Inclusional Complexation by Cyclodextrin−Polymer Conjugates in Organic Solvents†

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ABSTRACT: β-Cyclodextrin (β-CD) was modified with poly(*N*-isopropylacrylamide) (PIPA, $M_n = 2.5 \times 10^3$) or poly(ethylene glycol) (PEG, $M_n = 5.15 \times 10^3$) chains (PIPA−β-CD or PEG−β-CD, respectively). In aqueous solutions of PIPA−β-CD and PEG−β-CD, an inclusion of 2-anilino-6-naphthalenesulfonic acid (2,6-ANS) into a cavity of the modified cyclodextrin increased the fluorescence intensity similarly to a free β-CD−2,6-ANS system. The association constants for 2,6-ANS−PIPA-β-CD and 2,6-ANS−PEG−β-CD systems evaluated were, however, slightly smaller than that for the free β-CD−2,6-ANS system, due to a steric hindrance by the polymer chains on the rim of CD. The PIPA−β-CD and PEG−β-CD could be easily dissolved in a tetrahydrofuran−phosphate buffer (*M*/30, pH 7.2) mixture (99:1), and the fluorescence intensity of 2,6-ANS in this medium was decreased by the presence of PIPA−β-CD and PEG−β-CD. A similar tendency was observed in the case of PEG−β-CD dissolved in a 1,4-dioxane−buffer mixture (99:1). This phenomenon might be attributed to the inclusion of 2,6-ANS in the *less* nonpolar environment of the cavity of CD−polymer conjugates than that of bulk solution (tetrahydrofuran−buffer and 1,4-dioxane−buffer, 99:1), which is consistent with the fact that the peak positions of both fluorescence spectra of 2,6-ANS and 8-anilino-1-naphthalenesulfonic acid (1,8-ANS) and electronic spectra of *p-tert*-butylphenol in aqueous β-CD solution were similar to those in polar organic solvents such as glycerol.

Introduction

Recently, molecular recognition phenomena in vivo, where specific binding of guest molecules and subsequent highly efficient catalysis are pursued by host molecules, have been of interest for many scientists, including organic chemists.[1] One of the goals in such a host−guest chemistry is a construction of artificial enzymes that can recognize only a specific type of substrate and accelerate its reaction enormously under mild conditions.

Cyclodextrins (CDs), cyclic α-1,4-oligoglucopyranosides, have served as the mainstays in this field of chemistry since the pioneering works of Bender et al.[2] It has been frequently reported that the unique cyclic structure of cyclodextrins with a nonpolar cavity can recognize many compounds containing hydrophobic groups by including the compounds specifically in their cavities.[3] CDs have been applied industrially to stabilizers for various compounds, solubilizers for slightly water-soluble compounds, etc., and biologically to design receptor model, analysis of catalytic function to mimic enzymes, separation of amino acids or nucleic acids, etc.[4−6] Furthermore, an enhancement and inhibition of the catalytic effect of CD by the presence of various water-soluble polymers were previously investigated, and the dissociation constants for the complex of α- and β-CDs with various polymers were determined for the first time.[7] Recently the preparation of a molecular necklace of cyclodextrins ("rotaxane") has been examined.[8] The photoresponsive catalysis in ester hydrolysis was pursued by using thymine−CD conjugates.[9] Taking these previous results into consideration, we examined here the molecular recognition by cyclodextrin−polymer conjugates in organic solvent systems.

As mentioned above, the cavity of CD is considered to be more nonpolar than the exterior surface, and it has been believed that many organic compounds are included in the cavity by the *hydrophobic* interaction. If CD can be dissolved in organic solvent, what will be changed in the inclusion behavior of organic compounds? This simple question is the first motif for beginning this research. For that purpose we modified CD with polymer chains such as poly(ethylene glycol) and poly(*N*-isopropylacrylamide), both of which are soluble in organic solvents such as alcohols, tetrahydrofuran (THF), 1,4-dioxane, etc., and examined the effect of introduction of polymer chains on the inclusion behavior of fluorescence dyes such as 2-anilino-6-naphthalenesulfonic acid (2,6-ANS) into the cavity of the modified CD, both in aqueous and organic solutions. Since many enzymes such as lipase do their roles in nonpolar environment,[10] basic study on specific catalysis in organic solvent systems might be very important, not only for industrial application but also for basic research of biological processes.

