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1:1:1 Inclusion Compounds of β-Cyclodextrin with Fluorene and Alcohols or Nitriles in Aqueous Solution

Sanyo HAMAI
Department of Physics, Miyazaki Medical College, Kiyotake, Miyazaki 889-16
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Fluorene forms a 1:1 inclusion compound (FC) with β-cyclodextrin (CDx) in aqueous solution. From electronic absorption and emission spectra, FC has been found to further associate with alcohol (from ethanol to 1-pentanol) or nitrile (from propionitrile to hexanenitrile) to form a 1:1:1 inclusion compound. An equilibrium constant (K_3) for the formation of the 1:1:1 inclusion compound from FC and alcohol, and that (K_4) from fluorene and a 1:1 inclusion compound of CDx with alcohol have been determined. Upon going from ethanol to 1-pentanol, the value of K_4 increases, reaches a maximum at 1-propanol, and then decreases while K_3 monotonously increases with an increase in the alkyl-chain length of alcohol. For nitriles, K_3 and K_4 have also been evaluated. In this case, the largest K_4 value is seen for propionitrile and butyronitrile, while K_3 increases with increasing the alkyl-chain length of nitrile.

In the β-cyclodextrin (CDx)–alcohol–pyrene and CDx–alcohol–acenaphthene systems, we have found that association occurs between alcohol and a 1:1 inclusion compound of CDx with pyrene or acenaphthene.[1,2] From fluorescence lifetime measurements, Nelson et al. have proposed that 2-methyl-2-propanol is associated with a CDx–pyrene 1:1 inclusion compound.[3] Such behavior of the 1:1 inclusion compound of pyrene or acenaphthene seems to be reasonable since these aromatic guests are too large to bind deeply into the CDx cavity. From comparisons of equilibrium constants for the formation of a ternary 1:1:1 inclusion compound, it was concluded that 1-propanol, among primary alcohols, fits most snugly into the void interior space of the CDx cavity which already accommodates pyrene or acenaphthene.[1,2] These results suggest that the fit of two guest molecules of different kinds into the CDx cavity plays an important role in forming the 1:1:1 inclusion compound.

From a stereochemical point of view, the whole body of a fluorene molecule is anticipated to be deeply incorporated into the cavity, thereby the interior dimension of the cavity being thoroughly occupied by fluorene. Under such circumstances, it seems to be difficult for a 1:1 inclusion compound of CDx with fluorene to associate with an additional alcohol molecule. Thus, in order to further clarify the nature of 1:1:1 inclusion compounds of CDx, we investigated whether or not the CDx–fluorene 1:1 inclusion compound complexes with additional alcohol or nitrile by means of measurements of absorption and fluorescence spectra.

Experimental

Fluorene purchased from Tokyo Kasei was recrystallized twice from ethanol. β-Cyclodextrin (CDx) from Nakarai was recrystallized three times from water. Alcohols (from methanol to hexanol) and nitriles (from acetonitrile to hexanenitrile) were distilled under atmospheric or reduced pressure. Fluorene aqueous solutions were prepared according to the same method as that used for pyrene.[4] The fluorene concentration of sample solutions was (6—7)$\times10^{-6}$ mol dm^{-3}. Aerated sample solutions were employed throughout this work. Absorption spectra were run on a Shimadzu UV-260 spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-501 spectrofluorometer equipped with a cooled Hamamatsu R-943 photomultiplier, and were corrected for the spectral response of the fluorometer. All measurements were made at 25±0.1 °C, unless otherwise noted.

