Fluorescence Polarization of Inclusion Complexes of β-Cyclodextrin with 6-Anilino-2-naphthalenesulfonate and 8-Anilino-1-naphthalenesulfonate in Aqueous D-Glucose Solutions

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In aqueous and aqueous D-glucose solutions, 6-anilino-2-naphthalenesulfonate (2,6-ANS) and 8-anilino-1-naphthalenesulfonate (1,8-ANS) form 1:1 inclusion complexes with β-CD. The molecular volume of 2,6-ANS bound to the β-CD cavity has been evaluated to be 4.7 nm^3 from the viscosity dependence (Perrin plot) of the 2,6-ANS fluorescence polarization. This value indicates that 2,6-ANS incorporated into the β-CD cavity rotates as the inclusion complex on a fluorescence lifetime scale. The molecular volume of 2,6-ANS in solution without β-CD has been evaluated to be 0.88 nm^3, which provides additional evidence for the whole rotation of the β-CD–2,6-ANS inclusion complex. The molecular volume of 1,8-ANS bound to the β-CD has been evaluated to be 2.7 nm^3, which is comparable to the calculated molecular volume (1.9 nm^3) of β-CD, indicating that 1,8-ANS most likely rotates as the inclusion complex. The fact that the molecular volume of the 1,8-ANS inclusion complex is smaller than that of the 2,6-ANS inclusion complex may be attributed to the differences in the binding site of β-CD and the substitution positions of anilino and sulfonato groups.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of more than five D-glucopyranose residues.[1] CDs having six, seven, and eight D-glucopyranose units are called α-, β-, and γ-CD, respectively. Because there is a relatively hydrophobic cavity in the molecular center, CDs and their derivatives accommodate various kinds of organic compounds into their cavities to form inclusion complexes in aqueous solutions. Due to the formation of the inclusion complexes of CDs with guest molecules, spectroscopic properties, such as electronic absorption, fluorescence, and phosphorescence, of guest molecules are varied to some extents.[2—8]

Recently, the behavior of guest molecules within CD inclusion complexes has been investigated by means of emission-polarization techniques.[9—12] Rotational relaxation times of resorufin, oxazine-118, and oxazine-725 complexed to β-CD have been estimated on the basis of time-resolved polarization spectroscopy.[13] However, the molecular volume of an inclusion complex has not been estimated except for the β-CD–1-chloronaphthalene system. In a previous paper, we have estimated the molecular volume of the 1-chloronaphthalene excimer in a 2:2 β-CD–1-chloronaphthalene inclusion complex, analyzing the fluorescence polarization of the 1-chloronaphthalene excimer as a function of solution viscosity (Perrin plot).[14] The evaluated molecular volume corresponds to the molecular volume of the 1-chloronaphthalene excimer itself, indicating that it independently rotates within the β-CD cavities on a time scale of its fluorescence lifetime. The result concerning the molecular volume of the 1-chloronaphthalene excimer located within the β-CD cavities implies relatively weak interactions of β-CD with 1-chloronaphthalene. Consequently, the interactions between CD and a guest can be explored through the estimation of the molecular volume of a guest bound to the CD cavity. Although there are many studies concerning inclusion complexes of CDs, the interactions between CD and a guest molecule are not thoroughly understood. The inquiries in the interactions of CDs are important from standpoints of the improvement and use of the molecular-recognition ability of CDs as well as the intermolecular interactions between CD and a guest molecule. As previously noted, the molecular volume obtained from a Perrin plot provides a means of estimating the interaction between CD and a guest molecule, which cannot be obtained from the estimation of other physical quantities. Thus, we conducted our further study on the molecular volumes of inclusion complexes. In this article, we report the molecular volumes of 6-anilino-2-naphthalenesulfonate and 8-anilino-1-naphthalenesulfonate which are bound to the β-CD cavity.