Experimentals

Materials. β-Cyclodextrin was from Nacalai Tesque, Kyoto, Japan. *N*-Isopropylacrylamide from Eastman Kodak, Rochester, NY, was purified by recrystallization from *n*-hexane−acetone. 8-Anilino-1-naphthalenesulfonic acid, ammonium salt (1,8-ANS), was from Wako Pure Chemicals, Osaka, Japan. 2-Anilino-6-naphthalenesulfonic acid (2,6-ANS) was from Molecular Probes Inc., Eugene, OR. Other reagents were commercially available. Deionized water was distilled to prepare sample solutions.

Preparation of Amino Group-Carrying β-CD. β-Cyclodextrin (19.9 g) was coupled with *p*-toluenesulfonyl (tosyl) chloride (42.4 g) in dry pyridine (70 mL) for 2 h at 25 °C.[5d,f,g] After evaporation of pyridine, water (50 mL) was added to the reaction mixture. The precipitate was filtrated, washed several times with water to remove pyridine, and dried in vacuo (tosyl−β-CD, 24.8 g). ^{1}H NMR (dimethyl-d_7 sulfoxide (DMSO-d_7)) δ 7.49 (20H, aromatic protons), 5.60 (7H, C(1)*H*). IR (KBr, cm^{-1}): 3400 (ν_{OH}), 3050 (ν_{CH}), 2950 (ν_{as,CH_2}), 1360, 1180 ($\nu_{S(=O)_2}$), 1000, 920, 820 (ν_{SOC}), 750, 670 (ρ_{CH_2}). Anal. Calcd

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for (tosyl)$_5$−β-CD$_5$·H$_2$O: C, 46.33; H, 5.56. Found: C, 46.24; H, 5.25.

The tosyl−β-CD (6.56 g) was reacted with an excess amount of ethylenediamine (EDA, 20 mL) at 40 °C for 46 h, and precipitated in cold acetone (30 mL). A 2-aminoethylamino group-carrying β-CD was finally obtained (EDA−β-CD, 4.2 g).^{1}H NMR (D$_2$O): δ 5.02 (7H,C(1)*H*), 2.90 (10H,−C*H*$_2$NH−β-CD), 1.70 (−C*H*$_2$NH$_2$). IR (KBr, cm^{-1}): 3350 (ν_{NH}), 1060 (ν_{CN}). Anal. Calcd for (EDA)$_5$−β-CD: C, 46.43; H, 7.44; N, 10.42. Found: C, 46.57; H, 7.47; N, 9.96.

Preparation of Poly(*N*-isopropylacrylamide) with a Carboxyl Group. N-Isopropylacrylamide (IPA, 23.7 g, 209 mmol) was polymerized by using 2,2′-azobis(isobutyronitrile) (AIBN, 349 mg, 2.1 mmol) and β-mercaptopropionic acid (MPA, 1.11 g, 10.4 mmol) as initiator and chain transfer reagent, respectively, in MeOH (40 mL) at 70 °C for 7 h.[5c,e] After evaporation of the solvent, the poly(*N*-isopropylacrylamide) (PIPA) obtained was dissolved in a small amount of acetone and precipitated in *n*-hexane. The polymer product was dissolved in water and ultrafiltrated by using a Diaflo membrane (Amicon YM-3; cutoff molecular weight for globular molecules, 3000) and lyophilized (PIPA, 19.9 g). The degree of polymerization (DP) of PIPA was determined to be 22 ($M_n = 2.5 \times 10^3$) by the conductometric titration of the carboxyl group at the end of the polymer. This is consistent with the M_n value (2.45×10^3; $M_w/M_n = 1.09$) estimated by using a matrix-assisted laser desorption−ionization time-of-flight mass spectrometer (MALDI-TOF MAS, Voyager RP, PerSeptive Biosystems).