Results and Discussion

1:1 Inclusion Compound of CDx with Fluorene. Figure 1 shows the absorption spectra of fluorene aqueous solutions containing various concentrations of CDx. The addition of CDx resulted in red shifts of absorption peaks and the appearance of isosbestic points at 294 and 299 nm. The spectral change is attributed to the formation of a 1:1 inclusion compound (FC) of CDx with fluorene:

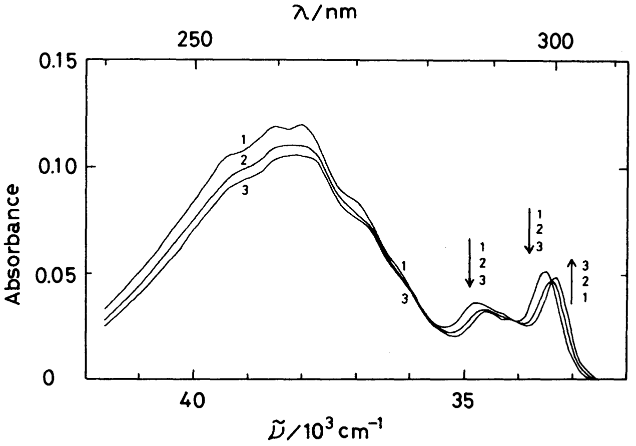

Fig. 1. Absorption spectra of fluorene (ca. 6×10^{-6} mol dm^{-3}) in the presence of CDx. Concentration of CDx: (1) 0 mol dm^{-3}; (2) 8.4×10^{-4} mol dm^{-3}; (3) 4.2×10^{-3} mol dm^{-3}.

$$\mathrm{FL} + \mathrm{CDx} \overset{K_1}{\rightleftharpoons} \mathrm{FC}, \tag{1}$$

where FL represents fluorene, and K_1 is an equilibrium constant for the formation of FC. From the absorbance change of fluorene,[5] K_1 has been determined to be 1200±100 mol^{-1} dm^3. This value is nearly twice that for naphthalene (685 mol^{-1} dm^3)[5] and one to two orders of magnitude greater than those for pyrene (7.6 mol^{-1} dm^3)[1] and acenaphthene (76±5 mol^{-1} dm^3).[2] Since pyrene and acenaphthene cannot bind deeply into the CDx cavity owing to the steric hindrance, smaller K_1 values are obtained. A naphthalene ring fully enters the cavity,[6] resulting in the relatively large value of K_1. A comparison between these K_1 values suggests that in FC the hydrophobic interaction occurs between CDx and fluorene, i.e., as expected above, the whole body of a nearly prolate fluorene molecule deeply penetrates into the CDx cavity. An examination of a space-filling molecular model supports such a picture of FC. When CDx is added to a fluorene aqueous solution, the intensity ratio of the 304-nm peak to the 312-nm peak in the fluorene fluorescence is enhanced. This change in the fluorescence spectra also shows the complex formation between CDx and fluorene. From the fluorescence intensity change caused by the addition of CDx, a K_1 value (1100±100 mol^{-1} dm^3) similar to that obtained from the absorption method was evaluated.[7]

1:1:1 Inclusion Compounds of CDx with Fluorene and Alcohols. Figure 2 illustrates the absorption spectra of fluorene aqueous solutions containing both CDx (4.2×10^{-3} mol dm^{-3}) and varying amounts of 1-butanol (Bu). Isosbestic points are observed at 256, 261, 286, 290, 298, and 301 nm, and a new shoulder appears at ca. 303 nm, which locates at longer wavelengths than the 0–0 band of fluorene in water or in water containing CDx. Upon cooling to 5 °C, this shoulder becomes a sharp peak at 304 nm. Such spectral changes have never been observed in the absence of CDx. Consequently, the absorption change shown in Fig. 2 can be interpreted in terms of the formation of a ternary inclusion compound composed of CDx, fluorene, and Bu. This conclusion is consistent with Scypinsky and Cline Love's suggestion that CDx, 1,2-dibromoethane, and fluorene forms a ternary complex.[8] Figure 3 exhibits the normalized fluorescence of fluorene in aqueous solutions contain-

