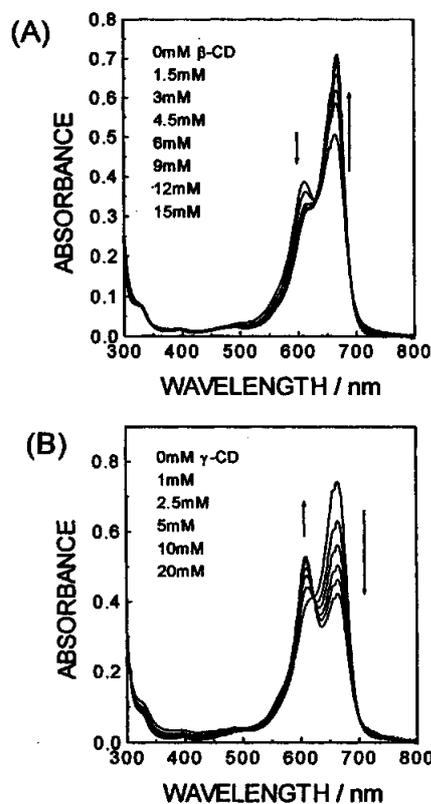


Fig. 1 Effects of CDs on absorption spectra of MB: (A) for 5.1×10^{-5} M MB in the presence of β -CD with $b = 0.2$ cm; (B) for 1.0×10^{-5} M MB in the presence of γ -CD with $b = 1$ cm. Arrows point the direction of increasing concentration of CDs.

near 660 nm whereas $(\text{MB})_2$ exhibits absorption maxima near 610 nm.⁶⁻⁸ Figs. 1-3 shows the effects of β - and γ -CDs on the spectrum of MB, TH and DMMB solutions, respectively.

The spectra taken in the absence of CDs (Fig. 1) shows the mixture of MB and $(\text{MB})_2$. Addition of α -CD cause little spectral change suggesting that α -CD is not effective on the dimerization of MB (not shown) because the size of MB is too large to fit

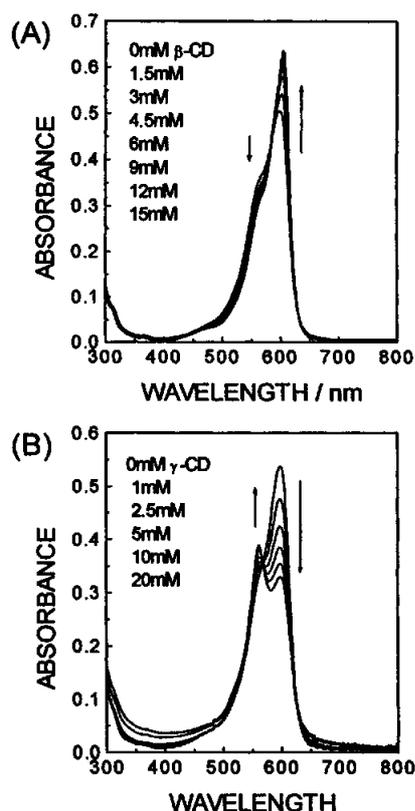


Fig. 2 Effects of CDs on absorption spectra of TH: (A) for 5.0×10^{-5} M TH in the presence of β -CD with $b = 0.2$ cm; (B) for 9.5×10^{-6} M TH in the presence of γ -CD with $b = 1$ cm. Arrows point the direction of increasing concentration of CDs.

inside α -CD cavity. A large increase in monomer peak (664 nm) and decrease in dimer peak (610 nm) are observed with increasing concentration of β -CD (Fig. 1A). This is interpreted as suppression of the dimer formation by inclusion of MB monomer into the β -CD cavity preferentially. The opposite tendency is observed with γ -CD (Fig. 1B), which is attributed to the stabilization of the dimer by inclusion of dimer in the cavity of γ -CD.

The effects of β and γ -CDs on the spectrum of TH (Fig. 2) show similar tendency as in MB with the parallel interpretations. However, remarkable differences of the effects of CDs are found in DMMB (Fig. 3) compared with MB and TH. A small but noticeable increase of monomer peak (652 nm) is observed with β -CD. Increase of monomer peak in spectra is more enhanced with γ -CD (Fig. 3B), which suggests that the size of $(\text{DMMB})_2$ is too large to be included selectively into the γ -CD cavity while that of DMMB monomer is large enough to form inclusion complex-

