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Association of Inclusion Compounds of β-Cyclodextrin in Aqueous Solution

Sanyo Hamai

Department of Physics, Miyazaki Medical College, Kiyotake, Miyazaki 889-16

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Inclusion compounds in the systems naphthalene–β-cyclodextrin (CDx) and 2-methoxynaphthalene (MN)–o-dicyanobenzene (DB)–β-cyclodextrin in aqueous solution were studied by means of absorption and fluorescence spectra. In the former system a naphthalene excimer fluorescence was observed. By the analyses of the concentration dependence of the absorption spectra and the excimer fluorescence intensities, the species responsible for the excimer fluorescence is identified as a 2:2 inclusion compound which is formed by association of two 1:1 naphthalene–CDx inclusion compounds, not a 2:1 inclusion compound in which two naphthalene molecules enter into the cavity of one CDx molecule. In the latter system, absorption and fluorescence spectra due to a charge transfer complex of MN with DB were observed. By analyses similar to the naphthalene case, the existence of a 1:1:2 association compound formed between a MN–CDx inclusion compound and a DB–CDx inclusion compound is confirmed. Fluorescence quenching by I^- and IO_3^- was also studied for the inclusion compounds in both systems.

Recently, much attention has been focused on cyclodextrin as a model compound for enzymatic processes. It is interesting to note that such a relatively small molecule can promote a stereospecific reaction although in organisms much larger molecules act catalytically. This catalytic action is based on the fact that cyclodextrin forms inclusion compounds with many kinds of organic compounds in aqueous solution. The component ratios of substrates to β-cyclodextrin are 1:1 for most compounds.[1–5] However, 1:2 or 2:1 inclusion compounds have been reported for α-, β-, γ-, and capped cyclodextrins.[6–8] Ueno *et al.*[9] suggested that γ-cyclodextrin forms a 2:1 inclusion compound with 1-naphthyl acetate in aqueous solution by observing an excimer fluorescence of 1-naphthyl acetate. For some systems, formation of a 2:2 inclusion compound by association of two 1:1 inclusion compounds has been speculated,[10,11] but ambiguity remains as to whether such an association really occurs. By study of fluorescence quenching in the system in which two kinds of 1:1 inclusion compounds can exist, Kano *et al.*[12] predicted the existence of a 1:1:1 inclusion compound. Thus it appears that the compositions of the inclusion compounds other than the 1:1 compounds depend critically on the types of cyclodextrin and the physicochemical properties of the substrates involved.

In the present study, we have investigated inclusion compounds in the systems naphthalene–β-cyclodextrin and 2-methoxynaphthalene–o-dicyanobenzene–β-cyclodextrin in aqueous solution by analyses of the absorption and fluorescence spectra.

Experimental

β-Cyclodextrin (CDx) purchased from Nakarai Chemical Co. was recrystallized three times from water and the molecular weight of CDx was calculated as the dodecahydrate.[13] Naphthalene (Nakarai Chemical Co.) and 2-methoxynaphthalene (MN) (Tokyo Kasei Kogyo Co.) were recrystallized three times from ethanol. o-Dicyanobenzene (DB) (Tokyo Kasei Kogyo Co.) was recrystallized first from water and then from cyclohexane. Potassium iodide (Wako Pure Chemical Industries) and potassium iodate (Nakarai Chemical Co.) were used without further purification. Water was doubly distilled.

Absorption spectra were measured with a Hitachi 124 spectrophotometer and fluorescence spectra with a Shimadzu RF-501 fluorescence spectrophotometer (modified by employing a cooled EMI 9789QA photomultiplier). Both instruments were equipped with thermostatically controlled sample compartments. Fluorescence was detected at right angles to the direction of excitation. Fluorescence spectra were corrected for the spectral sensitivity of the detection system.[14]

Absorption and fluorescence spectra were measured at (25±0.1) °C unless otherwise stated. Aerated sample solutions were used throughout the present work because nitrogen bubbling caused considerable decreases in the concentrations of naphthalene and MN in aqueous solution (containing no CDx).

Fluorescence decay times were determined by means of an Ortec 9200 nanosecond decay time fluorescence spectrometer. Light from a nanosecond light pulser (filled with H_2 or air) was passed into a 0.3 m Ritsu MC-30 monochromator and the 270 nm (for naphthalene–CDx system, H_2 gas) or the 316 nm (for MN–DB–CDx system, air) exciting light was isolated. Fluorescence from sample solutions was collected through appropriate filters.

Results

Inclusion Compounds in Naphthalene–β-Cyclodextrin System. Absorption spectra of naphthalene aqueous solutions containing varying concentrations of CDx are shown in Fig. 1. At concentrations of CDx between 0 and $\approx 3\times10^{-3}$ M (1 M=1 mol dm^{-3}), isosbestic points are observed at 267, 271, 276, and 283 nm, indicating the following 1:1 equilibrium in this concentration range:

$$\mathrm{N} + \mathrm{CDx} \overset{K_1}{\rightleftharpoons} \mathrm{NC}, \tag{1}$$

where N and NC represent naphthalene and the 1:1 inclusion compound of CDx with naphthalene, respectively, and K_1 is the equilibrium constant for the formation of NC. The Benesi-Hildebrand relation for such an equilibrium is[1]

$$\frac{1}{A-A_0} = \frac{1}{(\varepsilon_1-\varepsilon_0)[\mathrm{N}]_0} + \frac{1}{(\varepsilon_1-\varepsilon_0)[\mathrm{N}]_0K_1}\frac{1}{[\mathrm{CDx}]_0}, \tag{2}$$

where A and A_0 are the absorbances per cm of the naphthalene aqueous solutions in the presence and absence of CDx, ε_1 and ε_0 are the molar absorption coefficients of NC and naphthalene, and $[\mathrm{N}]_0$ and $[\mathrm{CDx}]_0$

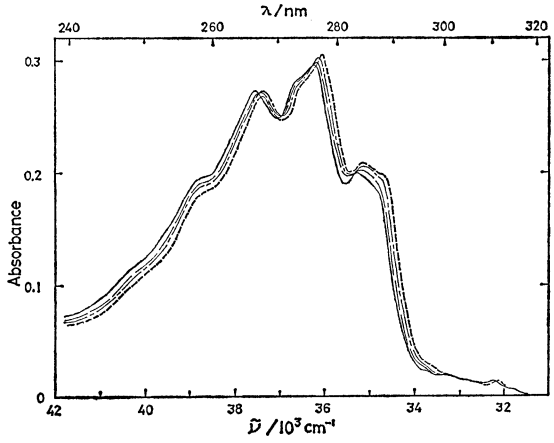

Fig. 1. Absorption spectra of naphthalene (5.80×10^{-5} M) in aqueous solution in the presence of CDx. Initial concentration of CDx, ——: 0 M, — — —: 8.40×10^{-4} M, — — — —: 2.52×10^{-3} M, - - - -: 8.40×10^{-3} M.