Modification of β-CD with PEG. Monomethoxypoly(ethylene glycol) (2.5 g, $M_n = 5.15 \times 10^3$, $M_w/M_n = 1.01$) was coupled with carbonyldiimidazole (CDI, 800 mg) in dry 1,4-dioxane (10 mL) for 3 h at 25 °C, and after evaporation, the oily mixture was stirred with dry diethyl ether to give a white precipitate (PEG−CDI).[11,12] After filtration and drying in vacuo, PEG−CDI (2 g) was coupled with EDA−β-CD (87 mg) in dry dimethylformamide (DMF) (20 mL) in the presence of dry triethylamine (TEA, 1 mL) at 25 °C for 72 h. After evaporation, the oily mixture was precipitated in ethyl acetate (solvent of PEG). The slightly yellowish powder obtained was dissolved in water, dialyzed against water (Seamless-cellulose tube; cutoff molecular weight, 13 000; Wako Pure Chemicals) for 7 days, and lyophilized (PEG−β-CD, 1.2 g, $M_n = 8.42 \times 10^3$, $M_w/M_n = 1.17$; the most probable structure, (PEG−EDA)$_2$−β-CD). ^{1}H NMR (D$_2$O): δ 4.98 (7H, C(1)*H*), 4.01 (4H, −C*H*$_2$CO−EDA−β-CD), 3.56 (898H, −CH$_2$C*H*$_2$O−), 1.18 (6H, −C*H*$_3$). IR (Nujol mull method, cm^{-1}): 1650 (ν_{CO}), 1670 (δ_{NH}), 1365, 1345 (ν_{CN}). Anal. Calcd for (PEG−EDA)$_2$−β-CD·18H$_2$O: C, 53.51; H, 8.87; N, 0.92. Found: C, 53.84; H, 8.79; N, 0.38.

Modification of β-CD with PIPA. The modification of EDA−β-CD (0.522 g) with PIPA (5.64 g) using a water-soluble carbodiimide (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, WSC, 435 mg) was carried out as reported previously (PIPA−β-CD, 2.21 g, $M_n = 5.20 \times 10^3$, $M_w/M_n = 1.16$; the most probable structure, (PIPA−EDA)$_2$−β-CD).[5c,e] ^{1}H NMR (D$_2$O) δ 4.97 (7H, C(1)*H*), 3.52 (5H, −C*H*$_2$CO−EDA−β-CD), 2.61 (10H, −C*H*$_2$S−), 1.99 (37H, C*H*CONH−), 1.59 (70H, −CHC*H*$_2$CH−), 1.18 (7H, −C*H*$_3$). IR (Nujol mull method, cm^{-1}): 1645 (ν_{CO}), 1540 (δ_{NH}), 1380, 1360 (ν_{CN}). Anal. Calcd for (PIPA−EDA)$_2$−β-CD·18H$_2$O: C, 56.63; H, 8.63; N, 10.46. Found: C, 56.45; H, 8.90; N, 11.00.

Turbidity Measurements. The turbidity of solutions of the PIPA derivatives at 500 nm was followed by using a UV−visible spectrophotometer (Ubest 35, Japan Spectroscopic Co., Tokyo, Japan). The observation cell was thermostated by a Peltier device.

Absorbance Measurements. The absorbance of *p-tert*-butylphenol was observed by a Shimadzu UV-1600 spectrophotometer (Kyoto, Japan) at 25 °C. The observation cell was thermostated by using a Neslab waterbath (RTE-111).

Fluorescence Measurements. The fluorescence of 1,8- and 2,6-ANS solutions in the presence of cyclodextrins was observed by using a fluorescence spectrophotometer (FP-777, Japan Spectroscopic). The observation cell was thermostated by using the Neslab waterbath. The excitation wavelengths for 1,8-ANS and 2,6-ANS were 350 nm, while the emission wavelengths were from 370 to 650 nm. The spectral bandpasses were 1.5 nm. Integration of the peak area gave relative fluorescence intensity (arbitrary unit). Concentration of 1,8-ANS or 2,6-ANS was 0.1 mM unless otherwise noted.

