Interaction of Cyclodextrin-Containing Polymers with Fluorescent Compounds

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ABSTRACT: The interaction of poly(acryloyl-β-cyclodextrin) (poly-β-CD-A) with some fluorescent dyes was studied by the fluorescent technique and was compared with that of β-cyclodextrin (β-CD). Poly-β-CD-A (10^{-3} M) was found to cause much greater enhancement (571-fold) and larger blue shift of the fluorescence of potassium 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) (2×10^{-5} M) than β-CD (25-fold), acryloyl-β-CD (27-fold), and *N*-acrylyl-6-aminocaproyl-β-CD (29-fold). Dissociation constants and stoichiometries of the complexes were determined by the fluorometric titration and the continuous variation method. The polymer complex showed exclusively 2:1 stoichiometry, whereas the complex of β-CD showed 1:1 stoichiometry at low concentrations of β-CD and 2:1 stoichiometry at high concentrations. The 2:1 complex of poly-β-CD-A was much more stable and fluorescent than the 2:1 complex of β-CD. A homopolymer of acryloyl-β-CD caused larger fluorescence enhancement than copolymers with acrylic acid and with acrylamide. Thus, two cyclodextrin units on the polymer chain are considered to cooperate in binding one TNS molecule. The structural requirements for the fluorescence enhancement of TNS are discussed.

Cyclodextrins (CD) are known to form noncovalent inclusion complexes with various organic molecules in aqueous solution.[1] The inclusion properties of CD's resemble those of proteins,[2] so that CD's have been used as models of enzymes[3] and proteins.[4]

In the preceding papers we reported the preparation of some polymers containing cyclodextrins[5] and their catalytic and inclusion behaviors.[6] Polymers containing β-cyclodextrin were found to bind substrates which were too large to be accommodated in a single CD cavity more efficiently than β-CD but to bind small substrates which could be completely included in a single cavity less efficiently than β-CD. We suggested that these polymer effects on the formation of complexes with large substrates were caused by cooperation of two adjacent β-CD moieties on a polymer chain.

In this work, the interaction of some water-soluble fluorescent compounds (especially fluorescent dyes which have been widely used as microenvironmental probes[7]) with polymers containing cyclodextrin was studied by fluorescence techniques. Data were compared with the case of cyclodextins in order to make clear the cooperative effects in binding and moreover to investigate in detail how this cooperation affects the guest molecules. The relationships between the properties of the fluorescent dyes and the structure of the binding site are discussed in terms of the structural requirement for binding and for fluorescence change.

The interaction between cyclodextrins and fluorescent dyes was studied by Cramer et al.,[8] Kondo et al.,[9] and Seliskar et al.[10a]

Polymeric units used are shown in Figure 1.

Experimental Section

Materials. Cyclodextrins were kindly supplied from Hayashibara Biochemical Laboratories Inc. and were purified as described previously.[5] Poly-β-CD-A and poly-β-CD-NAC were prepared as described previously.[5] The molecular weights of the polymers were estimated to be 10^4–10^5. Potassium 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) was purchased from Sigma Chemical Co. Its purity was checked by thin layer chromatography on silica gel G (Merk). Sodium 1-anilinonaphthalene-8-sulfonate (ANS), dimethylaminonaphthalenesulfonylphenylalanine (DNS-Phe), Rhodamine B, Auramine O, β-naphthol, methyl α-glucoside, maltose, amylose, and dextran were obtained commercially. Pullulan was kindly supplied from Hayashibara Biochemical Lab.

Fluorescence Measurements. Fluorescence measurements were carried out in 0.1 M phosphate buffer (pH 5.9) using a Hitachi MPF-3 spectrofluorometer. Excitation of TNS fluorescence was at 366 nm, and the fluorescence intensity was monitored at 460 nm for the TNS–β-CD system and at 438 nm for the poly-β-CD-A–TNS system,[10b] which were the corresponding emission maxima. The band width for excitation was 8 nm and was usually 10 nm for emission. The concentration of TNS was determined spectrophotometrically assuming that the molecular absorptivity $\epsilon_{TNS} = 4300$ at 366 nm. When the absorbance exceeded 0.1 at the wavelength of excitation, corrections for self-absorption of incident and emitted light were applied to the emission intensities.

Absorption spectra were measured using a Hitachi spectrophotometer, Model 124.