Experimental

β-Cyclodextrin (β-CD) obtained from Nacalai Tesque was recrystallized twice from water. Sodium 6-anilino-2-naphthalenesulfonate (2,6-ANS) obtained from Molecular Probes and ammonium 8-anilino-1-naphthalenesulfonate (1,8-ANS) obtained from Tokyo Kasei Kogyo were used as received. D-Glucose from Wako Pure Chemical Industries was used without further purification. Aqueous D-glucose solutions of 2,6- and 1,8-ANS were prepared using a method similar to that used for those of 1-chloronaphthalene.[14] Concentrations of 2,6- and 1,8-ANS were 5.0×10^{-5} mol dm^{-3}.

Absorption spectra were recorded on a Shimadzu UV-260 spectrophotometer. Fluorescence spectra were taken with 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 and a POLAX-32N polarizer from Luceo were employed for excitation and emission light, respectively. The degree of polarization, P, is defined as

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

where $I_{//}$ and $I_{\perp}$ are the intensities of emitted light polarized parallel and perpendicular to the exciting light, respectively. According to Azumi and McGlynn, the degree of polarization for fluorescence was calculated.[15] Fluorescence lifetimes were measured with a Horiba NAES-550 nanosecond fluorometer. An Andover P/N:340FS10-25 optical interference filter and a Toshiba L-42 filter were used for excitation and emission light, respectively. The fluorescence decays of 2,6- and 1,8-ANS in β-CD solutions were analyzed as a bi-exponential function. The χ^2 values for the fluorescence lifetimes of 2,6- and 1,8-ANS were in the range of 1.09—1.21 and 1.29—2.22, respectively. Spectroscopic measurements were made at 25±0.1 °C. Viscosities of D-glucose solutions were measured with a Tokimec ELD rotating viscometer at 25±0.2 °C.

Results and Discussion

Fluorescence Properties of 2,6-ANS in Aqueous Solutions. Figure 1 shows absorption spectra of 2,6-ANS in aqueous solutions containing various concentrations of β-CD. When β-CD is added to aqueous 2,6-ANS solution, the absorption peaks of 2,6-ANS at 265 and 318 nm are slightly shifted to shorter wavelengths, accompanied by an isosbestic point at 368 nm. At the same time, the absorption intensities at the peaks are weakened. The blue shift of the absorption bands implies that 2,6-ANS experiences a less polar environment compared to bulk water phase. These findings indicate the formation of a 1:1 inclusion complex of β-CD with 2,6-ANS. Figure 2 exhibits fluorescence spectra of 2,6-ANS in aqueous solutions containing various concentrations of β-CD. With an increase in the β-CD concentration, the fluorescence intensity of 2,6-ANS is significantly enhanced, accompanied by the blue shift of the fluorescence peak. From the fluorescence intensity change, an equilibrium constant (K) for the formation of the 1:1 inclusion complex of β-CD with 2,6-ANS can be evaluated:[8a]

$$1/(I_f - I_f^0) = 1/a + 1/(aK[\beta\text{-CD}]_0). \quad (2)$$

Here, I_f, I_f^0, a, and $[\beta\text{-CD}]_0$ are the fluorescence intensity in the presence of β-CD, that in the absence of β-CD, a constant, and the initial concentration of β-CD, respectively. Figure 3 depicts a plot of $1/(I_f - I_f^0)$ for 2,6-ANS against $1/[\beta\text{-CD}]_0$, which gives a K value of 1730±20 mol^{-1} dm^3. This K value for 2,6-ANS is slightly smaller than the reported one (2080±20 mol^{-1} dm^3).[16]

Figure 4 shows absorption spectra of 2,6-ANS in aqueous solutions in the absence and presence of D-glucose (0.5 g cm^{-3}). Addition of D-glucose results in a red-shift of the absorption. For naphthalene, a red-shift of absorption peaks has similarly been observed.[17] Consequently, the in-