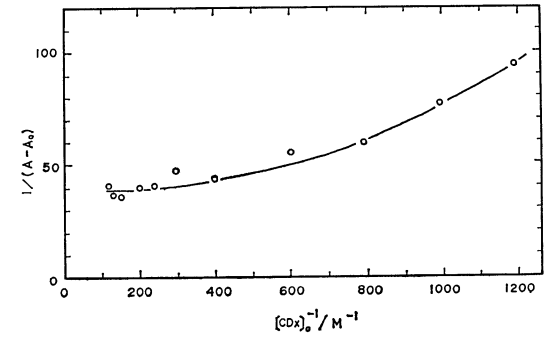

Fig. 2. Benesi-Hildebrand plot for absorbance changes at 277 nm of naphthalene aqueous solutions containing varying concentrations of CDx. $[N]_0=5.80\times10^{-5}$ M.

are the initial concentrations of naphthalene and CDx, respectively. We choose 277 nm as the analyzing wavelength for the absorbance changes. A plot of $1/(A-A_0)$ *vs.* $1/[CDx]_0$ gives a concave upward curve as shown in Fig. 2. This combined with the fact that the spectral curves no longer pass through the isosbestic points at higher CDx concentrations (see Fig. 1) suggests the existence of another species other than NC in the naphthalene–CDx system.

Figure 3 shows the fluorescence spectra of naphthalene in the absence and presence of CDx in aqueous solution. By addition of CDx the fluorescence band at around 330 nm is enhanced, and at the same time a broad structureless band having λ_{max} at 410 nm appears. This new band grows in intensity at the expense of the 330 nm band with decreasing temperature as shown in Fig. 4. The enhancement of the 330 nm band seen in Fig. 3 can be interpreted as due to the formation of NC having the higher fluorescence yield than naphthalene. The new band is assignable to the well-known naphthalene excimer fluorescence.[15,16] The appearance of this band indicates the existence of another species beside NC because the excimer cannot be formed from uncomplexed naphthalene molecules at the concentrations as low as $\approx6\times10^{-5}$ M.

As the species responsible for the excimer fluorescence, two kinds of inclusion compounds represented by the following two mechanisms can be considered:

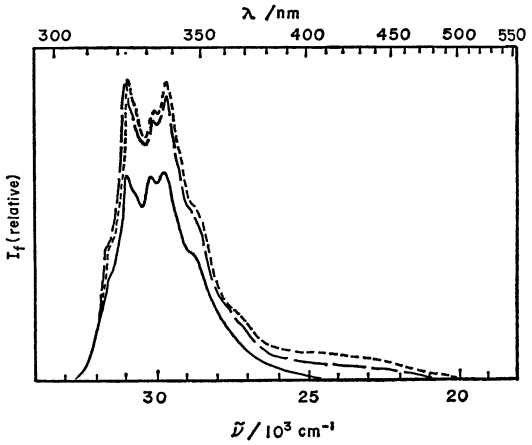

Fig. 3. Fluorescence spectra of naphthalene (5.80×10^{-5} M) in aqueous solution in the presence of CDx. Initial concentration of CDx, ——: 0 M, — — —: 2.52×10^{-3} M, - - - -: 8.40×10^{-3} M. $\lambda_{exc}=283$ nm.

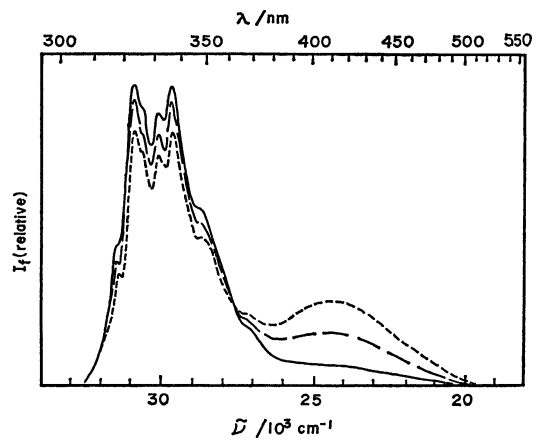

Fig. 4. Temperature dependence of the fluorescence spectra of naphthalene (4.90×10^{-5} M) in aqueous solution in the presence of CDx (8.40×10^{-3} M). ——: 25 °C, — — —: 12 °C, - - - -: 3 °C. $\lambda_{exc}=283$ nm.

Mechanism 1) $$N + NC \overset{K_2'}{\rightleftharpoons} N_2C, \quad (3)$$

Mechanism 2) $$NC + NC \overset{K_2}{\rightleftharpoons} N_2C_2. \quad (4)$$

One of these is a 2:1 inclusion compound N_2C which is composed of two naphthalene molecules entering into the cavity of a CDx molecule. The other is a 2:2 inclusion compound N_2C_2 which is formed by association of two 1:1 inclusion compounds. For Mechanism 1, the relation that the concentration of the uncomplexed naphthalene [N] should satisfy is

$$2K_1K_2'[CDx]_0[N]^2 + (1+K_1[CDx]_0)[N]-[N]_0 = 0, \quad (5)$$

whereas for Mechanism 2, it is

$$2K_1^2K_2[CDx]_0^2[N]^2 + (1+K_1(CDx]_0)[N]-[N]_0 = 0. \quad (6)$$

The concentration of N_2C or N_2C_2 can be evaluated after obtaining [N] from Eq. 5 or 6. To solve these equations for [N] we must first know the equilibrium constant K_1. The value of K_1 was determined as following.