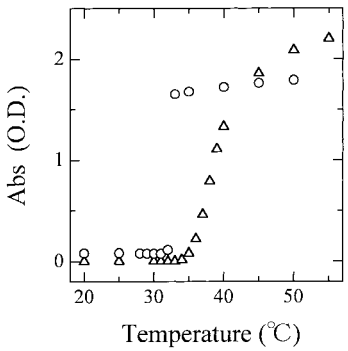

Figure 1. Temperature effect on the turbidity of the solution of PIPA derivatives. Wavelength, 500 nm. ○, PIPA−β-CD. △, PIPA.

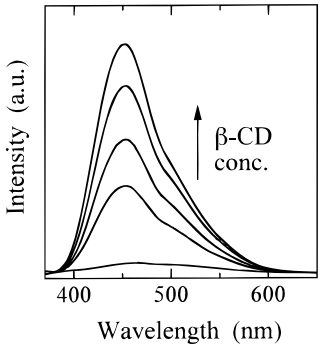

Figure 2. Fluorescence spectra of 2,6-ANS in the presence of various concentrations of β-CD at 25 °C. pH 7.2 phosphate buffer (*M*/30). [β-CD] (from bottom to top) = 0, 0.2, 0.4, 0.8, and 1.6 mM.

Results and Discussion

A. Turbidity of Aqueous Solutions of PIPA Derivatives. Turbidity of aqueous solutions of both PIPA and PIPA−β-CD was largely increased above 34 (PIPA) and 32 °C (PIPA−β-CD) (Figure 1). This turbidity change corresponds to the thermoreversible coil−globule transition of PIPA chains in aqueous media in response to small changes in temperature at about 32 °C,[13−17] which is called a lower critical solution temperature (LCST). PIPA chains hydrate to form expanded structures in water when the solution temperature is below its transition temperature, whereas they become compact structures by dehydration when heated above the transition temperature.[16] The observed transition temperature for PIPA is slightly higher than that reported (32 °C),[13,16] because the molecular weight of PIPA examined here was smaller than those previously examined (>50 000). The dependence of the transition temperature on the molecular weight of PIPA was previously reported.[17]

B. Fluorescence of ANS in CD Solutions. Next we examined fluorescence spectra of 2,6-ANS. It is well-known that fluorescence of 2,6-ANS is largely affected by the polarity of the microenvironment.[18] By the presence of β-CD, the fluorescence (emission 450 nm) was largely increased (Figure 2), showing that 2,6-ANS was captured in the nonpolar cavity of CD. Assuming

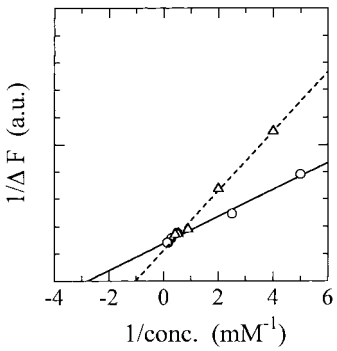

Figure 3. Double reciprocal plot of the increase in fluorescence intensity of 2,6-ANS and concentration of β-CD or PIPA−β-CD in the phosphate buffer at 25 °C. ○, β-CD; △, PIPA−β-CD.

Table 1. Association Constants for Various Systems[a]

system	K (M^{-1}) phosphate buffer[b]	THF−buffer[b] (99:1)	1,4-dioxane−buffer[b](99:1)
2,6-ANS + β-CD	2.7×10^3 [c]		
2,6-ANS + PIPA−β-CD	1.0×10^3	1.4×10^3	
2,6-ANS + PEG−β-CD	1.7×10^3	2.1×10^3	1.1×10^3

