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Fluorescence Polarization Studies of Inclusion Complexes between β-Cyclodextrin and 1-Chloronaphthalene in Aqueous and Aqueous D-Glucose Solutions

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In aqueous and aqueous D-glucose solutions, 1-chloronaphthalene (1CN) has been found to form a 2:2 inclusion complex with β-cyclodextrin (β-CD), which is generated by the self-association of 1:1 β-CD−1CN inclusion complexes. The 2:2 inclusion complex is responsible for the excimer fluorescence of 1CN. Equilibrium constants for the formation of the 1:1 and 2:2 inclusion complexes in aqueous solutions have been evaluated from a simulation of the 1CN excimer fluorescence intensity which is a function of β-CD concentration. When a wavelength of 315 nm has been selected as an excitation light, the degree of polarization has been maximized at the wavelengths of the 1CN monomer fluorescence band, and is decreased at the 1CN excimer fluorescence band. The decrease in the degree of polarization is due to the long lifetime of the excimer fluorescence. The results of the fluorescence polarization are consistent with the theory that the transition moment of the 1CN excimer fluorescence is parallel to that of the monomer fluorescence. On the basis of a Perrin plot, the molecular volume of the 1CN excimer has been estimated to be 0.20 nm^3, which corresponds to the molecular volume of the sandwich-type excimer of 1CN; the 1CN excimer does not rotate as the 2:2 inclusion complex but rotates as the guest within the β-CD cavities.

Introduction

Cyclodextrins (CDs) are toroidally shaped cyclic oligosaccharides composed of D-glucopyranose units linked by α(1→4) bonds.[1] CDs having six, seven, and eight D-glucopyranose units are called α-, β-, and γ-CDs, respectively. Due to the hydrophobic cavity, CDs can accommodate organic molecules to form inclusion complexes.

It is known that 1:1 inclusion complexes of CD with a guest molecule frequently self-associate to form a 2:2 inclusion complex.[2−11]

In the low concentration range (below about 6×10^{-6} mol dm^{-3}) of naphthalene, addition of β-CD to an aqueous naphthalene solution results in an enhancement of the naphthalene monomer fluorescence.[2] At high concentrations of naphthalene such as 6×10^{-5} mol dm^{-3}, however, the naphthalene excimer fluorescence appears upon the addition of β-CD. The naphthalene excimer fluorescence is due to a 2:2 β-CD−naphthalene inclusion complex, which is formed by the self-association of two 1:1 β-CD−naphthalene inclusion complexes. Besides naphthalene, there are many examples of 2:2 inclusion complexes; 2:2 inclusion complexes of β-CD−1-cyanonaphthalene,[3] β-CD−2-chloronaphthalene,[4] β-CD−sodium 2-naphthalenesulfonate,[5] β-CD−sodium 2-anthracenesulfonate,[5,6] β-CD−sodium 2-anthracenecarboxylate,[5] γ-CD−pyrene,[7] γ-CD−sodium 1-pyrenebutyrate,[8] γ-CD−sodium 1-pyrenesulfonate,[8] β-CD−1-methylnaphthalene,[9] β-CD−2-methylnaphthalene,[10] γ-CD−2-methylnaphthalene,[10] and 6-*O*-α-D-glucosyl-β-CD−4-(dimethylamino)benzonitrile.[11]

Interactions between a guest molecule and CD have been investigated by means of absorption, fluorescence, NMR spectroscopy, etc. When a guest molecule is incorporated into the CD cavity, the absorption band of a guest is generally shifted to longer wavelengths, accompanied by a sharpening of vibrational bands. The fluorescence band of a guest is also shifted with a sharpening of vibrational bands upon the addition of CD. The fluorescence intensity of the guest is usually enhanced in the presence of CD. In ^{1}H NMR spectra, proton signals of a guest generally exhibit higher-field shifts as the CD concentration is increased. In addition, signals of H-3 and H-5 of CDs, which form a ring inside the CD cavity, are higher-field shifted with an increase in the guest concentration. Recently, a fluorescence polarization technique has been used to acquire information concerning the molecular motions of an inclusion complex.[12,13] The formation of an inclusion complex restricts molecular movements of a guest within the CD cavity to a large extent, leading to the observation of the polarized fluorescence of the guest.

We have found that β-CD induces the excimer fluorescence of 1-chloronaphthalene (1CN) in aqueous and aqueous D-glucose solutions. Thus, we tried to investigate the interactions between a guest molecule (1CN) and the β-CD cavity by means of a fluorescence polarization technique as well as fluorescence spectroscopy. The degree of polarization for the monomer and excimer fluorescence of 1CN was examined as a function of excitation and observation wavelengths. In addition, the molecular volume of the 1CN excimer within the β-CD cavities has been estimated on the basis of a Perrin equation.