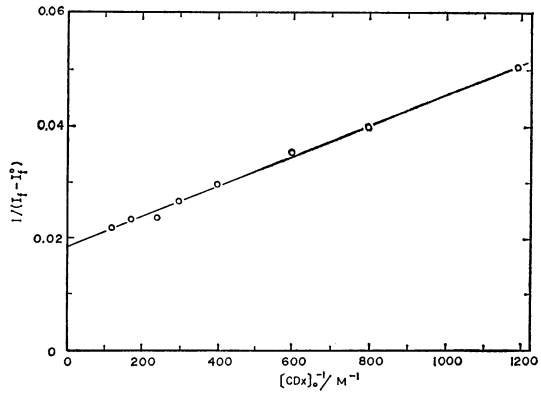

Fig. 5. Benesi-Hildebrand plot for fluorescence increments at 336 nm of naphthalene aqueous solutions containing varying concentrations of CDx. $[N]_0=6.10\times10^{-6}$ M.

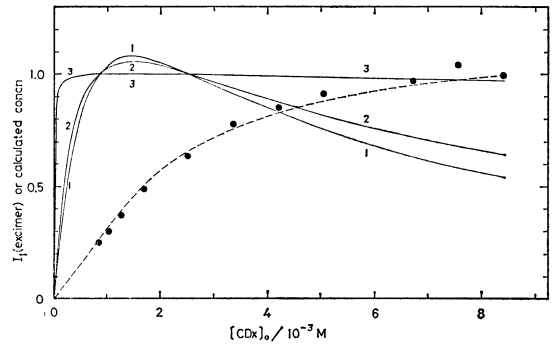

Fig. 6. Comparison of the observed excimer intensities, I_f(excimer), with calculated concentrations $[N_2C]$ and $[N_2C_2]$.
●: I_f(excimer), ——: $[N_2C]$ calculated with $K'_2=1\ M^{-1}$ (curve 1); $K'_2=10^4\ M^{-1}$ (curve 2); $K'_2=10^7\ M^{-1}$ (curve 3), - - - -: $[N_2C_2]$ calculated with $K_2=4000\ M^{-1}$. $[N]_0=5.80\times10^{-5}$ M. I_f(excimer) and $[N_2C_2]$ are normalized to unity at the highest concentration of $[CDx]_0$, and $[N_2C]$ at the lowest concentration of $[CDx]_0$.

The fluorescence intensity was measured at a naphthalene concentration of 6×10^{-6} M at which no excimer fluorescence appears and thus only NC exists as the inclusion compound. For our experimental geometry in the fluorescence measurements, the observed intensity was always proportional to the concentration of the emitting species. Under these conditions, the following relation holds:[7]

$$\frac{1}{I_f-I_f^0}=\frac{1}{a[N]_0}+\frac{1}{a[N]_0K_1}\frac{1}{[CDx]_0}, \tag{7}$$

where I_f and I_f^0 are the fluorescence intensities of the 330 nm band in the presence and absence of CDx, and a is a proportionality constant. A plot of $1/(I_f-I_f^0)$ *vs.* $1/[CDx]_0$ yields a good straight line as shown in Fig. 5, and from the intercept-to-slope ratio $K_1=685\ M^{-1}$ is obtained.

The value of [N] for Mechanism 1 can then be calculated from Eq. 5 for an assumed value of K'_2 from which the concentration of N_2C can be evaluated. The concentration of N_2C_2 will be similarly evaluated for an assumed value of K_2 through Eq. 6. Figure 6 shows the dependence of the observed excimer fluorescence intensity on CDx concentration and the calculated concentrations of N_2C and N_2C_2 using various values of K'_2 or K_2. As seen in Fig. 6 even when K'_2 is varied from 1 M^{-1} to 10^7 M^{-1}, none of the concentration curves for N_2C fit the intensity curve of the excimer fluorescence. On the other hand, the calculated curve for N_2C_2 fits the data quite well when K_2 is taken equal to 4000 M^{-1}. These findings strongly support Mechanism 2. We will therefore assume in the following that N_2C_2 is the species responsible for the excimer emission in the naphthalene–CDx system.

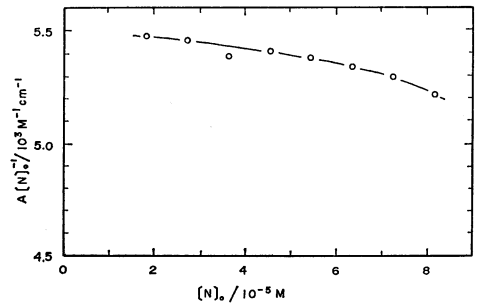

Fig. 7. Plot of $A/[N]_0$ at 277 nm *vs.* $[N]_0$. $[CDx]_0=8.40\times10^{-3}$ M.

So far, we have dealt with the spectroscopic data which were obtained at a fixed concentration of naphthalene with varying amounts of CDx. We will now examine the dependence of the naphthalene concentration (at a fixed CDx concentration) on the absorption and fluorescence spectra. No appreciable spectral change was observed in the absorption spectra when the varying concentrations of naphthalene were added to the solution containing a fixed concentration of CDx. However, as displayed in Fig. 7, the apparent molar absorption coefficient ($A/[N]_0$) at 277 nm decreases with increasing $[N]_0$. Obviously, the molar absorption coefficient of N_2C_2 per naphthalene molecule, $\varepsilon_2/2$, must be smaller than ε_1.