[a] At 25 °C. Uncertainties of the association constants were within 20%. [b] *M*/30, pH 7.2. [c] In water, $K = 1.9 \times 10^3$ M^{-1}. At pH 6.0, $K = 2.5 \times 10^3$ M^{-1}.[19]

that 2,6-ANS makes a 1:1 inclusion complex with CD (eq 1),[19] the association constant of 2,6-ANS−CD com-

$$\text{2,6-ANS} + \text{CD} \overset{K}{\rightleftharpoons} \text{2,6-ANS−CD} \qquad (1)$$

plex, K, could be estimated from the double reciprocal plot of [CD] vs the increase in the relative fluorescence intensity (ΔF) of ANS using Benesi−Hildebrand eq 2 (Figure 3, Table 1).

$$\frac{1}{\Delta F} = \frac{1}{[\text{CD}]}\frac{1}{[\text{ANS}]}\frac{1}{K} + \frac{1}{[\text{ANS}]} \qquad (2)$$

The association constant that was obtained (2.7×10^3 M^{-1}) was similar to that reported before (2.5×10^3 M^{-1} at pH 6.0).[19] As the driving forces for the association of CD with guest molecules, several intermolecular interactions have been proposed:[3a] (1) hydrophobic interaction, (2) van der Waals interaction, (3) hydrogen bonding interaction, (4) relief of high-energy water from the CD cavity upon inclusion of a guest, and (5) relief of the conformational strain in a vacant CD (CD−water complex) upon inclusion of a guest. Especially the hydrophobic interaction between the cavity and the solutes has been emphasized.

In 1967 it was reported that the microenvironment of the CD cavity is comparable to that of 1,4-dioxane (dielectric constant $\epsilon = 2.2$), because the absorption peak of *p-tert*-butylphenol in a α-CD solution was similar to that in 1,4-dioxane.[2b] To reconfirm this, we examined the UV absorption of *p-tert*-butylphenol in various solvents (Figure 4a and Table 2). The absorbance peak of the phenol shifted to longer wavelength with a decrease in polarity (E_T^N,[20] and ϵ) of the solvents. Furthermore, in cyclohexane ($\epsilon = 2.02$), the absorption peak shifted to shorter wavelength again, showing that the peak shift of *p-tert*-butylphenol is not highly reliable in estimating the micropolarity of the CD cavity. If we neglect the result in cyclohexane, we can say that the

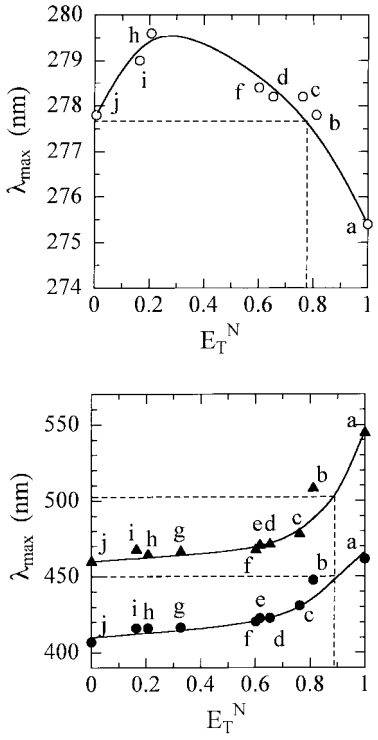

Figure 4. (a, top) Solvent effect on UV absorption of 0.1 mM *p-tert*-butylphenol at 25 °C. (b, bottom) Solvent effect on fluorescence of 2,6-ANS (●, 0.02 mM) and 1,8-ANS (▲, 0.04 mM) at 25 °C. a, water; b, glycerol; c, methanol; d, ethanol; e, 1-propanol; f, 1-butanol; g, acetone; h, tetrahydrofuran; i, 1,4-dioxane; j, cyclohexane (saturated solutions of 2,6-ANS or 1,8-ANS were used). The dotted lines in the figures correspond to the peak positions in β-CD solution.