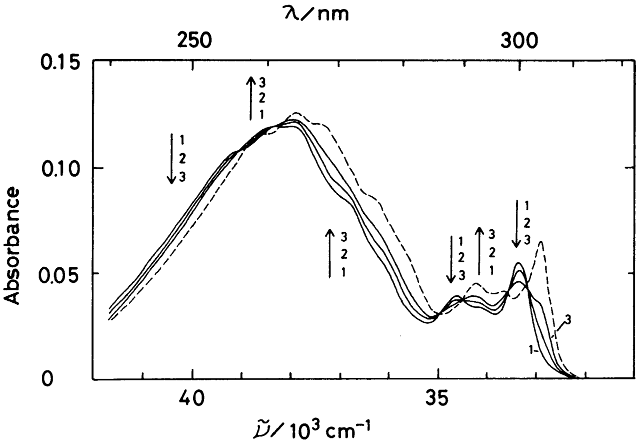

Fig. 2. Absorption spectra of fluorene (ca. 6×10^{-6} mol dm^{-3}) aqueous solutions containing CDx (4.2×10^{-3} mol dm^{-3}) and varying concentrations of Bu at 25 °C and the absorption spectrum (----) of a fluorene solution containing CDx (4.2×10^{-3} mol dm^{-3}) and Bu (1.09×10^{-1} mol dm^{-3}) at 5 °C. Concentration of Bu: (1) 0 mol dm^{-3}; (2) 3.28×10^{-2} mol dm^{-3}; (3) 1.09×10^{-1} mol dm^{-3}.

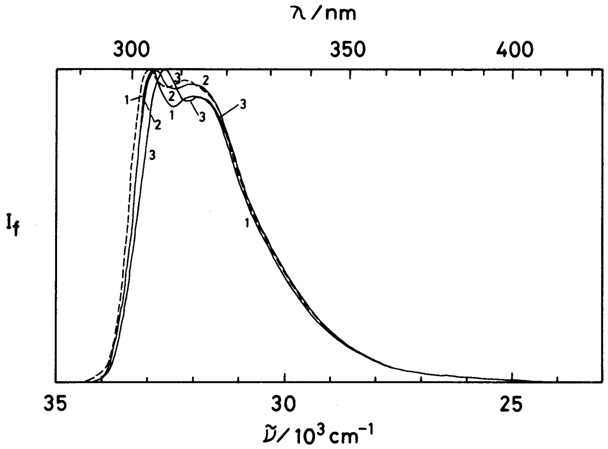

Fig. 3. Normalized fluorescence spectra of fluorene (ca. 6×10^{-6} mol dm^{-3}) aqueous solutions containing CDx (4.2×10^{-3} mol dm^{-3}) and varying concentrations of Bu. For comparison, the normalized fluorescence spectrum (----) of fluorene in aqueous solution is also shown. Concentration of Bu: (1) 0 mol dm^{-3}; (2) 2.19×10^{-2} mol dm^{-3}; (3) 1.09×10^{-1} mol dm^{-3}. λ_{ex}=298 nm.

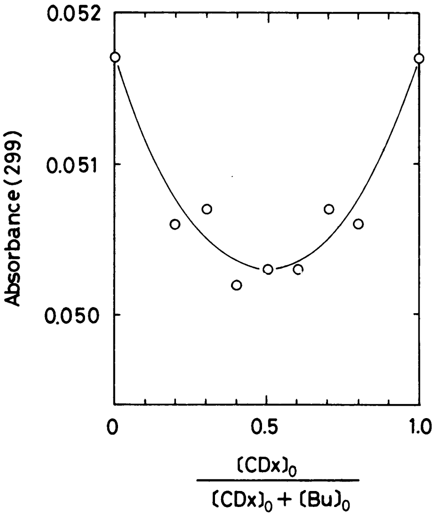

Fig. 4. Continuous variation plot under the condition of [CDx]$_0$+[Bu]$_0$=1×10^{-2} mol dm^{-3}. The observed wavelength is 299 nm. The fluorene concentration is ca. 6.5×10^{-6} mol dm^{-3}.