The data shown in Fig. 7 can further be analyzed as following. The absorbance A at 277 nm is given by

$$A=\varepsilon_0[N]+\varepsilon_1[NC]+\varepsilon_2[N_2C_2], \tag{8}$$

which can be rewritten using the equilibrium constants K_1 and K_2 as

$$\frac{A}{[N]}=(\varepsilon_0+\varepsilon_1K_1[CDx]_0)+\varepsilon_2K_1^2K_2[CDx]_0^2[N]. \tag{9}$$

The concentration of uncomplexed naphthalene [N] can be calculated from Eq. 6 as functions of $[N]_0$ and $[CDx]_0$ since K_1 and K_2 have already been determined. A plot of $A/[N]$ *vs.* [N] gives a straight line as presented in Fig. 8, and from this plot the values $\varepsilon_1=5690\ M^{-1}\ cm^{-1}$ and $\varepsilon_2=9160\ M^{-1}\ cm^{-1}$ are obtained using the known value of ε_0 (4870 $M^{-1}\ cm^{-1}$). As expected, $\varepsilon_1>\varepsilon_0$ and $\varepsilon_2/2<\varepsilon_1$. These relationships amongst ε_0, ε_1, and $\varepsilon_2/2$ explain the temperature variation of the absorption spectra given in Fig. 9 which shows the decrease of the absorbance at 277 nm on cooling. As is apparent from the temperature depend-

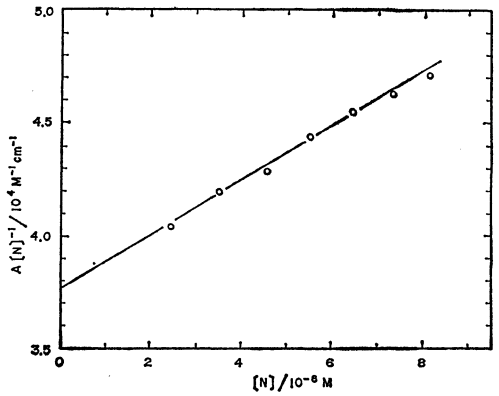

Fig. 8. Plot of $A/[N]$ at 277 nm *vs.* [N]. [N] is calculated from Eq. 6.

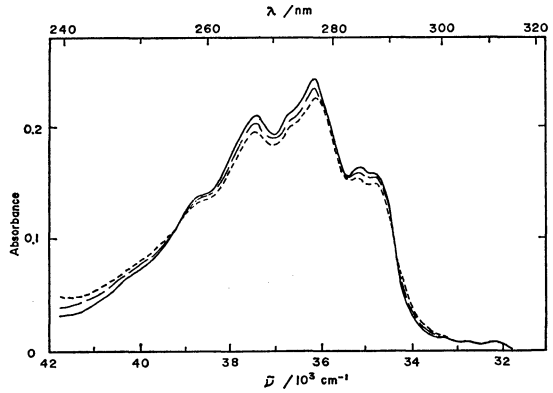

Fig. 9. Temperature dependence of the absorption spectra of naphthalene (4.90×10^{-5} M) in aqueous solution in the presence of CDx (8.40×10^{-3} M). ——: 25 °C, — —: 12 °C, - - - -: 3 °C.

ence of the fluorescence spectra (Fig. 4), $[N_2C_2]$ increases on lowering temperature, accompanied by the decrease in [NC].

The good linearity obtained in Fig. 8 indicates that any aggregates higher than a 2:2 adduct (N_nC_n, $n>2$) do not exist at least in the concentration range we have examined. That the species other than NC in the naphthalene–CDx system is indeed the 2:2 inclusion compounds, and is not a higher aggregate can also be shown from the dependence of the excimer intensity, I_f(excimer), on the naphthalene concentration at a fixed CDx concentration. For Mechanism 2,

$$I_f(\text{excimer}) = b'[N_2C_2] = b'K_1^2K_2[\text{CDx}]_0^2[N]^2 = b[N]^2, \tag{10}$$

where b' is an instrumental constant. Taking logarithm of both sides of Eq. 10,

$$\log I_f(\text{excimer}) = 2\log[N] + \log b. \tag{11}$$

A plot of $\log I_f$(excimer) *vs.* $\log[N]$, [N] being calculated from Eq. 6, gives a good straight line with the slope 1.9 ($\simeq$2) as shown in Fig. 10, indicating the square dependence of I_f(excimer) on [N].

By measuring I_f(330 nm) over the temperature range from 3 to 25 °C and obtaining the value of K_1 at each temperature from the plot as shown in Fig. 5, the enthalpy and entropy changes for the equilibrium constant K_1 were determined to be -19 kJ mol^{-1} and

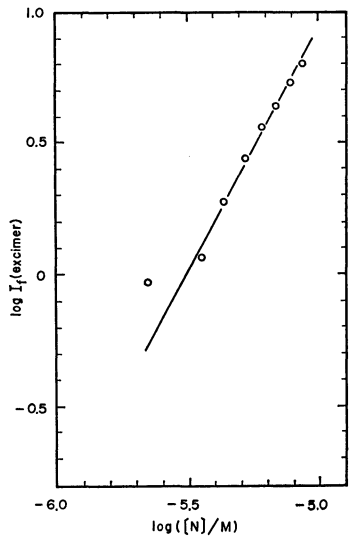

Fig. 10. Plot of $\log I_f$(excimer) *vs.* $\log[N]$.

-10 J mol^{-1} K^{-1}, respectively. These thermodynamic quantities are similar to those obtained for other 1:1 inclusion compounds with CDx.[1,17] Unfortunately, these quantities could not be obtained for K_2 since the shape of the I_f(excimer) curve shown in Fig. 6 was relatively insensitive to the temperature change.

Fluorescence decay times and fluorescence quenching by I^- and IO_3^- were also studied for the three components in the naphthalene–CDx system. An aqueous solution of naphthalene alone for the uncomplexed naphthalene and a solution of naphthalene (6.0×10^{-5} M) and CDx (8.4×10^{-3} M) for NC and N_2C_2 were examined. With the 283 nm excitation the quenching of the 330 nm fluorescence bands for N and NC and the 410 nm band for N_2C_2 was studied by adding varying amounts of KI or KIO_3. Strictly speaking, the 330 nm band of the naphthalene–CDx solution contains the fluorescence from the uncomplexed naphthalene, but since its contribution was calculated to be only about 10%, no corrections were applied for the quenching data for NC. The results were analyzed by the usual Stern-Volmer relation:

$$\frac{I_f^0}{I_f} = 1 + k_q\tau'[Q], \tag{12}$$

where I_f^0 and I_f represent the fluorescence intensities in the absence and presence of a quencher Q (using the same notations for N, NC, and N_2C_2 for simplicity), k_q is the rate constant for quenching, and τ' is the fluorescence lifetime of each species in aerated solution. As an example, the Stern-Volmer plots for quenching by IO_3^- are shown in Fig. 11. In Table 1 are summarized the fluorescence lifetimes and the rate constants k_q.[18,19] The reduction of the quenching efficiencies by forming the 1:1 inclusion compounds has already been shown for many other systems and has been attributed to the CDx molecule partly preventing the quencher molecule to penetrate into its cavity.[10,20] The further reduction of the quenching efficiencies for N_2C_2 indicates that it is more difficult for the quencher molecule to approach the naphthalene molecules contained in the two CDx molecules