Table 2. Relationship between λ_{max} of *p-tert*-Butylphenol and Dielectric Constant of the Solvents Examined[a]

solvent	λ_{max}	dielectric constant (F/m)
water	275.4	78.5
glycerol	277.8	42.5
methanol	278.2	32.6
ethanol	278.2	24.3
1-butanol	278.4	17.7
THF	279.6	7.39
1,4-dioxane	279.0	2.21
cyclohexane	277.8	2.02
α-CD[b]	277.8	
β-CD[c]	277.6	
γ-CD[d]	276.8	

[a] At 25 °C. [*p-tert*-butylphenol] = 0.1 mM. [b] 25 mM in H_2O. [c] 10 mM in H_2O. [d] 25 mM in H_2O.

peak wavelength in the presence of both α- and β-CD was quite similar to that in glycerol ($\epsilon = 42.5$), and *not* in 1,4-dioxane ($\epsilon = 2.2$).

As for the fluorescence of 2,6-ANS and 1,8-ANS, we plotted the emission peak versus polarity (E_T^N) of various organic solvents (Figure 4b). In the figure, the emission peaks of 2,6-ANS and 1,8-ANS in the presence of β-CD (7.6 and 9.9 mM, respectively) in water (450 and 508 nm, respectively) were also shown with a broken line. From these results it can be said that the microenvironment of the CD cavity was comparable to that in highly polar organic solvents such as glycerol (dielectric constant $\epsilon = 42.5$) and not so nonpolar as widely believed hitherto. Nigam and Durocher reported that the polarity value (ϵ) of 10 mM aqueous β-CD solution is similar to 41 (MeOH−water = 80:20),[21] which is in good agreement with our results (glycerol, $\epsilon = 42.5$).

There are extremely large discrepancies in the literature among the data on the polarity of the cyclodextrin cavity with the effective equivalent dielectric constants ranging from 2.2 to 55.[22] Among them Van Etten et al.[2b] and Hamai[23] reported that the microenvironment was similar to 1,4-dioxane ($\epsilon = 2.2$). Hamai, furthermore, reported in another paper that the polarity is similar to 1-propanol ($\epsilon = 20.1$).[24] Our present data concerning the peak shifts of *p-tert*-butylphenol, 1,8-ANS, and 2,6-ANS suggested that the cavity was more nonpolar than water, but not very much and as low as glycerol at the most.

It should be mentioned here that the *K* value for 2,6-ANS with *β*-CD (2.7×10^3 M^{-1}) is much higher than that for 1,8-ANS (80 M^{-1}),[5c] which means that the steric hindrance to penetrate into and dissociate from the cavity of CD is very important in deciding the *K* value, because the polarity of 2,6-ANS seems not to be so largely different from that of 1,8-ANS. It has been often reported that many inorganic ions can be included in the CD cavity. For example, the *K* values for the complexation of I_3^- ion with α- and *β*-CD were reported to be 2.2×10^5 and 4.5×10^2 M^{-1}, respectively.[25] These values are much larger than that for the complexation of *β*-CD with 1,8-ANS (80 M^{-1}). The *K* values for the complexation of α-CD with $HClO_4$ and $NaClO_4$ were 40 and 20 M^{-1}, respectively,[26] and comparable to that of the 1,8-ANS−*β*-CD system.

It was reported that PEG forms a complex with α-CD, but *not* with *β*-CD.[8] There seem to be no difficulties for PEG molecules to penetrate into the *β*-CD cavity. Therefore, it is probable that the friction for PEG molecules to pass through the α-CD cavity was much larger than that through the *β*-CD cavity (in other words, the dissociation step is very fast in the *β*-CD−PEG system), which might result in the highly stable complex of PEG−α-CD rather than PEG−*β*-CD complex to be easily observed as precipitates. It was previously reported that an introduction of bulky groups at the end of the PEG chain prevented the chain from passing through the cavity of *β*-CD and stabilized the complex.[27]

It has been recently reported that the addition of urea decreased the association constant of CD with various organic compounds.[28,29] However, there still remained the detectable inclusion phenomena even in the presence of 5 M urea in which both hydrogen bonding and hydrophobic interaction are much smaller than in water[30] (*K* for *β*-CD with 2-(*p*-(dimethylamino)phenyl)-3,3-dimethyl-5-carboethoxy-3*H*-indole, 1770 (in H_2O), 780 (in 3 M urea), and 540 M^{-1} (in 5 M urea)).[28]

These results also support that the major driving force to be included into the cavity of cyclodextrin is not always hydrophobic interaction, and the steric factor is very often more dominant over the *K* value than the hydrophobic interaction.