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Experimental Section

1-Chloronaphthalene (1CN) purchased from Tokyo Kasei Kogyo Co., Ltd. was purified by use of silica gel column chromatography. β-CD obtained from Nacalai Tesque, Inc., was recrystallized twice from water. D-Glucose from Wako Pure Chemical Industries, Ltd., was used as received. Aqueous solutions of 1CN were prepared by leaving several drops of purified 1CN in water in the dark for several days. In the preparation of dilute β-CD solutions containing 1CN, appropriate amounts of 1×10^{-2} mol dm^{-3} β-CD solution were transferred into 10-mL volumetric flasks, followed by the addition of 6 mL of aqueous 1CN solution. The flasks were then filled to the mark with water. For concentrated β-CD solutions of 1CN, weighed amounts of β-CD, which were added to 10-mL volumetric flasks, were dissolved upon warming. In preparation of dilute D-glucose solutions, weighed amounts of D-glucose, appropriate amounts of 1×10^{-2} mol dm^{-3} β-CD solution, and 1 mL of 1CN solution were added to 10-mL volumetric flasks. After dissolution of D-glucose upon warming, the mixtures were cooled to room temperature, followed by the introduction of 5 mL of 1CN solution and made up to volume with water. For a concentrated D-glucose solution of 1CN, such as 1.0 g/mL, a method similar to that previously described was employed.[14] Concentrations of 1CN in sample solutions were about 5×10^{-5} mol dm^{-3}. In the D-glucose solutions, the concentrations of D-glucose were equal to or less than 1.0 g/mL. Spectroscopic measurements were made immediately after sample preparation. The viscosities of the D-glucose solutions were measured with a Tokimec ELD rotating viscometer at 25 $\pm$ 0.2 °C.

Absorption spectra were recorded on a Shimadzu UV-260 spectrophotometer. Fluorescence spectra were run 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.

In the measurements of the fluorescence polarization, an HNP′B polarizer from Polaroid Co. Ltd., which has a single transmission of 16−27% in the wavelength range of 280−360 nm, and a POLAX-32N polarizer from Luceo Co. Ltd., which has a single transimission of 22−32% in the wavelength range of 350−700 nm and a crossed transmittance less than 0.07%, were employed for excitation and emission lights, respectively. The degree of polarization, P, is defined as

$$P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp}) \quad (1)$$

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of emitted light polarized parallel and perpendicular to the exciting light, respectively. According to Azumi and McGlynn,[15] the degree of polarization was calculated by

$$P = (I_{EE} - I_{EB}(I_{BE}/I_{BB}))/(I_{EE} + I_{EB}(I_{BE}/I_{BB})) \quad (2)$$

where the subscript E stands for a polarizer oriented to pass only light polarized with electric vector perpendicular to the plane formed by the excitation and observation beams, and the subscript B stands for a polarizer oriented to pass only light polarized in the plane.

Fluorescence lifetimes were measured with a Horiba NAES-550 nanosecond fluorometer. A nanosecond flash lamp was used for excitation. The excitation lamp profiles were collected from the reflection of a quartz plate. Time resolution of the fluorometer was 0.1 ns. An Andover P/N:300FS10-25 optical interference filter and a Toshiba L-37 filter were used for excitation and emission lights, respectively. The fluorescence decays for β-CD solutions of 1CN were deconvoluted with triexponential functions. The χ^2 values for the fluorescence lifetimes were in the range of 1.08−1.20.

Spectroscopic measurements were made at 25 $\pm$ 0.1 °C.

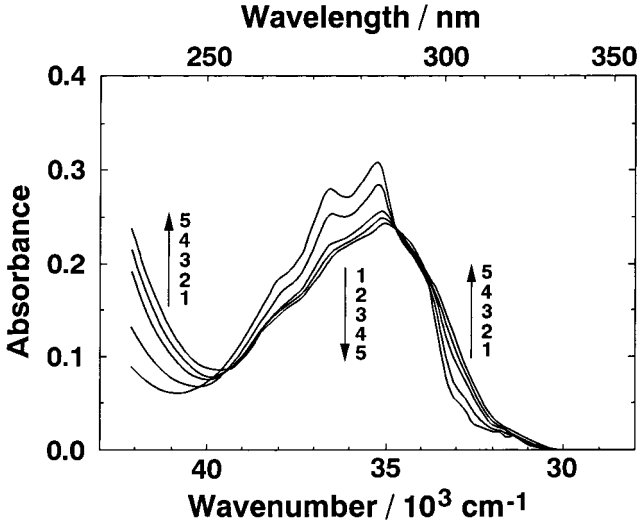

Figure 1. Absorption spectra of 1CN (5.0×10^{-5} mol dm^{-3}) in aqueous solutions containing varying concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 1.0×10^{-4}, (3) 1.0×10^{-3}, (4) 3.0×10^{-3}, and (5) 1.0×10^{-2} mol dm^{-3}.