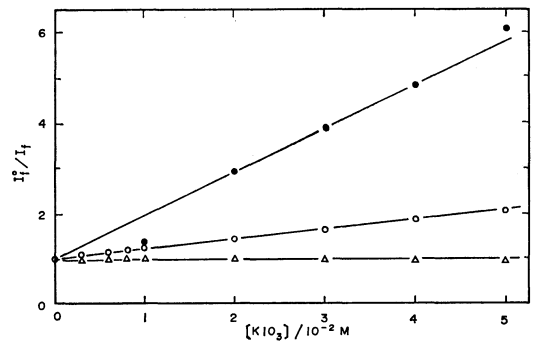

Fig. 11. Stern-Volmer plots for fluorescence quenching of naphthalene (●), NC (○), and N_2C_2 (△) by IO_3^-.

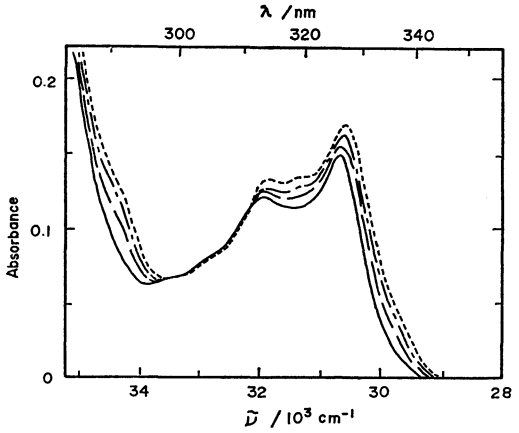

Fig. 12. Absorption spectra of MN (9.60×10^{-5} M) in aqueous solution in the presence of CDx.
Initial concentration of CDx, ——: 0 M, — —: 8.40×10^{-4} M, — — —: 2.52×10^{-3} M, - - -: 8.40×10^{-3} M.

Table 1. Fluorescence lifetimes (τ') in aerated solution and rate constants for fluorescence quenching (k_q) for N, NC, and N_2C_2 in the naphthalene-CDx system

		N	NC	N_2C_2
τ'/ns		40 (39)[a] (35)[b]	48	68
$k_q/10^9$ M^{-1} s^{-1}	I^-	6.0	3.9	1.8
	IO_3^-	2.4	0.45	0

a) R. R. Hautala, N. E. Schore, and N. J. Turro, *J. Am. Chem. Soc.*, **95**, 5508 (1973). b) M. Van Bockstaele, J. Gelan, H. Martens, J. Put, J. C. Dederen, N. Boens, and F. C. de Schrijver, *Chem. Phys. Lett.*, **58**, 211 (1978).

than in NC, the extreme case being that no quenching was observed by IO_3^- ion which is bulkier than I^-.

Inclusion Compounds in 2-Methoxynaphthalene–o-Dicyanobenzene–β-Cyclodextrin System. Absorption spectra of 2-methoxynaphthalene (MN) aqueous solutions containing varying concentrations of CDx are shown in Fig. 12. Isosbestic points observed at 310 nm and ≈300 nm indicate the following equilibrium:

$$MN + CDx \overset{K_3}{\rightleftharpoons} MC, \qquad (13)$$

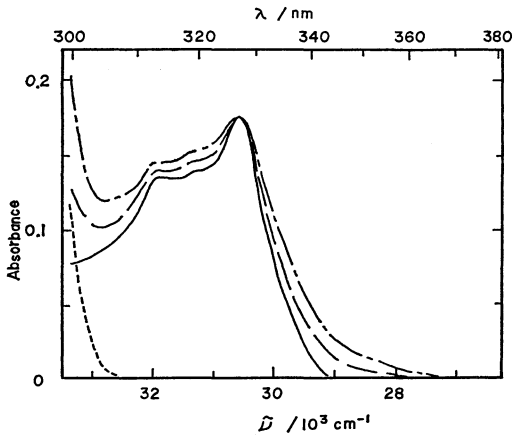

Fig. 13. Absorption spectra of MN (9.60×10^{-5} M)–CDx (8.40×10^{-3} M) in aqueous solution in the presence of DB.
Initial concentration of DB, ——: 0 M, — —: 7.0×10^{-4} M, — — —: 2.0×10^{-3} M. - - - -: absorption spectrum of DB (2.0×10^{-3} M) in the presence of CDx (8.40×10^{-3} M).

where MC is the 1:1 inclusion compound of CDx with MN and K_3 is the equilibrium constant for the formation of MC. The value of K_3 was determined from the Benesi-Hildebrand plot (Eq. 2) using the absorbance changes at 330 nm. $K_3=630$ M^{-1} was obtained.

Very little changes were observed in the absorption spectrum of an *o*-dicyanobenzene (DB) aqueous solution on adding CDx. However, the fluorescence intensity of DB ($\lambda_{max}=307$ nm) decreased with increasing the CDx concentration. We attribute this change as being due to the formation of a 1:1 inclusion compound, DC, *i.e.*,

$$DB + CDx \overset{K_4}{\rightleftharpoons} DC. \qquad (14)$$

From the Benesi-Hildebrand plot for the change in fluorescence intensity (Eq. 7), the value of the equilibrium constant K_4 was determined to be 15 M^{-1}.

Figure 13 shows the absorption spectra of the MN–CDx solutions containing varying amounts of DB. The appearance of a new band extending to longer wavelengths is seen on adding DB. This band is most plausibly due to an MN–DB charge transfer (CT) complex since MN is an electron donor and DB an electron acceptor. Such a CT band, however, could not be observed for the solutions containing only MN and DB in 1,4-dioxane, ethyl ether, and toluene (up to the DB concentrations of 0.2 M, 0.1 M, and 0.1 M, respectively) or in water ($<4\times10^{-3}$ M).