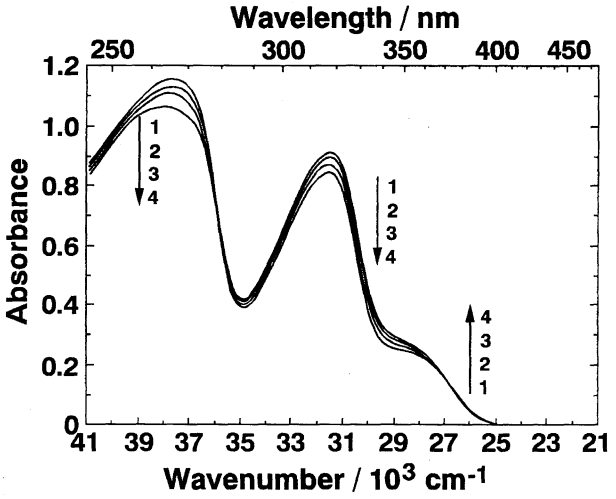

Fig. 1. Absorption spectra of 2,6-ANS in aqueous solutions containing various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 1.0×10^{-4}, (3) 3.0×10^{-4}, and (4) 1.0×10^{-3} mol dm^{-3}.

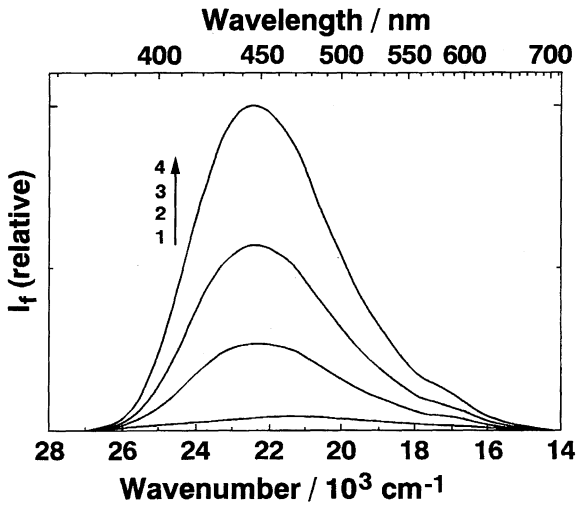

Fig. 2. Fluorescence spectra of 2,6-ANS in aqueous solutions containing various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 1.0×10^{-4}, (3) 3.0×10^{-4}, and (4) 1.0×10^{-3} mol dm^{-3}. λ_{ex} = 369 nm.

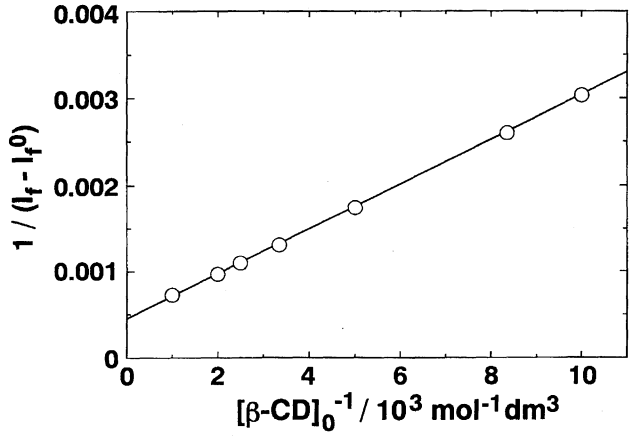

Fig. 3. Double reciprocal plot of the 2,6-ANS fluorescence intensity against the β-CD concentration. λ_{ex} = 369 nm. λ_{obs} = 450 nm.

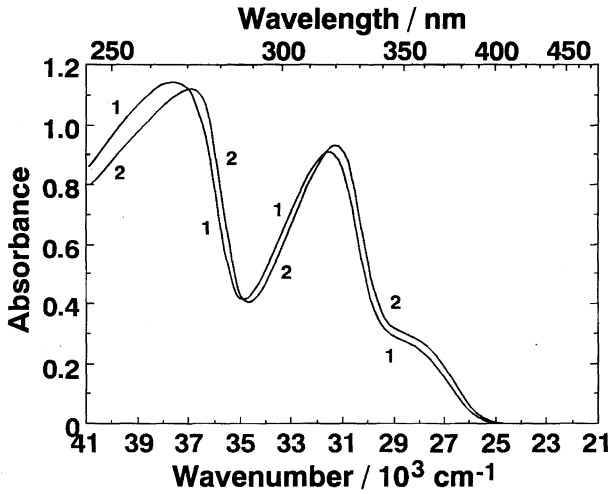

Fig. 4. Absorption spectra of 2,6-ANS in aqueous solutions in the absence and presence of D-glucose (0.5 g cm^{-3}).