Results and Discussion

Inclusion Complexes of β-CD with 1CN in Aqueous Solutions. Figure 1 shows absorption spectra of 1CN in aqueous solutions containing varying concentrations of β-CD. When β-CD is added to 1CN solution, the absorption maxima are shifted to longer wavelengths. As the β-CD concentration is increased, the absorption band is reduced in intensity and its vibrational structures are remarkably blurred. Although an isosbestic point is observed at 287.5 nm, it does not appear at around 255 nm, indicating the existence of at least two equilibria. One equilibrium is the following:

$$\beta\text{-CD} + 1\text{CN} \overset{K_1}{\rightleftharpoons} \beta\text{-CD}-1\text{CN} \quad (3)$$

where K_1 is the equilibrium constant for the formation of a 1:1 inclusion complex (β-CD−1CN) between β-CD and 1CN.

Figure 2 exhibits fluorescence spectra of 1CN in aqueous solutions containing varying concentrations of β-CD. Upon the addition of β-CD, the monomer fluorescence band of 1CN is slightly shifted from 337 nm to longer wavelengths, accompanied by the intensity reduction and the blurring of the vibrational bands. Furthermore, a new broad emission band appears at around 400 nm, with an increase in the β-CD concentration. This new emission band can be ascribed to the excimer fluorescence of 1CN.

As stated previously, the naphthalene excimer fluorescence is observed for aqueous naphthalene solutions containing β-CD, when the naphthalene concentration is higher than about 5×10^{-5} mol dm^{-3}.[2] The naphthalene excimer fluorescence is due to a 2:2 β-CD−naphthalene inclusion complex which is formed by the self-association of two 1:1 β-CD−naphthalene inclusion complexes. Such 2:2 CD−guest inclusion complexes have also been reported for the other systems of β-CD−guest. Therefore, it is most likely that a 2:2 β-CD−1CN inclusion complex ((β-CD−1CN)$_2$) is responsible for the 1CN excimer fluorescence shown in Figure 2:

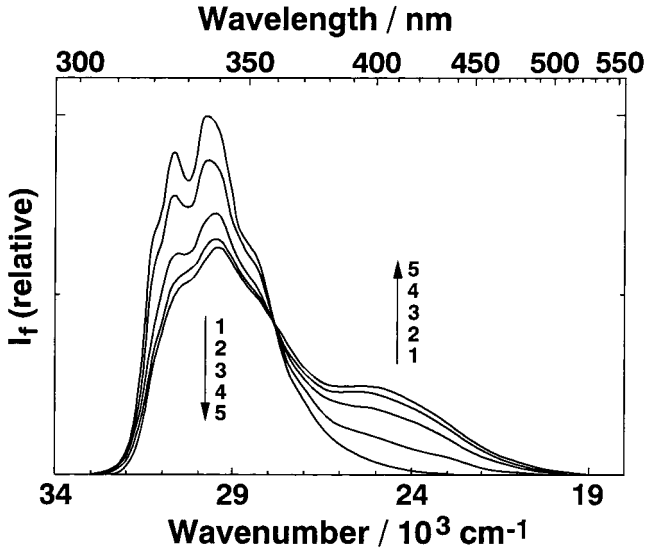

Figure 2. Fluorescence spectra of 1CN (5.0×10^{-5} mol dm^{-3}) in aqueous solutions containing varying concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 3.0×10^{-4}, (3) 1.0×10^{-3}, (4) 3.0×10^{-3}, and (5) 1.0×10^{-2} mol dm^{-3}. $\lambda_{ex} = 287.5$ nm.

$$\beta\text{-CD}-1\text{CN} + \beta\text{-CD}-1\text{CN} \overset{K_2}{\rightleftharpoons} (\beta\text{-CD}-1\text{CN})_2 \quad (4)$$

where K_2 is the equilibrium constant for the formation of the 2:2 β-CD−1CN inclusion complex. To confirm the existence of the 2:2 β-CD−1CN inclusion complex, we have simulated the 1CN excimer fluorescence intensity as a function of the β-CD concentration under the conditions that the 1CN excimer fluorescence intensity is proportional to the concentration of an emitting species.