Figure 14 shows the fluorescence spectra of the aqueous solutions of MN alone, MN with CDx, and MN with DB and CDx. The enhancement of the 350 nm band with the addition of CDx indicates that the fluorescence quantum yield of MC is higher than that of MN.[21,22] On adding DB, a structureless band having λ_{max} at 480 nm appears at the expense of the 350 nm band. The excitation spectrum of this band is found to coincide with the long wavelength tail of the absorption spectrum, and thus this band can be assigned to the fluorescence from the CT complex of

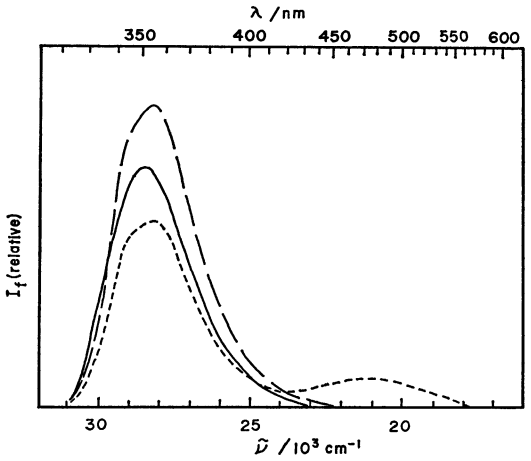

Fig. 14. Fluorescence spectra of MN (——), MN-CDx (— —), and MN–DB–CDx (- - - -) in aqueous solution.
$[MN]_0=9.60\times10^{-5}$ M; $[CDx]_0=8.40\times10^{-3}$ M; $[DB]_0=2.0\times10^{-3}$ M. $\lambda_{exc}=310$ nm.

MN and DB. This assignment is supported by our findings that the fluorescence from an exciplex formed between MN and DB in 1,4-dioxane, the dielectric constant of which is almost the same as that in the CDx cavity,[1] peaks at 480 nm.

Since, as mentioned above, MN and DB alone do not form a CT complex in their ground states in the solvents we have examined, the two substrate molecules are obviously forced to approach each other by virtue of the presence of CDx. Two alternatives can be considered as the species which forms the CT complex in the ground state and which emits the CT fluorescence in the excited state. One is a 1:1:1 inclusion compound, MDC, which is composed of one CDx molecule filled with an MN molecule and a DB molecule. The other is a 1:1:2 inclusion compound, MDC_2, formed by association of MC and DC. The formation of such species is represented by

Mechanism 3) $$MC + DB \overset{K'_5}{\rightleftharpoons} MDC, \quad (15)$$

$$MN + DC \overset{K'_6}{\rightleftharpoons} MDC, \quad (16)$$

and

Mechanism 4) $$MC + DC \overset{K_5}{\rightleftharpoons} MDC_2. \quad (17)$$

In the following, we will examine which of the two species, MDC or MDC_2, is the real species present in the MN–DB–CDx system using analyses somewhat similar to those employed for the naphthalene–CDx system. The fluorescence intensity of MC, I_f(MC), which is proportional to [MC] in the MN–DB–CDx system, should satisfy the following relations for Mechanism 3 and 4, respectively:[23]

$$\frac{1}{I_f(MC)}=\frac{K_3K'_5[CDx]_0}{c[MN]_0(1+K_4[CDx]_0)}[DB]_0 + \frac{(1+K_3[CDx]_0)}{c[MN]_0}, \quad (18)$$

and

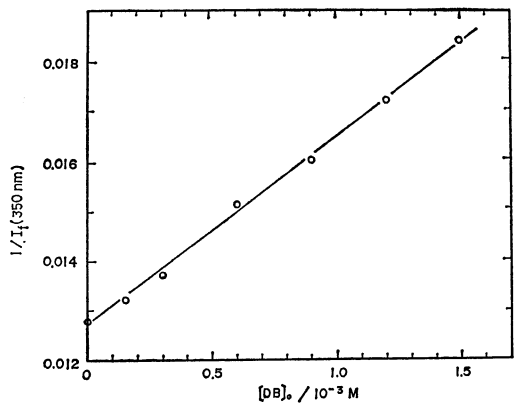

Fig. 15. Plot of $1/I_f$(350 nm) *vs.* $[DB]_0$.
$[MN]_0=9.60\times10^{-5}$ M; $[CDx]_0=8.40\times10^{-3}$ M.

$$\frac{1}{I_f(MC)}=\frac{K_4K_5[CDx]_0}{c[MN]_0(1+K_4[CDx]_0)}[DB]_0 + \frac{(1+K_3[CDx]_0)}{cK_3[MN]_0[CDx]_0}, \quad (19)$$

where $[MN]_0$, $[DB]_0$, and $[CDx]_0$ refer to the initial concentration of each substrate, and c is an instrumental constant. When I_f(MC) is measured as a function of the DB concentration at fixed concentrations of MN and CDx, both Eq. 18 and Eq. 19 predict the plots of $1/I_f$(MC) *vs.* $[DB]_0$ to be linear. We cannot, therefore, discriminate between the two mechanisms from such plots alone. However, we should be able to determine the values of K'_5 and K_5, since the equilibrium constants K_3 and K_4 have already been obtained ($K_3=630$ M^{-1}, $K_4=15$ M^{-1}). Our next step is the following. Whichever of the two mechanisms is operating, we should be able to calculate the expected concentration of MDC or MDC_2 as a function of the CDx concentration through the relation containing either K'_5 or K_5 with the common values of K_3 and K_4. We will then compare the measured intensity of the CT fluorescence, I_f(CT), with the calculated curves of [MDC] and [MDC_2] and see which of the concentration curves better reproduce the observed I_f(CT) curve.

As a first step of the analysis, the reciprocal of the fluorescence intensities of the 350 nm band, $1/I_f$(350 nm), is shown plotted against $[DB]_0$ in Fig. 15. Simple kinetics shows that I_f(350 nm), which contains the fluorescence from the uncomplexed MN too (amounting to $\approx10\%$ of I_f(350 nm)), is proportional to I_f(MC), and thus the analyses by Eqs. 18 and 19 are possible. The values we obtain from Fig. 15 are: $K'_5=400$ M^{-1}, $K_5=3200$ M^{-1}. We now advance to the second step.