ing both CDx (4.2×10^{-3} mol dm^{-3}) and varying concentrations of Bu. Upon the addition of Bu, the fluorescence maximum is shifted to longer wavelengths. The red shift in the fluorescence spectrum shown in Fig. 3 corresponds to that in the absorption spectrum in Fig. 2. In order to determine the stoichiometry of the ternary inclusion compound responsible for the red shifts in the absorption and fluorescence spectra, we applied a continuous variation method,[1,4] in which the absorbance at 299 nm was monitored as a function of the molar fraction of CDx under the condition that a sum of the initial concentrations of CDx and Bu was held constant at 1×10^{-2} mol dm^{-3} while the fluorene concentration was fixed. The experimental results are shown in Fig. 4. Although there is some scatter of the data, a minimum at a molar fraction of 0.5 evidently indicates a 1:1 stoichiometry concerning CDx and Bu. When Bu less than 5×10^{-2} mol dm^{-3} is added to a fluorene solution containing CDx (4.2×10^{-3} mol dm^{-3}), the absorbance at 305 nm is proportional to the Bu concentration, indicating that the ternary inclusion compound contains only one Bu molecule. From these results, the ternary inclusion compound can be assigned to a 1:1:1 inclusion compound (FBC) of CDx with fluorene and Bu. Such 1:1:1 inclusion compounds including alcohol have already been reported for the cases of pyrene and acenaphthene.[1,2] The formation of FBC is represented by the following equilibria:

$$\mathrm{Bu} + \mathrm{CDx} \overset{K_2}{\rightleftharpoons} \mathrm{BC}, \qquad (2)$$

$$\mathrm{FC} + \mathrm{Bu} \overset{K_3}{\rightleftharpoons} \mathrm{FBC}, \qquad (3)$$

and

$$\mathrm{FL} + \mathrm{BC} \overset{K_4}{\rightleftharpoons} \mathrm{FBC}, \qquad (4)$$

where BC is a 1:1 inclusion compound of CDx with Bu, and K_2, K_3, and K_4 are equilibrium constants for the formation of BC, FBC from FC and Bu, and FBC from fluorene and BC, respectively. As in the case of the CDx-alcohol-pyrene system,[1] K_3 can be determined from a comparison of the observed fluorescence intensities, which are a function of the Bu concentration, with curves calculated by assuming three parameters. By using an instrumental constant a, the fluorescence quantum yield ϕ, the molar absorption coefficient ε at the excitation wavelength, and subscripts 1, 2, and 3 which stand for fluorene, FC, and FBC, respectively, one can write the fluorene fluorescence intensity I_f as[1]

$$I_f = A - B/(C + [\mathrm{Bu}]_0), \qquad (5)$$

where $A=a\phi_3\varepsilon_3[\mathrm{FL}]_0$, $B=a\{\phi_3\varepsilon_3K_1K_3[\mathrm{CDx}]_0(1+K_1[\mathrm{CDx}]_0)(1+K_2[\mathrm{CDx}]_0)[\mathrm{FL}]_0/K_1K_3[\mathrm{CDx}]_0-(\phi_1\varepsilon_1+\phi_2\varepsilon_2K_1[\mathrm{CDx}]_0)(1+K_2[\mathrm{CDx}]_0)[\mathrm{FL}]_0/K_1K_3[\mathrm{CDx}]_0\}$, and $C=(1+K_1[\mathrm{CDx}]_0)(1+K_2[\mathrm{CDx}]_0)/K_1K_3[\mathrm{CDx}]_0$. Here, subscript 0 refers to the initial concentration. The least-squares fit of a simulation curve was obtained when $A=110$, $B=3.07$ mol dm^{-3}, and $C=0.0573$ mol dm^{-3} (Fig. 5). From this C value, the K_1 value determined in this study, and the K_2 value reported by Matsui and Mochida,[9] K_3 was evaluated to be 22±1 mol^{-1} dm^3. From a relationship $K_1K_3=K_2K_4$, K_4 was estimated to be 1900±200 mol^{-1} dm^3. These K_3 and K_4 values are given in Table 1.