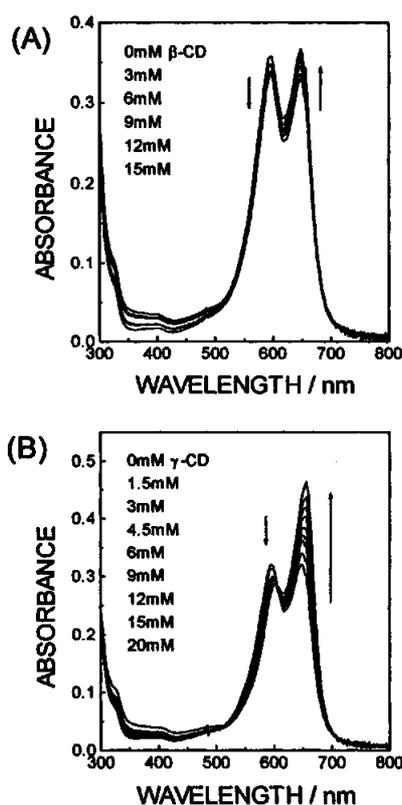


Fig. 3 Effects of CDs on absorption spectra of DMMB with $b = 1$ cm: (A) for 1.1×10^{-5} M DMMB in the presence of β -CD; (B) for 1.0×10^{-5} M TH in the presence of γ -CD. Arrows point the direction of increasing concentration of CDs.

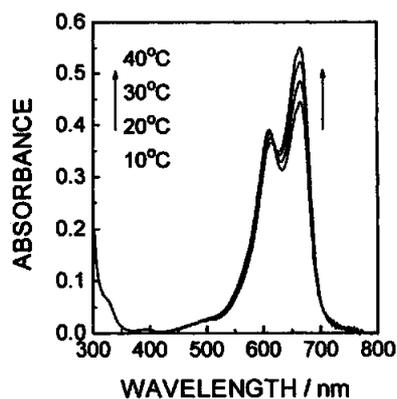


Fig. 4 Effects of temperature on absorption spectra of 5.0×10^{-5} M MB with $b = 0.2$ cm.

es with γ -CD. The effects of CDs on the monomer-dimer equilibria of the dyes are summarized in Table 1 qualitatively (see later part for quantitative

Table 2 Some spectroscopic parameters of Phenothiazine dyes.

	$\epsilon_i \times 10^4$ (λ , nm)	$\epsilon_m \times 10^4$ (λ , nm) ^a	$\epsilon_d \times 10^4$ (λ , nm) ^a	$K_D \times 10^{-3}$ (M ⁻¹)
TH	3.7(566)	6.1(598)	2.5(598)	3.2
		3.3(559)	8.0(559)	(3.2)
MB	3.6(620)	7.2(664)	2.5(664)	9.6
		2.8(604)	9.7(604)	(9.6)
DMMB	3.1(600)	6.0(648)	1.8(648)	160
		2.5(590)	7.0(590)	(150)

a. The longer wavelength is the absorption maximum of dimer and the shorter wavelength is that of monomer.

interpretations).

The position of the monomer-dimer equilibria of MB can also be shifted by changing temperature as shown Fig. 4. Here, isosbestic point of the monomer-dimer equilibrium, λ_i , is shown at 620 nm with ϵ_i of $3.6 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ as well as pronounced monomer peak, λ_m , at 664 nm (40 °C) and dimer peak, λ_d , at 604 nm (10 °C). Similarly, λ_i , ϵ_i , λ_m , and λ_d were obtained for TH and DMMB (Figs. not shown) and included in Table 2, ϵ_i values were used to determine total dye concentrations in solutions. The absorption maxima of the monomer (λ_m) and dimer (λ_d) were determined from the decomposed spectra (see following section).

Determination of K_D of Phenothiazine Dyes in the Absence of CDs

The spectrum of the mixtures of D and (D)₂ without CDs can be decomposed into individual monomer and dimer component by the linear combination of the respective spectra.^{9,11-14} For this, we obtained spectra of mixture of several different concentrations in the region of $1 - 10 \times 10^{-5}$ M for TH and MB and $1 - 10 \times 10^{-6}$ M for DMMB. Since the spectrum with higher dye concentration contains a larger fraction of monomer than that with lower one, the pure dimer spectrum can be obtained by subtracting the latter spectrum (multiplied by an adjustable factor) from the former spectrum. Similarly, the spectrum of monomer component in a monomer-dimer mixture spectrum is obtained by subtracting the dimer spectrum generated (multiplied by an adjustable factor) from the mixture spectrum, where the spectrum of 10^{-6} M dye at 40 °C is used as a reference monomer spectrum for comparison. For an example, the dimer spectrum of MB generated by this method is shown in Fig. 5: normalization of the resolved spectra obtained at different total concentra-

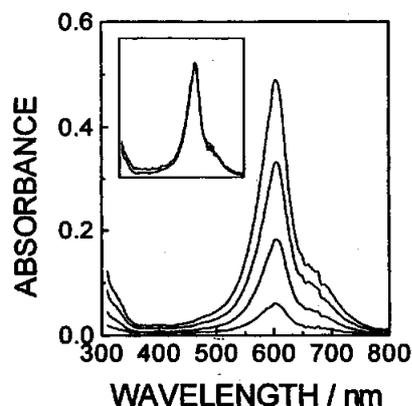


Fig. 5 Resolved dimer spectra obtained from 1×10^{-4} , 7.7×10^{-5} , 5.2×10^{-5} and 2.6×10^{-5} M MB solutions (from top to bottom). The inset shows the normalized spectra.

tion of MB indicates that the contribution of only one component to the spectra.