Determination of Dissociation Constants. The dissociation constants were determined as follows. If a cyclodextrin molecule forms a homogeneous inclusion complex with a TNS molecule, the dissociation constant of the cyclodextrin–TNS complex can be defined by eq 1.

$$\mathrm{CD} + \mathrm{S} \underset{K_d}{\rightleftharpoons} \mathrm{CD\text{–}S} \tag{1}$$

$$K_d = \frac{[\mathrm{CD}][\mathrm{S}]}{[\mathrm{CD\text{–}S}]} \tag{2}$$

where [CD], [S], and [CD–S] represent the concentrations of cyclodextrin, TNS, and the CD–TNS complex, respectively. When [CD] is in large excess of [CD–S],

$$\frac{1}{I} = \frac{K_d}{I_\infty}\frac{1}{[\mathrm{CD}]} + \frac{1}{I_\infty} \tag{3}$$

where I is the observed fluorescence intensity in arbitary units. I_∞ is the limiting value of the fluorescence intensity I.

Measurements of Fluorescence Polarization. Measurements of polarization of fluorescence were carried out with a Shimadzu spectrofluorometer RF 501 by obtaining $I_\parallel$ and $I_\perp$ and calculating the polarization P according to $P = (I_\parallel - I_\perp)/(I_\parallel + I_\perp)$.

Results

Interaction with Fluorescent Compounds. Table I shows the changes of fluorescence intensity and wavelength at maximum emission of several fluorescent compounds on addition of α-CD, β-CD, and poly-β-CD-A to aqueous buffer solutions. Addition of α-CD had little effect on the fluorescence spectrum of any of the compounds, whereas the additions of β-CD and poly-β-CD-A had large effects. The fluorescence intensity of Rhodamine B decreased to almost half on addition of β-CD. Auramine O, which binds to anionic polymers with emission (DNA,[11] PMA[12]), showed no fluorescence either in the absence or presence of CD's or poly-β-CD-A. The fluorescence spectrum of β-naphthol, which can be completely included in the β-CD cavity,[13] was affected to similar extents by β-CD and poly-β-CD-A. Among these dyes, large fluorescence change was observed with naphthylamine derivatives which had been widely used as microenvironmental probes, i.e., ANS,[14] TNS,[15] and DNS-Phe.[16] These compounds are virtually nonfluorescent in aqueous solutions, but fluoresce strongly when adsorbed to certain proteins[17] or

Table I
Effects of CD's and Poly-β-CD-A on the Fluorescence of Dyes[a]

Dye 10^{-5} M	I/I_0[b] +α-CD	I/I_0[b] +β-CD	I/I_0[b] +Poly-β-CD-A	λ_{max}^F None	λ_{max}^F +α-CD	λ_{max}^F +β-CD	λ_{max}^F +Poly-β-CD-A
Rhodamine B[c]	1.00	0.62	1.57	579	579	578	576
Auramine O[d]							
β-Naphthol	1.02	1.51	1.57	352	352	352	352
	0.98	0.89	0.82	408	408	405	405
ANS	2.2	10.4	70.0	515	510	495	475
TNS[e]	3.3	25.3	571	500	480	460	437
DNS-Phe	1.1	150	600	548	540	500	500

[a] pH 5.9 phosphate buffer, [CD] = 10^{-3} M. [b] I/I_0, relative fluorescence intensity. [c] *N*-[9-(2-Carboxyphenyl)-6-(diethylamino)-3*H*-xanthen-3-ylidene]-*N*-ethylethanaminium chloride. [d] 4,4′-Carbonimidoylbis[*N*,*N*-dimethylbenzenamine monohydrochloride]. [e] 2×10^{-5} M.

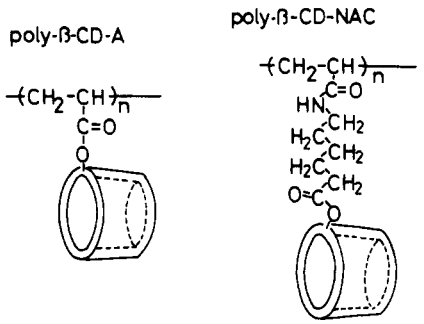

Figure 1. Cyclodextrin-containing polymers.