teractions of a substrate with D-glucose are to some extent different from those with water. When D-glucose was added to aqueous 2,6-ANS solution without β-CD, the fluorescence of 2,6-ANS was enhanced in intensity, accompanied by the shorter-wavelength shift of the peak. This enhancement of the 2,6-ANS fluorescence is due to the increase in the solvent viscosity by the addition of D-glucose.

Figure 5 exhibits fluorescence spectra of 2,6-ANS in aqueous β-CD (1.0×10^{-2} mol dm^{-3}) solutions containing various amounts of D-glucose. As the amount of D-glucose added is increased, the 2,6-ANS fluorescence is enhanced. This finding suggests that the solvent viscosity affects the fluorescence properties of the guest bound to the β-CD cavity.

In D-glucose (0.5 and 1.0 g cm^{-3}) solutions, the K values were evaluated to be 880±10 and 760±100 mol^{-1} dm^3, respectively, which are less than that for aqueous solution without D-glucose. Because, in aqueous solution containing D-glucose, an environment sensed by a guest molecule is somewhat close to an environment within the CD cavity, the K values may be less than that in solutions without D-glucose. For the α-CD–2-chloronaphthalene system, the magnitudes of the K values for aqueous and aqueous D-glucose (1.0 g cm^{-3}) solutions have a trend similar to those of 2,6-ANS. The 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 inclusion complex and α-CD in D-glucose solution is greater than that (364 mol^{-1} dm^3) in aqueous solution without D-glucose.[8f]

Fluorescence Polarization of 2,6-ANS in Aqueous D-Glucose Solutions containing β-CD. Figure 6 illustrates the degree of polarization for the fluorescence, observed at 450 and 550 nm, of 2,6-ANS in aqueous solution containing both β-CD (1.0×10^{-2} mol dm^{-3}) and D-glucose (0.5 g cm^{-3}) as a function of excitation wavelength. For both observation wavelengths, the degree of polarization remains constant in the wavelength range above about 300 nm, although the degree of polarization at 550 nm is slightly lower than that at 450 nm. The slightly lower degree of polarization at 550 nm may imply that, as pointed out by Bright et al., 2,6-ANS is not in a single discrete environment within the β-CD cavity but in an array of β-CD-cavity environments all in equilibrium with one another.[18] In this study, the viscosity is very high, since the solvent used contains D-glucose. Consequently, the reorientation of solvent molecules around 2,6-ANS bound to the β-CD cavity may be partly responsible for the different environments. Figure 7 shows a plot of the degree of polarization for the fluorescence, excited at 369 nm, of 2,6-ANS in aqueous solutions containing β-CD (1.0×10^{-2} mol dm^{-3}) and D-glucose (0.5 g cm^{-3}) against the observation wavelength. The degree of polarization is decreased with an increase in the observation wavelength. This finding is consistent with the results shown in Fig. 6, suggesting that there is an array of the inclusion complexes

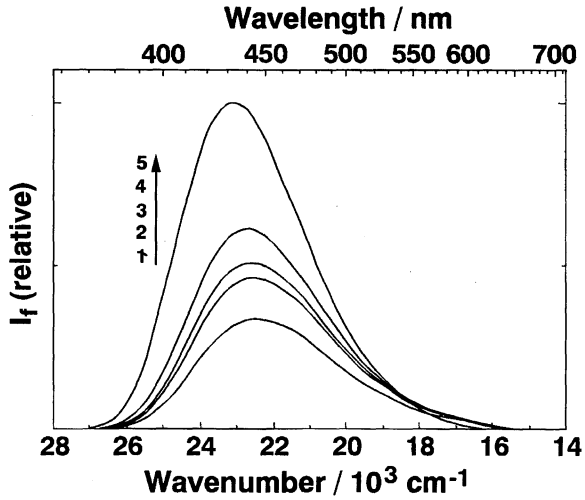

Fig. 5. Fluorescence spectra of 2,6-ANS in aqueous β-CD solutions containing various concentrations of D-glucose. Concentration of D-glucose: (1) 0, (2) 0.3, (3) 0.5, (4) 0.7, and (5) 1.0 g cm^{-3}. λ_{ex} = 369 nm.