Because the β-CD concentration is significantly higher than the 1CN concentration, the β-CD concentration in 1CN solution is approximated as the initial β-CD concentration. On the basis of eqs 3 and 4, an equation concerning the concentration of free 1CN, [1CN], is expressed by

$$2K_1^2K_2[\beta\text{-CD}]_0^2[1\text{CN}]^2 + (1 + K_1[\beta\text{-CD}]_0)[1\text{CN}] - [1\text{CN}]_0 = 0 \quad (5)$$

where subscripts 0 represent the initial concentration. Assuming the K_1 and K_2 values, the concentration of free 1CN can be calculated from eq 5. The concentration of the 2:2 β-CD−1CN inclusion complex is represented by

$$[(\beta\text{-CD}-1\text{CN})_2] = K_1^2K_2[\beta\text{-CD}]_0^2[1\text{CN}]^2 \quad (6)$$

Consequently, the concentration of the 2:2 inclusion complex is calculated at a given concentration of β-CD. Figure 3 depicts the best fit concentration curve of the 2:2 inclusion complex, which is a function of the β-CD concentration, together with the data points of the excimer fluorescence intensity. In Figure 3, the observed excimer fluorescence intensity and the concentration of the 2:2 inclusion complex are normalized to 100 at a β-CD concentration of 1.0×10^{-2} mol dm^{-3}. The result shown in Figure 3 indicates that the 2:2 inclusion complex is responsible for the 1CN excimer fluorescence. To further confirm the existence of the 2:2 inclusion complex, we similarly simulated the concentration of a 1:2 β-CD−1CN inclusion complex which may emit the excimer fluorescence. However, the best fit curve of the 1:2 inclusion complex did not well reproduce the observed excimer fluorescence intensities. This finding provides additional evidence for the existence of the 2:2 inclusion complex.

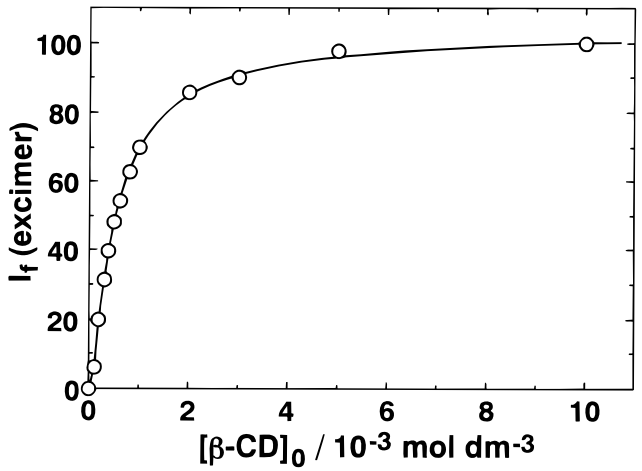

Figure 3. Comparison between the concentration curve of the 2:2 β-CD−1CN inclusion complex and the observed excimer fluorescence intensities (open circle). The best fit curve was calculated with an assumed K_1 value of 868 mol^{-1} dm^3 and an assumed K_2 value of 48400 mol^{-1} dm^3. Both the simulation curve and the observed excimer fluorescence intensities were normalized to 100 at a β-CD concentration of 1.0×10^{-2} mol dm^{-3}. $\lambda_{ex} = 287.5$ nm. $\lambda_{obs} = 420$ nm.

From the simulation shown in Figure 3, 868 and 48400 mol^{-1} dm^3 are obtained as the K_1 and K_2 values, respectively.[16] The K_1 values for naphthalene, 1-methylnaphthalene, 1-ethylnaphthalene, 1-(chloromethyl)naphthalene, and 1-cyanonaphthalene are reported to be 685, 340 ± 40, 630 ± 70, 190 ± 40, and 120 ± 10 mol^{-1} dm^3, respectively.[2,3,9] The K_1 value for 1CN is greater than those for naphthalene and the 1-substituted naphthalenes. This result seems to be due to the smaller steric hindrance of a chlorine atom in 1CN compared to the other substituents. The hydrophobicity of a chlorine atom seems to lead to the K_1 value greater than that for naphthalene. The K_2 values for naphthalene, 1-methylnaphthalene, and 1-cyanonaphthalene are 4000, 5820, and 70 000 mol^{-1} dm^3, respectively.[2,3,9] The K_2 value for 1CN is an order of magnitude greater than those for naphthalene and 1-methylnaphthalene, and is comparable to that for 1-cyanonaphthalene.