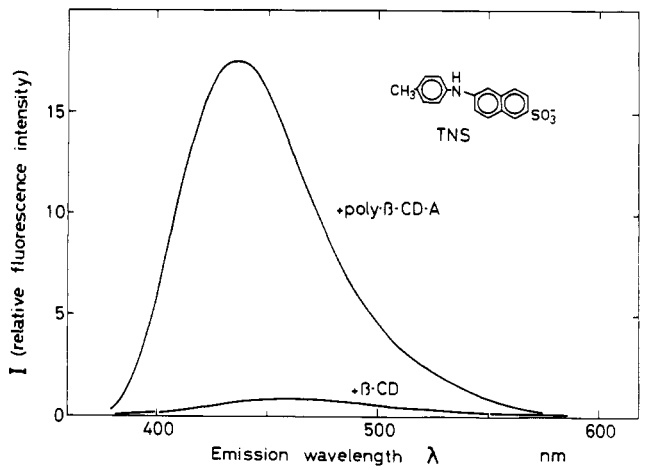

Figure 2. Fluorescence spectra of TNS in the presence of β-CD and poly-β-CD-A. [TNS] = 5×10^{-5} M, [β-CD] = [poly-β-CD-A] = 10^{-3} M, at pH 5.9. The emission spectra given here are not corrected for response of the photomultiplier and for transmission of the monochromator.

membranes.[18] β-CD caused a tenfold increase of fluorescence of ANS,[8] whereas poly-β-CD-A caused a 70-fold increase. In the case of TNS, the fluorescence intensity increased 25-fold on addition of β-CD[10] and almost 600-fold on addition of poly-β-CD-A. A similar tendency was observed in the case of DNS-Phe.

Interaction with TNS. The fluorescence spectra of TNS in the presence of β-CD and of poly-β-CD-A are shown in Figure 2. In this experiment the β-CD (or unit) concentration was in both cases 10^{-3} M, which was in large excess over the TNS concentration of 5×10^{-5} M, so that virtually all the TNS molecules added were bound to β-CD (or unit). TNS

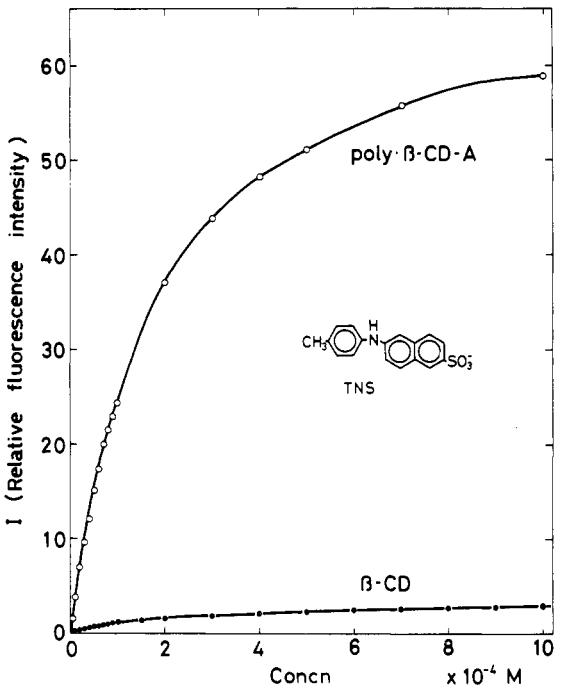

Figure 3. Effects of β-CD (●) and poly-β-CD-A (○) concentrations on the fluorescence intensity of TNS (10^{-5} M).

alone in buffer showed negligible fluoescence. In the presence of β-CD, TNS showed enhanced fluorescence with a blue shift of the emission maximum (see also Table I), and in the presence of poly-β-CD-A much greater fluorescence was observed with a significant blue shift.

The interactions of β-CD and poly-β-CD-A with TNS were studied quantitatively by fluorescence titration. The effects of concentration of β-CD and of poly-β-CD-A upon the fluorescence intensity at a fixed concentration of 10^{-5} M TNS are shown in Figure 3. Poly-β-CD-A caused strikingly larger increase in fluorescence than β-CD throughout the concentration range tested. The intensity showed saturation with increase in the CD concentration, the fact indicating that the fluorescence change was caused by formation of a complex.