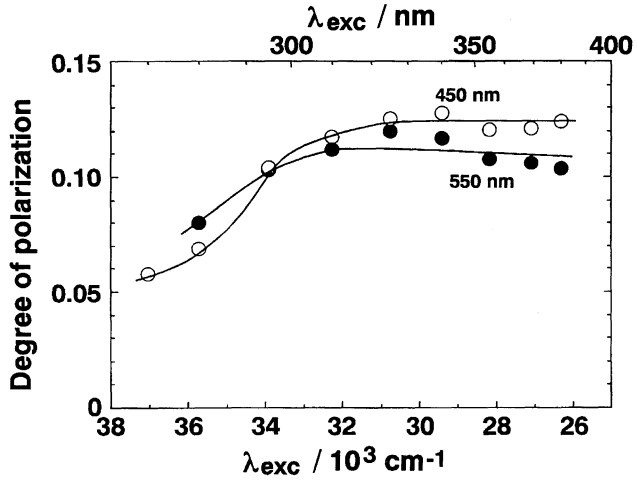

Fig. 6. Excitation-wavelength dependence of the degree of polarization for the fluorescence of 2,6-ANS in aqueous solutions containing both β-CD (1.0×10^{-2} mol dm^{-3}) and D-glucose (0.5 g cm^{-3}). Open circles: λ_{obs} = 450 nm. Closed circles: λ_{obs} = 550 nm.

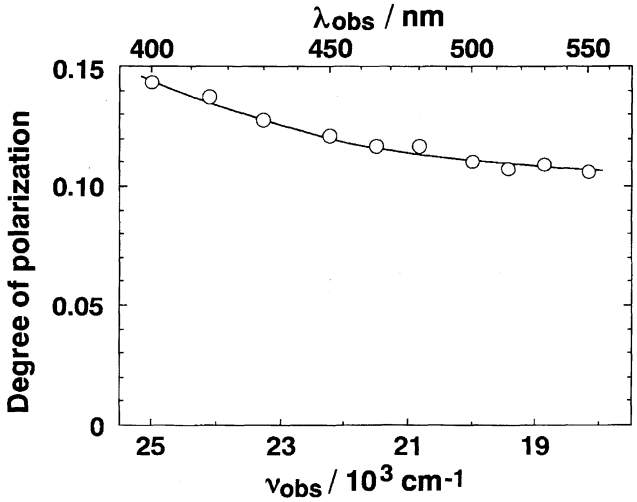

Fig. 7. Observation-wavelength dependence of the degree of polarization for the fluorescence of 2,6-ANS in aqueous solutions containing both β-CD (1.0×10^{-2} mol dm^{-3}) and D-glucose (0.5 g cm^{-3}). λ_{ex} = 369 nm.

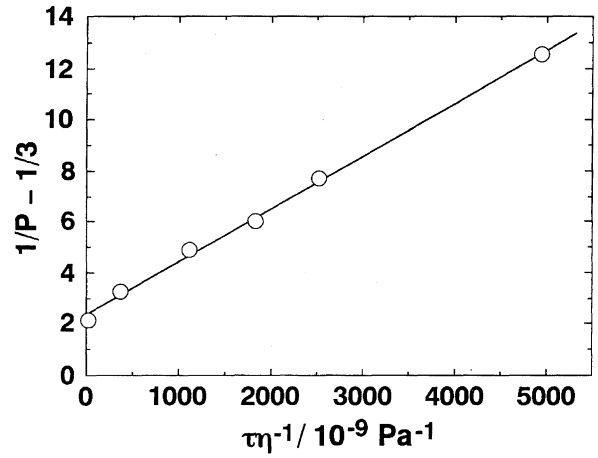

Fig. 8. Plot of $(1/P-1/3)$ against $\tau_f\eta^{-1}$ for the fluorescence of 2,6-ANS solutions containing β-CD (1.0×10^{-2} mol dm^{-3}) and D-glucose. λ_{ex} = 369 nm. λ_{obs} = 450 nm.

with slightly different relative host–guest geometries.