C. Fluorescence Spectra of ANS in PIPA−*β*-CD and PEG−*β*-CD Solutions. Figure 5 shows fluorescence spectra of 2,6-ANS in the presence of PIPA−*β*-CD in the phosphate buffer. Similar to *β*-CD, the fluorescence intensity increased and the fluorescence peak shifted to lower wavelength with an increase in the concentration of PIPA−*β*-CD, showing that the microenvironment in the cavity of polymer-conjugated CD is relatively more nonpolar than that of the buffer, which is consistent with the tendency of free *β*-CD. The association constant for the 2,6-ANS−PIPA−*β*-CD system obtained by the double reciprocal plot was slightly

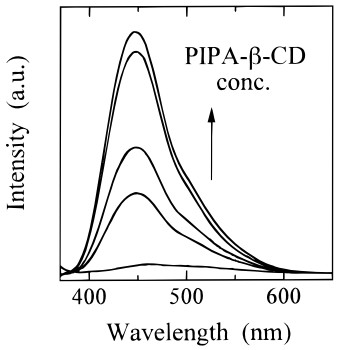

Figure 5. Fluorescence spectra of 2,6-ANS in the presence of various concentrations of PIPA−*β*-CD in the phosphate buffer at 25 °C. [PIPA−*β*-CD] (from bottom to top) = 0, 0.25, 0.5, 1.13, and 2.38 mM.

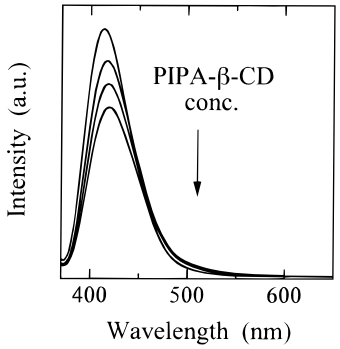

Figure 6. Fluorescence spectra of 2,6-ANS in the presence of various concentrations of PIPA−*β*-CD in THF−buffer (99:1) at 25 °C. [PIPA−*β*-CD] (from top to bottom) = 0, 0.17, 0.47, and 0.79 mM.

smaller than that for the 2,6-ANS−free *β*-CD system (Table 1), probably due to the steric effect of PIPA chains on the rim of CD for the complexation. A similar effect was observed for the 2,6-ANS−PEG−*β*-CD system (Table 1). It should be mentioned here that the PIPA alone did not increase the fluorescence intensity of 2,6-ANS significantly, probably because the molecular weight of PIPA ($M_n = 2500$) was not sufficiently high to capture 2,6-ANS molecules within its chain. Therefore, the *K* values for the PIPA−2,6-ANS complex could not be evaluated.

In a 99% (v/v) THF−buffer mixture, to the contrary, the fluorescence intensity of 2,6-ANS was *decreased* by the addition of PIPA−*β*-CD (Figure 6). It should be mentioned here that it was difficult to remove water from water-soluble CD−polymer conjugates completely. The remaining water would affect the complexation of 2,6-ANS with the CD residue in the polymers and cause considerable experimental error. We, therefore, added a predetermined amount of water (1% (v/v)) to the systems to reduce the error caused by the water of an uncontrollable amount. The result in Figure 6 suggests that the microenvironment of the cavity of polymer-conjugated CD is less nonpolar than that of bulk solution (99% THF−buffer). This is consistent with the results shown in Figure 4 and Table 2.