Figure 4 shows the double reciprocal plots of results on titration of TNS with β-CD and with poly-β-CD-A. If the binding sites are homogeneous and independent, the double reciprocal plot should give a straight line. The plot for β-CD is linear at low concentrations ($<10^{-3}$ M), but it deviates from linearity at higher concentrations and then gives another straight line with concentrations of above 5×10^{-3} M. This indicates that another type of binding occurs at higher β-CD

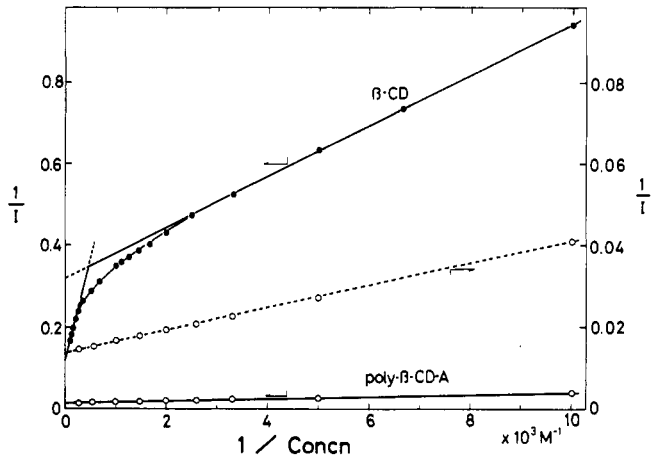

Figure 4. Double reciprocal plots of titration of TNS by β-CD (●) and poly-β-CD-A (○); the ordinate is enlarged ten times in the dotted line.

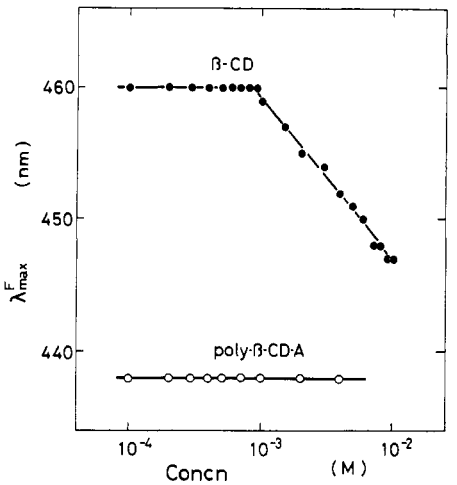

Figure 5. Effects of β-CD (●) and poly-β-CD-A (○) concentrations on the wavelength of maximum emission of TNS ([TNS] = 10^{-5} M).

concentrations. On the other hand, the plot for poly-β-CD-A is linear over all the concentration ranges examined, indicating the homogeneity of binding sites.

Besides the enhancement of fluorescence, there was also a change in the wavelength of the emission maximum (λ_{max}^F) on formation of TNS–β-CD complexes, as shown in Figure 5. The λ_{max}^F of the β-CD–TNS system remained constant at 460 nm until the β-CD concentration reaches 10^{-3} M. Above this concentration it gradually shifted to shorter wavelength. This change corresponds to that of the fluorescence intensity shown in Figure 4. On the other hand, the λ_{max}^F with poly-β-CD-A remained constant throughout the concentration range examined. These results indicate that a two-step binding occurs for the β-CD–TNS system, whereas a homogeneous binding occurs for the poly-β-CD-A–TNS system.

The continuous variation method was used to examine the stoichiometry. Figure 6a shows the continuous variation plots for the β-CD–TNS system and Figure 6b shows those for the poly-β-CD-A–TNS system. In this concentration range, the plot for the β-CD system showed a maximum at a molar fraction of 0.5, indicating 1:1 inclusion. On the other hand, the plot for the poly-β-CD-A–TNS system showed a maximum at 0.66, which corresponds to 2:1 (CD:TNS) stoichiometry. These results clearly indicate that two CD units on the polymer chain participate in the binding of one TNS molecule. The dissociation constants and fluorescence properties of complexes of TNS with β-CD and with poly-β-CD-A are summarized in Table II. At low concentrations ($<10^{-3}$ M) β-CD forms a 1:1 inclusion complex with a dissociation constant of 2.5×10^{-4} M. At higher concentrations, β-CD gives a 2:1 inclusion complex, the fluorescence intensity of which is three times that of the 1:1 complex. The maximum emission of the 2:1 complex is at a shorter wavelength than that of the 1:1 complex. On the other hand, poly-β-CD-A forms only a 2:1 inclusion complex with TNS with a dissociation constant of 10^{-4} M. The 2:1 poly-β-CD-A–TNS complex is remarkably more stable and is much more fluorescent than the 2:1 complex of β-CD. These results suggest that adjacent β-CD moieties on a polymer chain act cooperatively in binding.