Molecular Volume of 2,6-ANS Bound to the β-CD Cavity in Aqueous D-Glucose Solutions. The degree of polarization P is correlated to the solution viscosity η and the absolute temperature T. Assuming that the shape of a fluorescent solute is a sphere, P is given by the Perrin equation:[19]

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

where P_0 and k are the limiting polarization and the Boltzmann constant, respectively, and τ_f and V are the fluorescence lifetime and molecular volume of the fluorescent solute, respectively. From a plot of $(1/P-1/3)$ against τ_f/η (or $1/\eta$), the molecular volume of a fluorophore can be evaluated.[14,20] To change the solution viscosity, the amounts of D-glucose in solutions were varied in this study.

As shown in Fig. 2, the 2,6-ANS fluorescence in aqueous solution without D-glucose is significantly enhanced upon the addition of β-CD. In aqueous solution without D-glucose, therefore, the fluorescence of uncomplexed 2,6-ANS is negligible relative to the fluorescence of the β-CD–2,6-ANS inclusion complex. From the fluorescence intensity changes, the K values for D-glucose (0.5 and 1.0 g cm^{-3}) solutions have been evaluated to be 880±10 and 760±100 mol^{-1} dm^3, respectively. In D-glucose (0.5 and 1.0 g cm^{-3}) solutions, therefore, about 90 and 88% of 2,6-ANS form the inclusion complexes with β-CD, respectively. The fluorescence from uncomplexed 2,6-ANS in D-glucose solutions is also negligible relative to that from the inclusion complex. Consequently, the fluorescence from the β-CD–2,6-ANS inclusion complex in aqueous solutions with and without D-glucose is responsible for the observed degree of polarization. Figure 8 exhibits a plot of $(1/P-1/3)$ against τ_f/η for the fluorescence, observed at 450 nm, of 2,6-ANS in β-CD (1.0×10^{-2} mol dm^{-3}) solutions containing D-glucose (0—1.0 g cm^{-3}). The fluorescence lifetimes of 2,6-ANS in β-CD (1.0×10^{-2} mol dm^{-3}) solution were evaluated to be 5.3 and 7.5 ns when analyzed as a bi-exponential function. The longer-lifetime component, which is due to the β-CD–2,6-ANS inclusion complex, is shorter than the reported one (9.44 ns), although the shorter-lifetime component, which is assigned to uncomplexed 2,6-ANS, is nearly the same as the reported value (5.18 ns).[21] The lifetime of the longer-lifetime component becomes longer with an increase in the solution viscosity; in solution containing 1.0 g cm^{-3} of D-glucose, it is 10.3 ns, although the lifetime of the shorter-lifetime component almost remains constant.

As reported by Bright et al., 2,6-ANS incorporated into the β-CD cavity is described by a distributed lifetime process.[18] Consequently, the β-CD–2,6-ANS inclusion complex is represented as an ensemble of β-CD–2,6-ANS inclusion complexes with slightly different host–guest conformations. As an approximation, however, we used the fluorescence lifetimes analyzed with a bi-exponential function. The molecular volume estimated on the basis of Eq. 3 represents the molecular volume of 2,6-ANS, which has the evaluated fluorescence lifetime, located within the 1:1 β-CD–2,6-ANS inclusion complex.