From the resolved spectra using the ratio of absorbances at absorption maxima and λ_i , we calculated the ϵ values of monomers (ϵ_m) and dimers (ϵ_d) at wavelengths of absorption maxima. The results were summarized in Table 2. To determine K_D values, we first calculated the total concentration of dye from A_i values using Eq. (2). Then the concentrations of the monomer and dimer in a mixture were calculated using the combined relationships (2) and (3) at absorption maxima of monomer (λ_m) or dimer (λ_d),

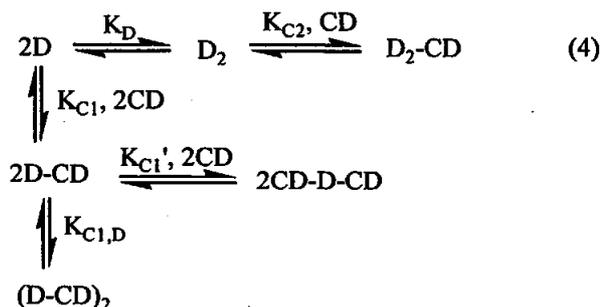
$$A_i/b\epsilon_i = c_t = c_m + 2c_d \quad (2)$$

$$A_\lambda = \epsilon_{m,\lambda} bc_m + \epsilon_{d,\lambda} bc_d \quad (3)$$

where c_t , c_m and c_d are the total, monomer, and dimer concentrations in the mixture (Table 2). For a given dye, the K_D values were determined at two different wavelengths, which were in good agreement

Determination of Association Constants of Dye Species with CDs

The monomer-dimer equilibria of dyes in the presence of CDs can be represented by the following scheme (Eq. (4)), where we considered only 1:1 complexation between the dimer (D₂) and CD.^{6,15,16} In Eq. (4), K_D is defined in Eq. (1). K_{C1} and K_{C1}' are the first and second association constant of dye monomer (D) with CD, respectively. K_{C2} is the association constant of D₂ with CD. $K_{C1,D}$ is the



dimerization constant of D-CD complexes forming 2:2 complexes.

The apparent dimerization constant (K_D') in the presence of CD is defined in Eq. (5).

$$K_D' = \frac{[D_2] + [D_2\text{-CD}] + [(D\text{-CD})_2]}{([D] + [D\text{-CD}] + [CD\text{-D-CD}])^2} \quad (5)$$

To evaluate K_D' from the decomposed spectra in the presence of CDs, we checked the effect of CD binding on the monomer spectra of dyes. A noticeable changes in spectra of 10^{-6} M MB was observed upon the addition of β -CD up to 10 mM, i.e., the narrower band width and ca. 3% increase in ϵ_{\max} presumably due to the change in the environment of MB upon binding with β -CD. Since the monomer is the predominant form at such a low concentration, the observed isosbestic point is that between the uncomplexed and CD-complexed monomers. Similar experiments were carried out for TH/ β -CD and DMMB/ γ -CD. The isosbestic points were found at the wavelengths same as λ_m in Table 2 (thus with same ϵ 's) except for DMMB at 648 nm with $\epsilon_m = 5.8 \times 10^4$ and $\epsilon_d = 1.8 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$. We could not observe any significant changes of ϵ_d of the resolved dimer spectra upon CD-complexation at the above isosbestic points. Using Eq. (3) with these values and Eq. (2), the K_D' values for each spectrum in Figs. 1 - 3 were calculated and the results are shown in Fig. 6 (symbols) as a function of $[\beta\text{-CD}]$ and $[\gamma\text{-CD}]$, respectively.

Rearrangement of Eq. (5) using the equilibrium constants shown in Eq. (4) gives Eq. (6).

$$K_D' = \frac{K_D + K_{C_2}K_D[CD] + K_{C_1}^2K_{C_1,D}[CD]^2}{(1 + K_{C_1}[CD])(1 + K_{C_1}'[CD])^2} \quad (6)$$

The thermodynamic parameters in Eq. (4) were determined from the dependence of K_D' on $[CD]$ by nonlinear least-square regression analysis.

In this analysis, we used K_D values (in Table 2) as a fixed parameter and others were fitting parameters.