Interaction of TNS with Various β-CD Derivatives. In order to confirm the assumption that the 2:1 inclusion complex with the polymer is due to the cooperative action of two neighboring β-CD residues on a polymer chain, the fluorescence spectrum of TNS was measured in the presence of various β-CD derivatives (Table III). The monomers, β-CD-A and β-CD-NAC, had similar effects to β-CD on the fluorescence of TNS. The fluorescence intensity in the presence of homopolymers of β-CD-A and of β-CD-NAC was larger than that of the copolymer of β-CD-A with acrylic acid or with acrylamide. Furthermore, the fluorescence enhancement of the polymer increased with a decrease in the distance between neighboring CD units on the polymer chain. It should be noted that both poly(acrylic acid) and polyacrylamide do not change the TNS fluorescence at all.

Interaction of TNS with Analogous Polymers. The effects of a variety of carbohydrates and some analogous synthetic polymers on the fluorescence intensity of TNS are shown in Table IV. No fluorescence enhancement was observed on addition of methyl α-glucoside, maltose, or maltotriose. However, addition of amylose (DP 13) resulted in a small but definite fluorescent enhancement. Dextran (α-1,6 with branch), pullulan (α-1,4–α-1,4–α-1,6), curdlan (β-1,3), PVA, and poly(ethylene oxide) had essentially no effect. The fluorescence enhancement by poly-β-CD-A is very unusual compared with other polymers composed of hydroxyl groups and/or ether linkages.

Polarization of Fluorescence. The fluorescence of TNS bound to either β-CD or poly-β-CD-A shows substantial polarization. A plot of $1/p$ against T/η was shown in Figure 7, where T is the absolute temperature and η is the solvent viscosity. The poly-β-CD-A–TNS system shows higher polarization of fluorescence than β-CD does.

Discussion

Binding Properties of CD's and Poly-β-CD-A. As shown in Table I, α-CD has slight effects on the fluorescence properties of a series of dyes, whereas β-CD has large effects. The only difference in the structure between α-CD and β-CD is the size of the cavity.[19] The dyes are too large to be included in a α-CD cavity, so that the fluorescence changes on the addition of β-CD must be due to formation of inclusion complexes. The binding by β-CD is competitive with that by *p*-hydroxybenzoic acid as shown in Figure 8, and this also indicates that the dye is included in the cavity of β-CD.

The fluorescence change with poly-β-CD-A is considered to be due to the same inclusion as the case of β-CD, and the fluorescence titration and the comparison with other analogous polymers support this idea.

As shown in Figures 4–6 and Table II, β-CD forms both 1:1 and 2:1 complexes, whereas poly-β-CD-A forms only a 2:1 complex. Examination of molecular models of β-CD and TNS indicates that TNS is too large to be completely included in a single β-CD cavity, which can accommodate only one aro-