In order to make a comparison with the naphthalene–CDx system easier, it will be preferable to keep the concentrations of the two kinds of inclusion compound, MC and DC, approximately the same at each CDx concentration. We therefore employed the system in which $[MN]_0$ was fixed but $[DB]_0$ was varied according to the relation: $[DB]_0/[MN]_0=K_3/K_4(1+K_3[CDx]_0)$. (If the equilibriums involving MN–CDx and DB–CDx stop at the first stage of forming 1:1 inclusion compounds, [MC]=[DC] when $[DB]_0$ is varied by this relation.)

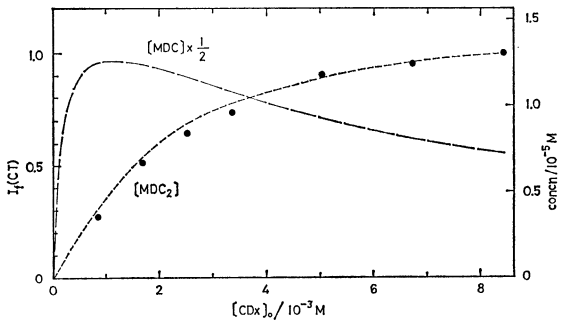

Fig. 16. Comparison of the observed CT fluorescence intensities, I_f(CT), with calculated concentrations [MDC] and [MDC_2].
●: I_f(CT), ——: [MDC], - - - -: [MDC_2].
$[MN]_0=5.0\times10^{-5}$ M. I_f(CT) is normalized to match the calculated [MDC_2] at the highest concentration of $[CDx]_0$.

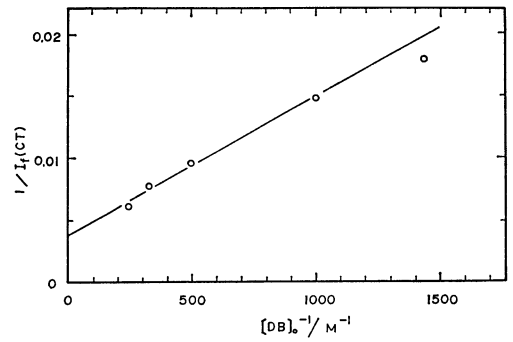

Fig. 17. Plot of $1/I_f$(CT) *vs.* $1/[DB]_0$.
$[MN]_0=9.60\times10^{-5}$ M; $[CDx]_0=8.40\times10^{-3}$ M.

The comparison of the variation of the experimentally observed I_f(CT) with $[CDx]_0$ under such conditions with the calculated curves of [MDC] and [MDC_2] is displayed in Fig. 16. It is immediately apparent that the calculated [MDC_2] curve reproduces the I_f(CT) curve fairly well, whereas the [MDC] curve nowhere matches the experimental curve. We are therefore led to conclude that MDC_2, not MDC, is the species responsible for the CT absorption and CT fluorescence in the MN–DB–CDx system.

Once Mechanism 4 is established, a simpler analysis of the CT fluorescence than that used above can be made. Namely, if we measure the CT fluorescence intensities as a function of $[DB]_0$ at fixed concentrations of MN and CDx, I_f(CT) can be expressed by

$$\frac{1}{I_f(CT)}=\frac{(1+K_3[CDx]_0)(1+K_4[CDx]_0)}{dK_3K_4K_5[MN]_0[CDx]_0^2}\frac{1}{[DB]_0}+\frac{1}{d[MN]_0}, \quad (20)$$

where d is an instrumental constant. A plot of $1/I_f$(CT) *vs.* $1/[DB]_0$ should give a linear relationship. Figure 17 shows such a plot, and with the known values of K_3 and K_4, the value $K_5=3700$ M^{-1} is obtained.[24] The K_5-value can also be determined from the absorbance of the CT absorption band, A(CT), of the same system which should have the same dependence on $[DB]_0$ as in Eq. 20. The value of K_5 evaluated from this type of analysis was 3300 M^{-1}.

Table 2. Equilibrium constants K_3, K_4, and K_5 at 25 °C, and enthalpy changes (ΔH) and entropy changes (ΔS) for K_3, K_4, and K_5 in the MN-DB-CDx system

	M^{-1} (at 25 °C)	ΔH / kJ mol^{-1}	ΔS / J mol^{-1} K^{-1}
K_3	630	−17	−5.0
K_4	15	−11	−16
K_5	3200[a]	−41	−16

a) The value obtained from I_f(MC) by Eq. 19.

These values of K_5 are close to the equilibrium constant obtained for the association of the 1:1 inclusion compounds in the naphthalene–CDx system ($K_2=4000$ M^{-1}). The previously determined value of K_5(=3200 M^{-1}) from Eq. 19 is solely from the intensity variation of the MC fluorescence (at 350 nm). The fairly good agreement between this K_5 and those obtained above from the CT bands implies that an exciplex type fluorescence is not involved in the observed 480 nm band which we have attributed to the CT type fluorescence.

The temperature dependence of the equilibrium constants, K_3, K_4, and K_5 was examined by measuring, respectively, the absorption spectra of the MC–CDx system, the fluorescence intensities of the DB–CDx system, and I_f(MC) of the MC–DB–CDx system in the temperature range from 3 to 25 °C, and by obtaining at each temperature the equilibrium constants as was done at 25 °C. The enthalpy changes ΔH and the entropy changes ΔS for K_3, K_4, and K_5 obtained from such measurements are summarized in Table 2.

It will be noted that the values of both $-\Delta H$ and ΔS for K_4 are smaller than those for K_3. This is consistent with the much smaller value of K_4 than K_3. The relatively inefficient formation of DC from DB and CDx may be due probably to the fact that the solubility of DB in water is about an order of magnitude larger than that of MN, and also that the steric effect of the two ortho-substituted linear cyano groups of DB may hinder the efficient complexing with CDx. The smaller value of $-\Delta S$ for K_3 than those for K_4 and K_5 indicates the less rigid structure of MC than DC and MDC_2.