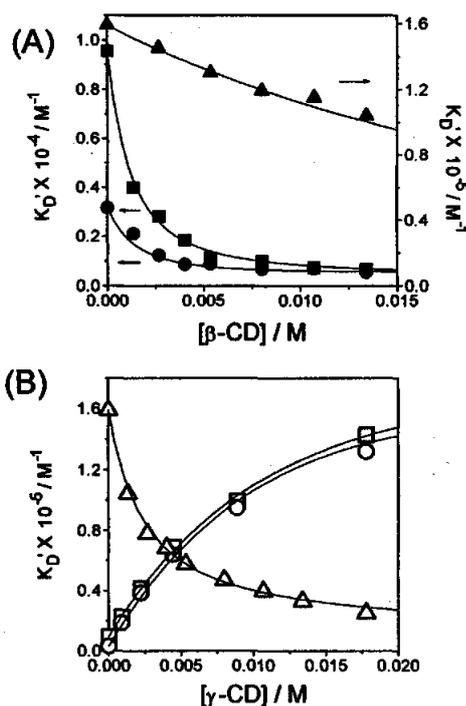


Fig. 6 The plot of K_D' against initial concentration of $[CD]$, $[CD]_{\text{initial}}$, for TH (circle), MB (square) and DMMB (triangle) with β -CD (A: closed symbols) and γ -CD (B: open symbols). Symbols are from experimental data in Figs. 1 - 3. Solid lines are the fitted lines according to Eq. (6) using parameters in Table 3.

Table 3 K_{C_1} , K_{C_2} and $K_{C_1,D}$ values with β -CD and γ -CD (for symbols, see Eq. (4)).^a

	$K_{C_1} (\text{M}^{-1})$	$K_{C_2} (\text{M}^{-1})$	$K_{C_1,D} (\text{M}^{-1})$
<i>with β-CD</i>			
TH	3.0×10^2	1.1×10^1	7.6×10^2
MB	4.0×10^2	3.5×10^1	6.0×10^2
DMMB	2.0×10^1	- ^b	- ^b
<i>with γ-CD</i>			
TH	2.5×10^1	5.2×10^3	- ^b
MB	2.5×10^1	1.7×10^3	- ^b
DMMB	2.4×10^2	1.4×10^2	2.1×10^4

a. K_{C_1}' values were less than 5 M^{-1} for all dyes with β -CD or γ -CD.

b. Too small to have physical meaning.

The lines fitted to the experimental data using Eq. (6) are shown in Fig. 6 as well. The parameters obtained are arranged in Table 3.

Discussions about the parameters obtained other than K_{C_1} and K_{C_2} will be followed by this paragraph. For TH and MB with β -CD, K_{C_1} values are a order of magnitude larger than K_{C_2} values, which is parallel

with the fact that suppression of the dimer formation by β -CD as observed in Figs. 1A and 2A. For TH and MB with γ -CD, K_{C2} values are larger than K_{C1} values by two orders of magnitude, which appears with the pronounced enhancement of dimerization upon addition of γ -CD as in Figs. 1B and 2B. However, K_{C1} is larger than K_{C2} for DMMB/ γ -CD resulting in suppression of dimerization with increasing [γ -CD] (Fig. 3B) similar to the case of TH or MB with β -CD. It is thus probable that the size of DMMB monomer is better fitted to the cavity of γ -CD than the dimer. The small value of K_{C1} of DMMB/ β -CD compared with those of MB or TH implies that the size of DMMB may be too large to form stable inclusion complexes and only small part of the molecule is included possibly from the end to the front of methyl moiety on the ring. Decreasing order of K_{C2} / γ -CD is TH, MB and DMMB, which tells the size-selective inclusion clearly. Overall, the parameters in Table 3 are in good accordance with our spectral observations.

For all cases listed in Table 3, the K_{C1}' (the second association constant of dye monomer (D) with CD) values were too small to have physical meaning. This agrees with a report in oxazine/ γ -CD system.¹⁶ If the both ends of the symmetric dyes bind independently with CD, K_{C1}' should be $K_{C1}/2$ from the statistical point.¹² The discrepancy between observation and statistical expectation can be explained by that the induced-charge-localization would be developed on the protruded part of the dye molecules of the dye/CD complexes and makes a barrier for second complexation with CD. The association related with K_{C1}' in Eq. (4) could be omitted for simplicity in Table 3. Note, however, this is quite different from the assumption of Raj and Ramaraj,⁴ which was based on electrochemistry.

Interestingly, the fitted dimerization lines of TH is leveled off with increasing [β -CD] in Fig. 6A. This suggests that 1:1 complexes of TH with β -CD dimerizes forming a 2:2 complex. The limiting K_D' value obtained at high concentrations of β -CD can be regarded as the dimerization constant of TH-(β -CD) complexes, $K_{C1,D}$. This situation arises from relatively small K_D values of the dye.

Acknowledgments

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