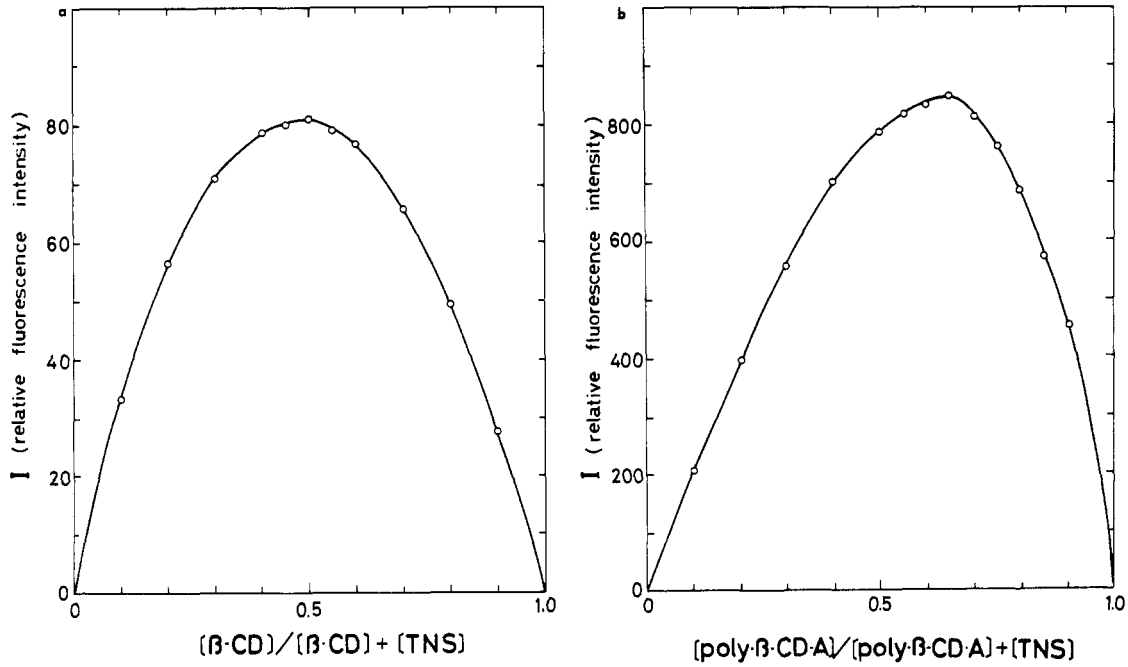

Figure 6. Continuous variation plots: (a) β-CD–TNS system, [TNS] + [β-CD] = 10^{-4} M; (b) poly-β-CD-A–TNS system, [TNS] + [poly-β-CD-A] = 10^{-4} M.

Table II
Dissociation Constants and Fluorescence Properties of Complexes of TNS with β-CD and Poly-β-CD-A

	CD:TNS	K_d, M^{-1}	λ_{max}^F, nm	Rel fluorescence inten
β-CD	1:1	2.5×10^{-4}	460	1
	2:1	5×10^{-2}	446	3
Poly-β-CD-A	2:1	1.0×10^{-4}	438	20

Table III
Fluorescence Enhancement of TNS[a] by the Addition of β-CD Derivatives

	Rel fluorescence inten	λ_{max}^F, nm
None	1	500
β-CD	25.3	460
β-CD-A	27.5	460
β-CD-NAC	29.6	458
Poly-β-CD-A	571	437
Poly-β-CD-NAC	576	436
β-CD-A–acrylamide[b] copolymer (1:4)	240	440
β-CD-A–acrylic acid[c] copolymer (1:8)	95	445

[a] pH 5.9 phosphate buffer, [TNS] = 2×10^{-5} M, [β-CD residue] = 10^{-3} M. [b] Polyacrylamide, no fluorescence enhancement. [c] Poly(acrylic acid), no fluorescence enhancement.

matic ring: either the toluidinyl group or the naphthalene ring can enter the β-CD cavity. Since the toluidinyl group may be more hydrophobic than the naphthalenesulfonate group, the first binding of TNS to CD could occur with the toluidinyl group, leaving the naphthalene group still exposed to solvent molecules (Figure 9a). Additional CD molecules associate

Table IV
Fluorescence Enhancement of TNS[a] with Carbohydrates and Some Polymers

	Fluorescence enhancement
Methyl α-glucoside	None
Amylose (DP 13)	5.0
α-CD	3.3
β-CD	25.3
Poly-β-CD-A	571
Dextran ($\bar{M}_w$ 20 000)	None
Pullulan ($\bar{M}_n$ 33 000)	1.6
Pullulan ($\bar{M}_n$ 580 000)	1.9
Curdlan	None
PVA	1.6
PEO	2.2

[a] pH 5.9 phosphate buffer, [TNS] = 2×10^{-5} M, [glucose residue] = 7×10^{-3} M.

together to form the 2:1 complex as shown in Figure 9b. The formation of such a ternary complex with a large substrate may be possible in the presence of considerable excess of β-CD. On the other hand, poly-β-CD-A forms only the 2:1 complex. In this case, when the toluidinyl group is included first vacant β-CD cavities will exist closely, so that the naphthalene group can be included easily, resulting in the formation of a 2:1 complex. The 2:1 complex of poly-β-CD-A–TNS is much more stable than the 2:1 complex of β-CD, indicating that both the toluidinyl group and the naphthyl group are included in two neighboring CD's cooperatively. Since two CD's are linked by the main chain, the cooperation makes the 2:1 complex difficult to dissociate. Energetically, rotational entropy is lost on formation of the poly-β-CD-A complex, but the larger entropy is lost on formation of the β-CD–TNS complex by the freezing of the translational motion of three components (2β-CD, TNS). This may be the deciding factor for the stabilization of the 2:1 complex of poly-β-CD-A and TNS relative to that of β-CD and TNS.