From the plot shown in Fig. 8, the molecular volume of 2,6-ANS bound to the β-CD cavity is estimated to be 4.7 nm^3, which is about 24 times greater than the molecular volume (0.20 nm^3) of the 1-chloronaphthalene excimer located within the two associating β-CD cavities.[14] In the case of the 2:2 β-CD–1-chloronaphthalene inclusion complex, the 1-chloronaphthalene excimer independently rotates inside the two β-CD cavities. The external diameter and height of β-CD are 1.54 and 0.79 nm, respectively.[22,23] When β-CD is assumed to be a sphere of 0.77 nm radius, its volume is calculated to be 1.9 nm^3, which may be slightly overestimated.[24] This value is about 40% of the molecular volume of 2,6-ANS bound to the β-CD cavity, which is evaluated from the fluorescence polarization. This finding indicates that the evaluated molecular volume of 2,6-ANS at least contains the molecular volume of β-CD. As shown in Fig. 3, a double reciprocal plot based on Eq. 2 shows a straight line, definitely

indicating the formation of the 1:1 β-CD–2,6-ANS inclusion complex. Therefore, the possibility of the formation of a 2:1 β-CD–2,6-ANS inclusion complex is denied. The evaluated molecular volume is attributed to the 1:1 β-CD–2,6-ANS inclusion complex. Since 2,6-ANS is not thoroughly encapsulated by β-CD, part of 2,6-ANS is extruded from the β-CD cavity; a naphthalene ring of 2,6-ANS is most likely incorporated into the β-CD cavity.[16] The extruded anilino and sulfonato moieties are expected to contribute to the increase in the molecular volume of the β-CD–2,6-ANS inclusion complex. In addition, the hydration of the extruded part of 2,6-ANS would increase the apparent molecular volume of the β-CD–2,6-ANS inclusion complex. Because the volume of a sphere is proportional to the cube of the radius, a slight increase in the radius would increase the molecular volume. For these reasons, the molecular volume (4.7 nm^3) of 2,6-ANS incorporated into the β-CD cavity, which is evaluated from the fluorescence polarization, may be greater than the calculated one (1.9 nm^3) for β-CD. At present, however, the large molecular volume of 2,6-ANS does not seem to be fully interpreted by the above descriptions. Further studies would be necessary to solve this. The evaluated molecular volume of 2,6-ANS encapsulated by β-CD indicates that 2,6-ANS rotates as the entire inclusion complex on a fluorescence lifetime scale. This suggests that the interactions between 2,6-ANS and β-CD are fairly strong; hydrogen bonding between them may be formed.[25] The strong interactions between 2,6-ANS and β-CD are in contrast to the weak interactions between 1-chloronaphthalene and β-CD; the 1-chloronaphthalene excimer located within the β-CD cavities does not rotate as the 2:2 inclusion complex but rotates independently.[14] A study on the rotational relaxation times of resorufin, oxazine-118, and oxazine-725 bound to the β-CD cavity has revealed that they rotate as the entire inclusion complex.[13] With respect to the molecular rotation of a guest located within the inclusion complex, the behavior of these dyes is analogous to that of 2,6-ANS. The whole rotation of the inclusion complex may occur for the guest compounds possessing a functional group such as an amino substituent, probably because they strongly interact with the cavity rim through hydrogen bonding.

To further examine the behavior of the β-CD–2,6-ANS inclusion complex, the molecular volume of uncomplexed 2,6-ANS was evaluated. A Perrin plot of $(1/P-1/3)$ against τ_f/η gave 0.88 nm^3 as its value (not shown). The length along the longitudinal axis of a 2,6-ANS molecule is calculated to be about 1.26 nm. When 2,6-ANS is assumed to be a sphere of 0.63 nm radius, the molecular volume of 2,6-ANS is calculated to be 1.0 nm^3, which is comparable to the evaluated molecular volume (0.88 nm^3) of uncomplexed 2,6-ANS. This finding provides additional evidence that, in the inclusion complex, 2,6-ANS rotates in phase together with β-CD.

Molecular Volume of 1,8-ANS Bound to the β-CD Cavity in Aqueous D-Glucose Solutions. The inclusion mode of the 1:1 β-CD–1,8-ANS has been reported; an anilino moiety of 1,8-ANS is thoroughly enclosed in the β-CD cavity from the secondary hydroxy side of β-CD.[26] In the case of 2,6-ANS, on the other hand, a naphthalene ring is most likely encapsulated by β-CD.[16] Consequently, there may be a difference in molecular volume between the inclusion complexes of 2,6-ANS and 1,8-ANS. Thus, we further examined the molecular volume of 1,8-ANS incorporated into the β-CD cavity.