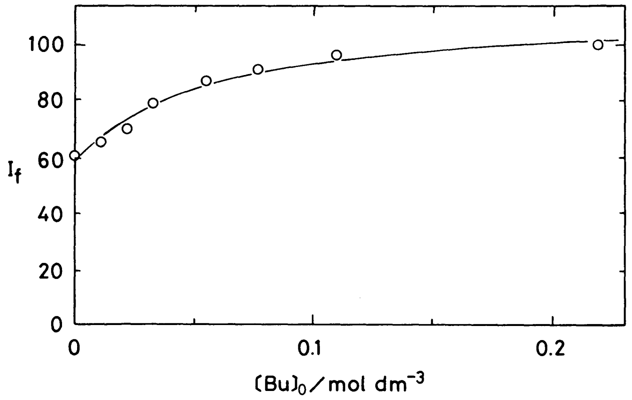

Fig. 5. Comparison between observed fluorescence intensities of fluorene (ca. 6×10^{-6} mol dm^{-3}) and a least-squares fit curve calculated with assumed parameters $A=110$, $B=3.07$ mol dm^{-3}, and $C=0.0573$ mol dm^{-3}. The maximum fluorescence intensity is normalized to 100. $[\mathrm{CDx}]_0=4.2\times10^{-3}$ mol dm^{-3}. $\lambda_{ex}=303$ nm.

The addition of alcohols from ethanol to 1-pentanol to fluorene aqueous solutions with CDx results in an absorption change similar to that shown in Fig. 2, indicating the formation of 1:1:1 inclusion compounds containing alcohol.[10] For alcohols other than Bu, K_3 and K_4 have similarly been evaluated, and are also summarized in Table 1. As the alkyl chain of alcohol is lengthened, K_3 monotonously increases like K_2. On the other hand, the largest K_4 is obtained for 1-propanol, and K_4 is reduced when the alkyl chain of alcohol is shortened or lengthened relative to that of 1-propanol. Such characteristics of K_3 and K_4 are quite similar to those obtained for the CDx–alcohol–pyrene and CDx–alcohol–acenaphthene systems.[1,2] In the 1:1 inclusion compound of pyrene or acenaphthene, the CDx cavity which accommodates pyrene or acenaphthene has a void space, while in the 1:1 inclusion compound of fluorene there is no void dimension inside the cavity since the cavity is fully occupied by fluorene. From the stereochemical standpoint, it is surprising that an additional guest, alcohol, can associate with the CDx–fluorene 1:1 inclusion compound. Therefore, owing to the formation of the 1:1:1 inclusion compound of fluorene, a fluorene molecule within the CDx cavity may protrude from the cavity to some extent to make a void space for an additional alcohol molecule. In the case of fluorene, the K_3 values are two to three orders of magnitude smaller than those for the pyrene or

Table 1. Equilibrium Constants for the Formation of 1:1 and 1:1:1 Inclusion Compounds

	K_2/mol^{-1} dm^3	K_3/mol^{-1} dm^3	K_4/mol^{-1} dm^3
Ethanol	0.93[a]	0.94±0.05	1400±100
1-Propanol	3.72[a]	7.9±0.4	3000±300
1-Butanol	16.6[a]	22±1	1900±200
1-Pentanol	63.1[a]	78±3	1800±200
Propionitrile	3.8±0.3	3.6±0.2	1300±200
Butyronitrile	16±1	15±1	1300±200
Valeronitrile	170±10	86±5	740±130
Hexanenitrile	510±30	120±10	340±60

a) Data of Matsui and Mochida (Ref. 9).

acenaphthene system,[1,2,11] indicating that the complexation reaction between the CDx–fluorene 1:1 inclusion compound and additional alcohol is not so favorable as that between a CDx–pyrene or CDx–acenahthene 1:1 inclusion compound and alcohol. The same is true for K_4.[12]