In the previous studies by equilibrium dialysis[6] we reported

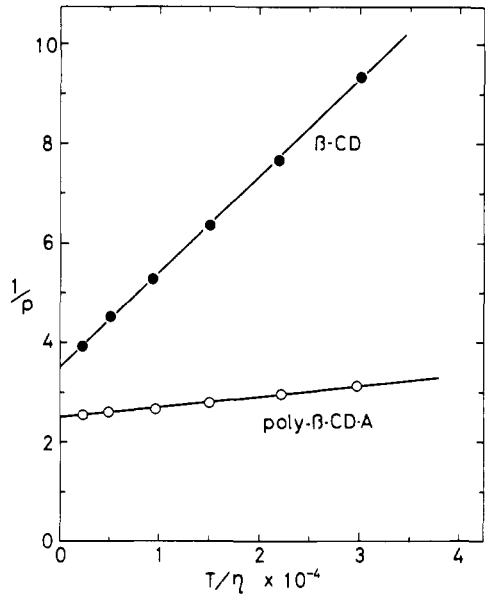

Figure 7. Fluorescence polarization of β-CD–TNS and poly-β-CD-A–TNS system.

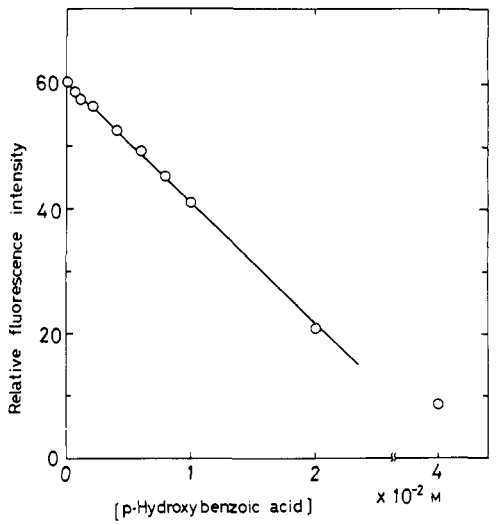

Figure 8. Effect of *p*-hydroxybenzoic acid on the fluorescence of TNS in the presence of β-CD, [β-CD] = 10^{-3} M.

that poly-β-CD-A had larger affinity for a large substrate than β-CD and formed a 2:1 complex with orange I.

Recently, various β-CD inclusion complexes have been examined by x-ray analysis. The results indicate that small substrates, such as methanol,[20] propanol,[21] and *p*-iodoaniline,[22] are included into a single α-CD cavity, whereas a large substrate Methyl Orange forms a 2:1 inclusion complex.[23] In the polyiodide–α-CD complex α-CD's are arranged head to head forming endless stacks in which the iodine chains are located.[24]

Fluorescence Change on Binding. As described above, polymers containing cyclodextrin were found to cause much greater fluorescence enhancement than β-CD. The "polymer effect" will be discussed below considering previous studies on TNS fluorescence. TNS and related fluorescent molecules are practically nonfluorescent in aqueous solutions. They become highly fluorescent with blue shift on addition of proteins which are known to possess hydrophobic binding sites,[25,26] and much less fluorescent in the presence of other proteins.[27] Accordingly, TNS has been widely used as a microenvironmental probe.[28] Fluorescence enhancement of TNS

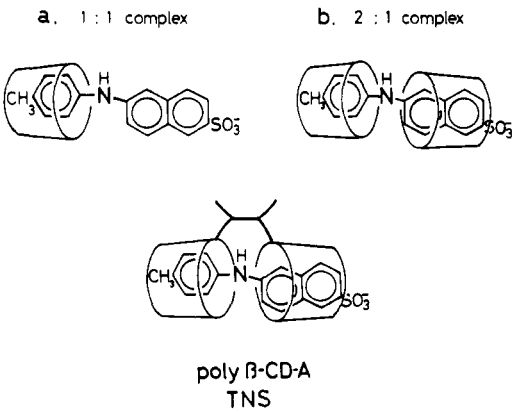

Figure 9. Binding mode of β-CD–TNS complex and poly-β-CD-A–TNS complex.

was generally thought to be caused by an environment of decreased polarity.[29] The effect of solvents on quantum yield has been interpreted in terms of conformation and rigidity of fluorophore, solvent relaxation, and existence of two excited singlet species. Intramolecular rotation of the fluorophore should promote radiationless transition to the ground state.[27,30] Solvent relaxation should decrease the singlet–triplet gap, facilitating the intersystem crossing and thereby quenching the fluorescence.[10,31] The existence of two emitting excited singlet species was reported: $S_{1,np}$, a species having a nonplanar structure whose emission is not sensitive to solvent, and $S_{1,ct}$, a species whose emission is markedly solvent dependent and which is formed from $S_{1,np}$ by an intramolecular charge transfer. Polar solvents favor the conversion of $S_{1,np}$ to $S_{1,ct}$ and also promote the radiationless deactivation of $S_{1,ct}$ to the ground state by a reverse intramolecular charge transfer.[32,33]