Inclusion Complexes of β-CD with 1CN in Aqueous D-Glucose Solutions. Absorption and fluorescence spectral changes in aqueous D-glucose solutions of 1CN were similar to those in aqueous solutions without D-glucose. Addition of β-CD to 1CN solution containing D-glucose results in the appearance of the 1CN excimer fluorescence. In the α-CD−2-chloronaphthalene system, the K_1 value for aqueous D-glucose solution is an order of magnitude less than that for aqueous solution without D-glucose, while an equilibrium constant (699 mol^{-1} dm^3) for the formation of a 2:1 α-CD−2-chloronaphthalene inclusion complex from a 1:1 α-CD−2-chloronaphthalene and α-CD in D-glucose solution is rather greater than that (364 mol^{-1} dm^3) in aqueous solution without D-glucose.[14] Although there are differences in the magnitude of the equilibrium constants of 2-chloronaphthalene for solutions with and without D-glucose, the inclusion modes of the guests are the same regardless of the presence of D-glucose. As in the α-CD−2-chloronaphthalene system, D-glucose seems to exert no effect on the inclusion modes of β-CD. Consequently, the excimer fluorescence of 1CN in aqueous D-glucose solutions is most likely due to the 2:2 β-CD−1CN inclusion complex which is formed by the self-association of the 1:1 β-CD−1CN inclusion complexes.

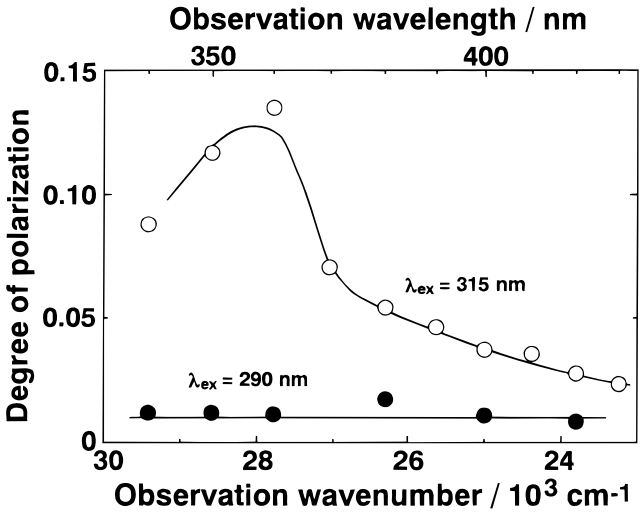

Figure 4. Observation-wavelength dependence of the degree of polarization for the fluorescence of 1CN (5.0×10^{-5} mol dm^{-3}) in aqueous solution containing β-CD (3.0×10^{-3} mol dm^{-3}). $\lambda_{ex} = 290$ (closed circle) and 315 nm (open circle).

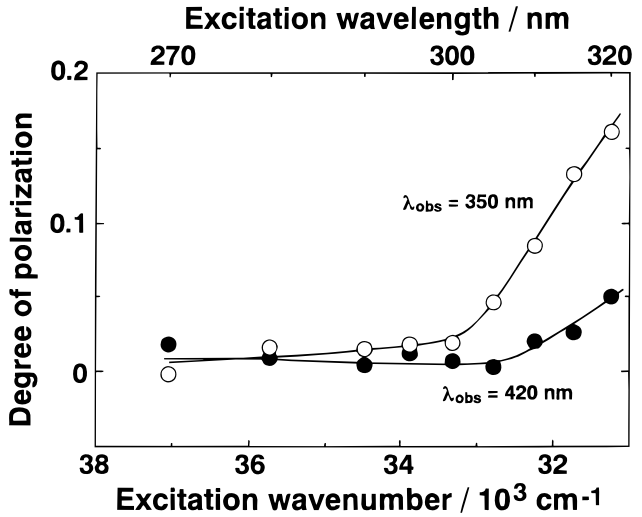

Figure 5. Excitation-wavelength dependence of the degree of polarization for the fluorescence of 1CN (5.0×10^{-5} mol dm^{-3}) in aqueous solution containing β-CD (3.0×10^{-3} mol dm^{-3}). $\lambda_{obs} = 350$ (open circle) and 420 nm (closed circle).