As mentioned above, at low temperatures the 0–0 absorption band of the 1:1:1 inclusion compound appears at 304 nm. This peak wavelength is rather abnormal because fluorene exhibits the peak of the 0–0 band at 301, 300, 300, 301, 300, 300, 299, and 298 nm in cyclohexane (ε=2.02), 1,4-dioxane (ε=2.21), ethyl acetate (ε=6.02), dichloromethane (ε=9.08), ethanol (ε=24.3), methanol (ε=32.6), acetonitrile (ε=37.5), and water (ε=78.5), respectively. For the S_2 absorption band of pyrene in the CDx–alcohol–pyrene 1:1:1 inclusion compound, the 0–0 band is also located at longer wavelengths than that in any solvent.[1] The abnormally large red shifts of the absorption peaks in the CDx–alcohol–fluorene and -pyrene 1:1:1 inclusion compounds may be interpreted in terms of a kind of microheterogeneous solvent effect. One part of a long fluorene molecule in the 1:1:1 inclusion compound is extruded out of the CDx cavity to the very polar water medium, and the other part of the fluorene molecule resides in the CDx cavity, being in contact with the alkyl chain of an alcohol molecule entering the cavity. Therefore, one end of the fluorene molecule experiences a very polar environment, and the other end of the fluorene molecule is in a relatively nonpolar environment owing to both the hydrophobicity of the inner wall of the CDx cavity and that of the alkyl chain of an alcohol molecule entering the CDx cavity. Under such circumstances, the excited state of fluorene may be lowered in energy relative to that in a uniform environment (neat solvent), since π electrons delocalize more easily in the excited state than in the ground state. As a consequence, the 0–0 band of fluorene in the 1:1:1 inclusion compound is shifted to the red relative to that in any neat solvent.

1:1:1 Inclusion Compounds of CDx with Fluorene and Nitriles. The association of FC with nitriles from propionitrile to hexanenitrile has been confirmed from the observations of absorption and fluorescence spectral changes similar to those for solutions containing alcohol.[13] This finding is consistent with Turro et al.'s suggestion that a ternary complex is formed among CDx, acetonitrile, and 1-halonaphthalene.[14] On the basis of a competition method,[2,9] K_2 values for the nitriles have been evaluated and are listed in Table 1. From comparisons between the observed fluorescence intensities and intensity curves which have been calculated according to Eq. 5, K_3 and K_4 for the nitriles have been determined (Table 1). Both K_2 and K_3 increase as the alkyl chain of nitrile is lengthened. These K_2 and K_3 values are several times greater than those for alcohols possessing the same alkyl group, indicating the stronger hydrophobicity of nitrile than that of alcohol. However, the K_4 values for the nitriles are rather small compared with those for the corresponding alcohols. The largest K_4 is seen both for propionitrile and butyronitrile, and K_4 decreases on going from butyronitrile to hexanenitrile. It should be noted that K_4 for propionitrile with a shorter alkyl chain than 1-propanol, whose K_4 is the largest in the alcohol series, has a maximum value in the nitrile series. These findings may imply that in the 1:1:1 inclusion compound the orientation and/or location of a nitrile molecule within the CDx cavity is slightly different from that of the alcohol molecule.

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8) S. Scypinsky and L. J. Cline Love, *Anal. Chem.*, **56**, 322 (1984).

9) Y. Matsui and K. Mochida, *Bull. Chem. Soc. Jpn.*, **52**, 2808 (1979).

10) No absorption and fluorescence spectral changes are observed when methanol is added to a fluorene solution with CDx.

11) The K_3 values in the CDx–1-propanol–pyrene and CDx–1-propanol–acenaphthene systems are 7600 and 1600 $mol^{-1} dm^3$, respectively (Refs. 1 and 2).

12) The K_4 values in the CDx–1-propanol–pyrene and CDx–1-propanol–acenaphthene systems are 15000 and 32000 $mol^{-1} dm^3$, respectively (Refs. 1 and 2).

13) Addition of acetonitrile to a fluorene aqueous solution containing CDx results in no changes in the absorption and fluorescence spectra.

14) N. J. Turro, J. D. Bolt, Y. Kuroda, and I. Tabushi, *Photochem. Photobiol.*, **35**, 69 (1982).