In nonpolar organic solvents, polar compounds might make self-association, as nonpolar compounds do in water.[31] Actually, the DLS data (hydrodynamic diameter of PIPA−*β*-CD $D_{hd} = 140$ and 46 nm in the buffer and in the THF−buffer mixture (99:1), respectively, at 25 °C) showed that PIPA−*β*-CD aggregates in these media (the average length of PIPA chains in all-trans

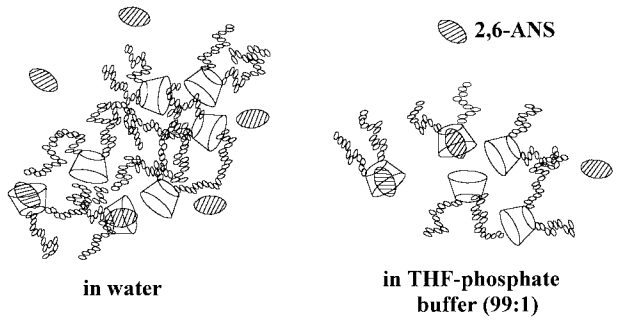

Figure 7. Susceptible situation of PIPA−β-CD molecules in the buffer and THF−buffer (99:1).

conformation is 5.5 nm). In the THF−buffer (99:1), PIPA−β-CD would make a self-association to form a *reversed micelle*-like structure in which the CD moiety might make a core region, and PIPA chains extend to an outer solvent phase (Figure 7). The small amount of water existing in the system (1% (v/v)) would be preferentially captured in the core region (CD moieties) of the aggregates. As mentioned above, the microenvironment in the cavity was similar to that in glycerol, and not to that in 1,4-dioxane. Therefore, the fluorescence intensity of fluorophores (hydrophobic probes) was decreased by the presence of CD−polymer conjugates.

A similar tendency was observed in the D_{hd} values of PEG−β-CD (in water and in the THF−buffer mixture (99:1), 141 and 32 nm, respectively). The length of PEG chains ($M_n = 5150$) was calculated to be 42 and 32 nm in the all-trans conformation and in a helical form as in crystal (the length of the PEG unit ($-CH_2CH_2O-$) in crystal, 0.276 nm),[32] respectively. The value of $(\langle R_g^2\rangle/M)^{0.5}$ of PEG in water was reported to be 0.034 nm ($\langle R_g^2\rangle$, unperturbed mean-square radius of gyration),[33] and consequently, the value of $\langle R_g^2\rangle^{0.5}$ for PEG with $M = 5150$ is calculated to be 2.44 nm in water. The unperturbed root-mean-square end-to-end distance r_o for PEG ($M = 5150$) was calculated to be 5.4 ± 0.2 nm in various solvents.[34] Therefore, PEG−β-CD might aggregate both in water and in the THF−buffer mixture. Taking account of hydrodynamic diameters of these CD−polymer conjugates, the degree of self-association of PEG−β-CD would be smaller than that of PIPA−β-CD in these media, because the length of PEG chains was much larger than that of PIPA chains as mentioned above (in all-trans conformation, 42 and 5.5 nm for PEG and PIPA, respectively).

PEG−β-CD could also be dissolved in 1,4-dioxane−buffer (99:1), where the environment is more nonpolar than in the THF−buffer mixture. The hydrodynamic diameter of PEG−β-CD in this medium was 54 nm, suggesting that PEG−β-CD aggregates similarly to that in the THF−buffer mixture. The tendency that the addition of PEG−β-CD to the system *decreased* the fluorescence intensity of 2,6-ANS was clear (data not shown), supporting the hypothesis that 2,6-ANS is captured in a cavity of PEG−β-CD, which is more polar than the bulk organic solution.

There would be a controversy that the structure of modified CD is not strictly the same as that of β-CD. However, the tendency that the fluorescence intensity of 2,6-ANS in the phosphate buffer was increased by the presence of PIPA−β-CD and PEG−β-CD supported that the inclusion mechanism of 2,6-ANS in the cavity of β-CD is similar to that in the cavity of the modified CD.

Such a system would be useful in clarifying the inclusion behavior of the guest into the host molecules, and at the same time, in preparing molecular receptors which can do their roles in organic solvents.

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