Fluorescence Polarization of 1CN. From an analogy of the assignments of the absorption bands of naphthalene, the 315-nm band of 1CN is attributable to the ^1L_b band, while the 290-nm band is assigned to the ^1L_a band. The transition moments of these two bands are likely almost perpendicular to each other, although a chlorine substituent in 1CN perturbs the directions of the transition moments to some extent. For aqueous 1CN solution without β-CD, the degree of polarization for the monomer fluorescence was equal to zero within experimental error. Figure 4 illustrates the degree of polarization for the fluorescence from aqueous 1CN solution containing β-CD (3.0×10^{-3} mol dm^{-3}) as a function of observation wavelength. Upon the excitation at 290 nm, the degree of polarization remains constant (about 0.01) irrespective of the observation wavelength, indicating that the transition moment of the 290-nm band is nearly perpendicular to those of the monomer and excimer fluorescence of 1CN. When the excitation wavelength of 315 nm is selected, on the other hand, the degree of polarization is maximized at an observation wavelength of around 360 nm which corresponds to the monomer fluorescence band of 1CN, and the degree of polarization is decreased with an increase in the observation wavelength. This finding suggests that the depolarization of the excimer fluorescence occurs during its longer lifetime compared to the monomer fluorescence, even when the directions of the transition moments of the monomer and excimer fluorescence are parallel to one another. For the 1CN excimer fluorescence, a lifetime of 8.3 ± 0.2 ns was obtained, whereas the lifetime of the 1CN monomer fluorescence from the inclusion complex was measured to be 2.8 ± 0.1 ns, which was about one-third of the lifetime of the excimer fluorescence. The lifetime data are consistent with the fluorescence polarization. At a β-CD concentration of 1.0×10^{-2} mol dm^{-3}, the degree of polarization is slightly enhanced over the whole observation wavelength range, although the degree of polarization exhibits curves similar to those for a β-CD concentration of 3.0×10^{-3} mol dm^{-3}. Consequently, the slightly smaller degree of polarization for the 1CN solution containing β-CD of 3.0×10^{-3} mol dm^{-3} is due to the existence of uncomplexed 1CN, compared to the 1CN solution containing β-CD of 1.0×10^{-2} mol dm^{-3}.

Figure 5 depicts the degree of polarization for the fluorescence from an aqueous 1CN solution containing β-CD (3.0×10^{-3} mol dm^{-3}) as a function of excitation wavelength. When 350 nm is selected as the observation wavelength of the monomer fluorescence, the degree of polarization is nearly zero at excitation wavelengths from 270 to 300 nm, but is increased at excitation wavelengths above 300 nm. This finding is consistent with the result of Figure 4, where the degree of polarization is plotted as a function of observation wavelength; not the ^1L_a band but the ^1L_b band is responsible for the 1CN monomer fluorescence. When the observation wavelength is at 420 nm (the excimer fluorescence), the change in the degree of polarization is similar to that observed at 350 nm, although the magnitude of the degree of polarization is about one-third. This implies that the depolarization occurs during the lifetime of the excimer fluorescence which is longer than that of the monomer fluorescence.

It is generally believed that excimers possess equilibrium configurations in which one molecule is superposed over the other, in a perfect sandwich structure. In excimers of such a configuration, fluorescence from the lowest excited singlet state is dipole-forbidden.[17] The torsional distortion from a perfect sandwich configuration induces dipole-allowed character to excimer fluorescence without greatly affecting its energy. The transition moment of excimer fluorescence is polarized in the molecular plane. Consequently, the transition moment of the 1CN excimer fluorescence is most likely parallel to the transition moment of the ^1L_b band. This is consistent with the experimental results shown in Figure 5; the degree of polarization observed at the wavelength of the 1CN excimer fluorescence exhibits a curve similar to that of the 1CN monomer fluorescence, although the degree of polarization for the 1CN excimer fluorescence is smaller than that for the 1CN monomer fluorescence because of the longer lifetime of the excimer fluorescence. Previously, induced circular dichroism spectra of CD inclusion complexes have been shown to be useful in determining the directions of the transition moments of guests.[14,18] In this study, analyses of the degree of polarization for the emissions of inclusion complexes have been proved to be utilized in acquiring information concerning the directions of transition moments of a guest.

Molecular Volume of the 1CN Excimer. The degree of polarization depends on the solution viscosity η and the absolute temperature T. Assuming that the shape of a fluorescent solute is a sphere, the degree of polarization is given by the equation derived by Perrin:[19,20]

$$1/P - 1/3 = (1/P_0 - 1/3)(1 + kT\tau_f/V\eta) \quad (7)$$

where P_0, k, τ_f, and V are the limiting polarization, the Boltzmann constant, the mean fluorescence lifetime, and the molecular volume of the solute, respectively. From the measurements of the degree of polarization for the fluorescence as a function of $1/\eta$ (or T/η), the molecular volume of a fluorophore can be evaluated.[21] To change the viscosity of solution, the amounts of D-glucose in solutions were varied in the concentration range of 0.77−1.0 g/mL. Because D-glucose decreased the solubility of β-CD to some extent, the concentrations of β-CD were set at 3.0×10^{-3} mol dm^{-3} in this experiment. At 420 nm, there is almost no overlap between the excimer and monomer fluorescence. Consequently, there is little or no influence of uncomplexed 1CN and the 1:1 inclusion complex on the degree of polarization observed for the excimer fluorescence. Figure 6 shows a plot of $1/P - 1/3$ against $1/\eta$ for the 1CN excimer. Since the lifetimes of the 1CN excimer fluorescence in aqueous D-glucose solutions containing β-CD (3.0×10^{-3} mol dm^{-3}) were nearly the same regardless of the amounts of added D-glucose,[22] the value of 8.3 ± 0.2 ns was used as τ_f.