As in the case of the naphthalene–CDx system, fluorescence decay times and fluorescence quenching by I^- and IO_3^- were studied for MN, MC, and MDC_2. Aqueous solutions of MN alone, MN–CDx, and MN–DB–CDx ($[MN]_0=9.6\times10^{-5}$M, $[CDx]_0=8.4\times10^{-3}$M, and $[DB]_0=2.0\times10^{-3}$ M) were examined. With the 316 nm excitation, the quenching of the 350 nm bands for MN and MC and of the 480 nm band for MDC_2 was measured by adding varying amounts of KI or KIO_3. No corrections were applied for quenching of the 350 nm band of MC which contains about 10% of MN fluorescence. The fluorescence lifetimes and the quenching rate constants for each species are presented in Table 3. One immediately finds upon comparison with Table 1 that the quenching behavior of these ions for MN, MC, and MDC_2 is very similar to that for the corresponding species in the naphthalene–CDx system. Thus, nearly the same structure of

TABLE 3. FLUORESCENCE LIFETIMES (τ') IN AERATED SOLUTION AND RATE CONSTANTS FOR QUENCHING (k_q) FOR MN, MC, AND MDC_2 IN THE MN-DB-CDx SYSTEM

		MN	MC	MDC_2
τ'/ns		11	12	39
$k_q/10^9\ M^{-1}\ s^{-1}$	I^-	6.0	2.6	1.7
	IO_3^-	2.8	0.60	−0.06

the 1:1:2 inclusion compound as that of the 2:2 inclusion compound (N_2C_2) is implicated from these quenching results.

Discussion

Sodium 1-naphthyl acetate (electron donor) and picric acid (electron acceptor) do not form a complex under usual conditions, but recently Kobayashi *et al.*[25] reported the formation of a charge transfer complex (CT) between them in the presence of γ-cyclodextrin but not significantly in the presence of β-cyclodextrin. They attributed these results to the capability of γ-cyclodextrin to include both electron donor and acceptor molecules in its cavity simultaneously.[26] What we have found in the MN–DB–CDx study is a similar promotion of the CT complex formation between MN and DB, but in this case by virtue of the association of two β-cyclodextrin molecules each of which accommodates one electron donor or acceptor molecule in its cavity. Much less efficient complex formation by β-cyclodextrin found in the Kobayashi *et al.*'s system appears to imply the incapability of the β-cyclodextrin molecule to hold the two molecules in its cavity, probably due to its smaller cavity size than in γ-cyclodextrin. Their results are thus consistent with our interpretation assigning the species responsible for the CT absorption and CT fluorescence to the 1:1:2 inclusion compound, not to the 1:1:1 inclusion compound.

Our results on the naphthalene–CDx system show that a similar type of association of two 1:1 inclusion compounds can occur when an aromatic hydrocarbon molecule is included in the CDx molecule. We did not find an excimer-type emission in the benzene–CDx system. Solubility restrictions prevented us from pursuing the same type of experiment for an anthracene–CDx or pyrene–CDx system. We therefore do not know at present whether the deciding factor for the formation of the 2:2 inclusion compound in an aromatic–CDx system is merely the molecular size of the substrate or if some other specific physicochemical properties of the substrate are involved. Such an association may be hindered for inclusion complexes containing a molecule larger than naphthalene by its protrusion out of the CDx cavity.

What remains to be discussed here is the problem of what kind of binding force brings the two 1:1 inclusion compounds together. One possible explanation is that the CDx molecules in aqueous solution (in the absence of any substrates) may be capable of forming a dimer by themselves. To the author's knowledge, however, there has been no experimental evidence pointing to the occurrence of such phenomena in cyclodextrin systems. Another explanation is to consider such an association as a first stage of the precipitation process. This idea comes from the fact that the solubility of the inclusion compounds is generally smaller than that of CDx. Once a 2:2 or 1:1:2 adduct is formed by adhesion of two 1:1 adducts in the course of precipitation, its unique structure of two CDx shells shielding completely the substrate molecules within them may prevent the attack of other 1:1 adducts to form a higher aggregate. That is, the first stage of the precipitation process may persist over a fairly wide range of concentration owing to the peculiar properties of the CDx inclusion compounds. Of course, this is only a speculation. Elucidation of further details in various aspects of 2:2 or 1:1:2 inclusion compounds will require more systematic studies using a variety of substrate materials and will have to wait for future study.

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18) CDx also forms inclusion compounds with inorganic anions. Rohrbach *et al.* (*J. Phys. Chem.*, **81**, 944 (1977)) reported that the equilibrium constant for the formation of an inclusion compound with I^- is 18.0 M^{-1} in aqueous so-

lution at 25 °C. They employed a competition method in which the indicator was an azo dye. We obtained, however, the values of 4.1 and 4.7 M^{-1} for I^- using neutral substances, 2-naphthol and 1,4-naphthoquinone, respectively, by a similar competition method. It is not apparent why such a discrepancy occurs between our values and their value. The equilibrium constant for IO_3^- was determined to be 1.0 M^{-1} using 1,4-naphthoquinone. Because of these small values of the equilibrium constants, formation of the inclusion compounds of CDx with I^- and IO_3^- does not seriously change the concentration of CDx in the quenching experiments.

19) The viscosity of water increases only about 2% on adding 8.4×10^{-3} M of CDx. Thus, the viscosity effect on the quenching constant k_q is negligible.

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21) The equilibrium constant K_3 for formation of MC can also be determined from the Benesi-Hildebrand plot for the fluorescence increment (Eq. 7). The value obtained is 660 M^{-1} which is close to 630 M^{-1} determined from the absorption spectra.

22) In more concentrated solutions of MN ($>5\times10^{-4}$ M), an excimer-like fluorescence appeared at around 420 nm. Although this emission is not as pronounced as in the case of naphthalene–CDx system, this observation suggests the possibility of the presence of a 2:2 inclusion compounds in the MN–CDx system in the high MN concentration region.

23) In deriving Eqs. 18 and 19, the fluorescence intensity of MC is regarded as that solely from the 1:1 inclusion compound MC, *i.e.*, a monomer-like emission from the MC part of MDC or MDC_2 is assumed not to occur. Since we define here MDC or MDC_2 as the species which exhibits the CT absorption, and the CT interaction is generally stronger in the excited state than in the ground state, this assumption seems reasonable.

24) The plots of $1/I_f(CT)$ *vs.* $1/[DB]_0$ shown in Fig. 17 can also be analyzed by Mechanism 3 which we have already discarded. Such an analysis gives $K'_5=8.7$ M^{-1} whereas the K'_5-value obtained from $I_f(MC)$ by Eq. 18 is 400 M^{-1} as stated before. This vast inconsistency further supports the inadequacy of Mechanism 3.

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