However, little is known about the relationship between the fluorescence change of TNS and the structure of the binding site of protein. To elucidate this relationship, it seemed desirable to investigate the interaction of this chromophore with well-defined binding sites. Recently, Beyer et al. studied cyclic peptides as models of proteins and reported that both ionic interaction and peptide self-association were required for the enhancement of fluorescence.[34] We used CD and its polymers, in which the environment of the binding region is well characterized. It was found that the fluorescence enhancement of the 2:1 complex of β-CD and TNS is three times larger than that of its 1:1 complex and that the fluorescence enhancement of the 2:1 complex of poly-β-CD-A is even greater. These results indicate that the requirement for fluorescence enhancement is to place the fluorophore in a rigid space. When the toluidinyl group and naphthyl group are included in cyclodextrin cavities cooperatively and are immobilized, internal rotation of TNS around the –NH– group may be restricted, hence the conversion from $S_{1,np}$ to $S_{1,ct}$ may be hindered, and moreover the inclusion may exclude the solvent relaxation.

As shown in Table III, β-CD-A and β-CD-NAC have similar effects to β-CD on TNS fluorescence, indicating the absence of any contribution from substituents.

Edelman et al. reported that the fluorescence intensity of TNS was affected by the bulk viscosity.[27] Poly-β-CD-A has a low intrinsic viscosity of 0.033 and the concentration of the polymer was very low (10^{-3} M). Dextran and pullulan which have much higher molecular weights had no effects on the TNS fluorescence at higher concentrations. These results indicate the absence of any contribution from macroscopic viscosity; probably "microscopic viscosity" may contribute to the fluorescence enhancement, i.e., rigidity of the environment.

Poly-β-CD-A shows higher polarization of fluorescence than β-CD. The polymer complex may rotate slowly and further the rigidity of the complex may contribute to increase the rotational relaxation time of TNS.

From our experimental results, it may be concluded that the fluorescence enhancement of TNS caused by binding is ascribable to the restriction of intramolecular rotation in the rigid environment and/or to the exclusion of solvent relaxation.

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LCST Behavior in Polymer Blends

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ABSTRACT: Lower critical solution temperature (LCST) behavior has been established for five miscible polymer blend systems, and for four of these the loci of cloud points observed on heating has been determined as a function of blend composition. These systems include: polycarbonate–poly(ε-caprolactone) and mixtures of poly(vinylidene fluoride) with poly(methyl acrylate), poly(ethyl acrylate), poly(methyl methacrylate), and poly(ethyl methacrylate). The effect of adding a compatible plasticizer, dimethyl phthalate, on the cloud point curve for the poly(methyl methacrylate)–poly(styrene-*co*-acrylonitrile) was also studied. The prior literature documents LCST behavior for only four polymer blend systems even though several theories predict this to be a general phenomenon in blends.

Recent theories of mixing, such as the "equation of state" approach by Flory[1–5] and Sanchez's[6–8] "lattice fluid" model, predict that polymer–polymer blends which are miscible at lower temperatures are likely to exhibit phase separation at higher temperatures[5,8] in contrast to the predictions of the classical Flory–Huggins theory.[9] However, to date apparently such lower critical solution temperature (LCST) behavior has been reported for only four blend systems, viz., poly(methyl methacrylate)–poly(styrene-*co*-acrylonitrile);[10,11] polystyrene–poly(vinyl methyl ether);[12–16] poly(ε-caprolactone)–poly(styrene-*co*-acrylonitrile);[5] and chlorinated rubber–poly(ethylene-*co*-vinyl acetate).[17] The question of whether LCST behavior is a general phenomenon of miscible blends or is a peculiar characteristic of the specific systems mentioned above led us to examine more closely several systems, whose miscibility at lower temperatures was under investigation in our laboratory, for any LCST type behavior they might exhibit. In our search for this behavior some of these blends were heated to temperatures higher than normally required or expected in conventional studies or sample preparation. This leads to certain problems of polymer thermal stability but nevertheless yields interesting and useful information. This preliminary investigation has resulted in the observation of LCST behavior for five additional blend systems as reported here. This return for our modest effort leads us to the conclusion that LCST behavior is rather common for blend systems as predicted.

Two different molecular weight samples of one of the