From a slope and an intercept of a straight line drawn in Figure 6, the molecular volume of the 1CN excimer is estimated to be 0.20 nm^3. If the 1CN excimer rotates as the 2:2 inclusion complex, the molecular volume of the excimer would be equal to that of two associating β-CD molecules. The external diameter and height of a β-CD molecule are 1.54 and 0.79 nm, respectively.[1,23] If we assume that the 2:2 inclusion complex has a head-to-head structure (barrel-type structure) with respect to β-CD and that the shape of the associating β-CD molecules is a sphere whose radius is 0.77 nm, the molecular volume of the 2:2 inclusion complex is calculated to be 1.9 nm^3. However, the molecular volume obtained from the analysis of the fluorescence polarization is an order of magnitude less than that calculated for the 2:2 inclusion complex. Therefore, the 1CN excimer does not rotate together with the two encapsulating β-CD molecules during its fluorescence lifetime; the 1CN excimer does not rotate as the 2:2 inclusion complex. The molecular dimensions of 1CN are 0.85 nm in length, 0.70−0.83 nm in height, and 0.33 nm in thickness. If we approximate the 1CN excimer as a sandwich structure, its thickness is 0.66 nm. When the 1CN excimer is assumed to be a sphere whose radius is 0.4 nm or a pillar whose radius and height are 0.43 and 0.66 nm, respectively, the molecular volume of the excimer is calculated to be 0.27 or 0.38 nm^3. Therefore, the molecular volumes calculated for the 1CN excimer are comparable to that estimated from the analysis of the fluorescence polarization. The 1CN excimer and the encapsulating β-CD molecules rotate independently during the time frame defined by the excimer fluorescence lifetime. Therefore, this finding implies that the interactions between 1CN and the wall of the β-CD cavity are weak. The degree of freedom of β-CD is decreased with an increase in viscosity of solution.

A fluorescence quenching study of the 2:2 β-CD−naphthalene inclusion complex indicates that a smaller I^- ion slightly quenches the excimer fluorescence of naphthalene included within the two β-CD cavities ($k_q = 1.8 \times 10^9$ mol^{-1} dm^3 s^{-1}), although the quenching of the naphthalene excimer fluorescence by a bulkier IO_3^- ion is completely suppressed.[2] A similar situation is expected for the 2:2 β-CD−1CN inclusion complex. Consequently, the 1CN excimer within the 2:2 inclusion complex of barrel-type likely senses the viscosity outside the cavity to some extent, since the 1CN excimer slightly interacts with the bulk environment through the open mouths of β-CD molecules. As an alternative explanation, there may be two types of rotation; one is the whole rotation of the 2:2 β-CD−1CN inclusion complex and the other is the rotation of the 1CN excimer relative to the β-CD cavities. The increased viscosity depresses only the first type of rotation, the whole rotation of the 2:2 inclusion complex. Therefore, the first type of rotation of the 1CN molecules (excimer) is also suppressed with the increase in viscosity, leading to the increased degree of polarization of the 1CN excimer fluorescence. However, the second type of rotation, the rotation of the 1CN excimer itself, still remains despite the increase in viscosity. Consequently, the estimated molecular volume may correspond to the molecular volume of the 1CN excimer.

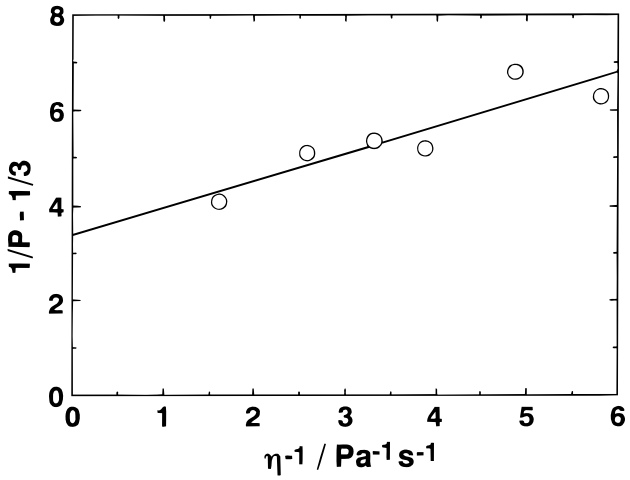

Figure 6. Plot of $(1/P - 1/3)$ against $1/\eta$ for the excimer fluorescence of 1CN (5.0×10^{-5} mol dm^{-3}) solutions containing β-CD (3.0×10^{-3} mol dm^{-3}) and D-glucose. The concentrations of D-glucose, which correspond to the data points from right to left, were 0.77, 0.81, 0.85, 0.90, 0.94, and 1.0 g/mL, respectively. $\lambda_{ex} = 315$ nm.