The fluorescence of 1,8-ANS in aqueous solution is enhanced upon the addition of β-CD.[27–30] The fluorescence enhancement is attributed to the formation of the 1:1 inclusion complex of β-CD with 1,8-ANS. The K value for the β-CD–1,8-ANS inclusion complex was evaluated to be 96±40 $mol^{-1}\ dm^3$ from the intensity change in the 1,8-ANS fluorescence (not shown). This K value is similar to the reported ones (65—110 $mol^{-1}\ dm^3$).[16,30–32] Because a considerable fluorescence enhancement has been observed by the formation of the inclusion complex with β-CD (not shown), the fluorescence of free 1,8-ANS in aqueous and aqueous D-glucose solutions is negligible relative to that of the β-CD–1,8-ANS inclusion complex, although the K value for D-glucose (0.5 $g\ cm^{-3}$) solution has been estimated to be 25±40 $mol^{-1}\ dm^3$, which is smaller than the K value for aqueous solution without D-glucose. The fluorescence lifetimes of the inclusion complex of 1,8-ANS were measured to be 4.2—5.5 ns in aqueous solutions containing D-glucose (0.3—1.0 $g\ cm^{-3}$). As in the case of 2,6-ANS, the fluorescence lifetime analyzed with a bi-exponential function is a typical lifetime of the β-CD–1,8-ANS inclusion complex, which is not a discrete one but an ensemble with various relative host–guest conformations.[18] Figure 9 shows a plot of $(1/P-1/3)$ against τ_f/η for 1,8-ANS in β-CD (1.0×10^{-2} $mol\ dm^{-3}$) solution containing D-glucose. From this plot, 2.7 nm^3 is evaluated as the molecular volume of 1,8-ANS bound to the β-CD cavity. This value is comparable to the molecular volume of β-CD, indicating that 1,8-ANS rotates together with a β-CD molecule as the entire inclusion complex. The molecular volume of uncomplexed 1,8-ANS was evaluated to be 1.1 nm^3, which is similar to that of uncomplexed 2,6-ANS. This value provides additional evidence for the above conclusion

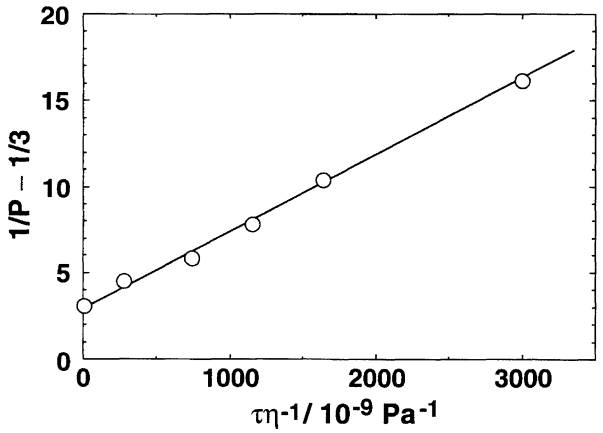

Fig. 9. Plot of $(1/P-1/3)$ against $\tau_f\eta^{-1}$ for the fluorescence of 1,8-ANS solutions containing β-CD (1.0×10^{-2} $mol\ dm^{-3}$) and D-glucose. λ_{ex} = 390 nm. λ_{obs} = 500 nm.

concerning the β-CD–1,8-ANS system. The result that the molecular volume of the β-CD–1,8-ANS inclusion complex is about 60% of that of the β-CD–2,6-ANS inclusion complex may be due partly to the difference in the binding site of 1,8- and 2,6-ANS; β-CD encapsulates an anilino moiety of 1,8-ANS, whereas it encapsulates a naphthalene ring of 2,6-ANS. Because of the incorporation of the anilino moiety, the shape of the β-CD–1,8-ANS inclusion complex seems to be more close to a sphere, compared to that of the β-CD–2,6-ANS inclusion complex. The difference in shape also may cause the difference in the molecular volumes of the inclusion complexes.

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