The ratio of the concentration of free 1CN to that of the 1:1 β-CD−1CN inclusion complex is controlled by the magnitude of the K_1 value (868 mol^{-1} dm^3) of eq 3, even if there is the other equilibrium (eq 4). At a β-CD concentration of 3.0×10^{-3} mol dm^{-3}, the ratio is calculated to be about 0.4 under the assumption of no effects of D-glucose on the K_1 value. Consequently, free 1CN fairly contributes to the monomer fluorescence of 1CN, although the fluorescence quantum yields of free 1CN and the 1:1 inclusion complex are likely different. We, therefore, did not try to estimate the molecular volume of the 1:1 inclusion complex.

Conclusions

In aqueous and aqueous D-glucose solutions, β-CD forms a 1:1 inclusion complex with 1CN, followed by the formation of a 2:2 β-CD−1CN inclusion complex which emits the 1CN excimer fluorescence. The equilibrium constants for the formation of the 1:1 and 2:2 inclusion complexes in aqueous solutions have been evaluated to be 868 and 48 400 mol^{-1} dm^3, respectively. The degree of polarization for the monomer and excimer fluorescence of 1CN in β-CD solutions has been measured as a function of excitation and observation wavelengths. Upon the excitation at 315 nm, the degree of polarization for the 1CN monomer fluorescence is greater than that for the 1CN excimer fluorescence, suggesting that the lifetime of the excimer fluorescence is longer than that of the monomer fluorescence even when the directions of the transition moments of the monomer and excimer fluorescence of 1CN are parallel. From a Perrin plot which is a function of solution viscosity,

the molecular volume of the 1CN excimer in β-CD solution has been estimated to be 0.20 nm^3, which corresponds to the molecular volume of the 1CN excimer itself, indicating that the 1CN excimer does not rotate as the 2:2 inclusion complex but as the excimer inside the β-CD cavities. From the viewpoint of the molecular motions, this implies the relatively weak interactions between 1CN and the β-CD cavities.

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References and Notes

(1) Saenger, W. *Angew. Chem., Int. Ed. Engl*. **1980**, *19*, 344.
(2) Hamai, S. *Bull. Chem. Soc. Jpn*. **1982**, *55*, 2721.
(3) Hamai, S. *J. Phys. Chem*. **1990**, *94*, 2595.
(4) Hamai, S.; Mononobe, N. *J. Photochem. Photobiol. A: Chem*. **1995**, *91*, 217.
(5) Tamaki, T.; Kokubu, T. *Tetrahedron* **1987**, *2*, 815.
(6) Tamaki, T.; Kokubu, T. *J. Inclusion Phenom*. **1984**, *2*, 815.
(7) Hamai, S. *J. Phys. Chem*. **1989**, *93*, 6527.
(8) Herkstroeter, W. G.; Martic, P. A.; Farid, S.; *J. Chem. Soc., Perkin Trans. 2* **1984**, 1453.
(9) Hamai, S.; Hatamiya, A. *Bull. Chem. Soc. Jpn*. **1996**, *69*, 2469.
(10) Hamai, S. *Bull. Chem. Soc. Jpn*. **1996**, *69*, 543.
(11) Nakamura, A.; Sato, S.; Hamasaki, K.; Ueno, A.; Toda, F. *J. Phys. Chem*. **1995**, *99*, 10952.
(12) Madrid, M. J.; Mendicuti, F. *Appl. Spectrosc*. **1997**, *51*, 1621.
(13) Pistolis, G.; Malliaris, A. *J. Phys. Chem. B* **1998**, *102*, 1095.
(14) Hamai, S. *J. Phys. Chem. B* **1997**, *101*, 1707.
(15) Azumi, T.; McGlynn, S. P. *J. Chem. Phys*. **1962**, *37*, 2413.
(16) Estimated errors for the K_1 and K_2 values are less than 10 and 15%, respectively.
(17) Chandra, A. K.; Lim, E. C. *J. Chem. Phys*. **1968**, *49*, 5066.
(18) Hamai, S. *J. Inclusion Phenom. Mol. Recogn. Chem*. **1997**, *27*, 57.
(19) Perrin, F. *J. Phys. Radium* **1926**, *7*, 390.
(20) Perrin, F. *Ann. Phys*. **1929**, *12*, 169.
(21) Hamai, S.; Kokubun, H. *Bull. Chem. Soc. Jpn*. **1974**, *47*, 24.
(22) The lifetime of the 1CN excimer fluorescence in aqueous D-glucose (1.0 g/mL) was estimated to be 8.7 $\pm$ 0.2 ns.
(23) Li, S.; Purdy, W. C. *Chem. Rev*. **1992**, *92*, 1457.