# Cyclodextrins Modified with Polymer Chains Which Are Responsive to External Stimuli†

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ABSTRACT: Amino-group-containing β-cyclodextrin (β-CD) was modified with poly(*N*-isopropylacrylamide) (PIPA; $\bar{M}_n$ = 4000) chains. The inclusion of 8-anilino-1-naphthalenesulfonic acid (ANS) into the cavity of the modified cyclodextrin was largely affected by the temperature due to the coil−globule transition of PIPA chains introduced to the β-CD molecule. The importance of cooperative binding of ANS by the PIPA chains and the cavity of β-CD was suggested.

Molecular recognition phenomena have been of interest in recent years.[1] Complexations of small organic compounds with polymeric compounds are very useful to construct artificial host−guest systems. These systems have been examined to mimic biological recognition, which are related to enzyme catalyses, transfer of genetic information, and transportation of information on surfaces of cell membranes.

In this paper we examined the construction of a molecular recognition system controlled by external stimuli. We chose cyclodextrin (CD) as a host, because of its capability to include diverse compounds into its apolar cavity.[2−4] We introduced poly(*N*-isopropylacrylamide) (PIPA) chains into primary OH groups of CD and then examined the effect of the presence of PIPA chains in the inclusion phenomena by CD molecules.

## Experimental Section

*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 (ANS) was from Wako Pure Chemicals, Osaka, Japan. Other reagents were commercially available. Deionized water was distilled to prepare sample solutions.

*Preparation of Poly(N-isopropylacrylamide) with a Carboxyl Group.* *N*-Isopropylacrylamide (5.09 g) was polymerized by using 4,4′-azobis(isobutyronitrile) (AIBN; 7.4 mg) and β-mercaptopropionic acid (MPA; 23.5 mg), as initiator and chain transfer reagents, respectively, in MeOH (30 mL) at 70 °C for 7 h. After evaporation of the solvent, poly(*N*-isopropylacrylamide) (PIPA) obtained was purified by dialysis against water (Visking seamless cellulose tube, size 30) and lyophilized (2.92 g). The degree of polymerization of PIPA was determined to be 34 ($\bar{M}_n$ = 4000) by the conductometric titration of a carboxyl group at the end of the polymer.

*Modification of Cyclodextrin.* β-Cyclodextrin (5.0 g) was coupled with *p*-toluenesulfonyl chloride (tosyl chloride, 11.4 g) in pyridine (88 mL) for 1.5 h at 25 °C (Figure 1).[5] After evaporation of pyridine, water (20 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, 7.2 g). $^1$H NMR (DMSO-$d_6$): δ 7.49 (22H, aromatic protons), 4.60 (7H, C(1)-*H*). IR (KBr, cm$^{-1}$): 3400 ($\nu_{OH}$), 3050 ($\nu_{CH}$), 2950 ($\nu_{CH}$), 1360, 1180 ($\nu_{S(=O)2}$), 1000, 920, 820, ($\nu_{SOC}$), 750, 670 ($\delta_{CH}$). Anal. Calcd for CD-(Tosyl)$_5$·5H$_2$O: C, 46.33; H, 5.56. Found: C, 46.24; H, 5.25.

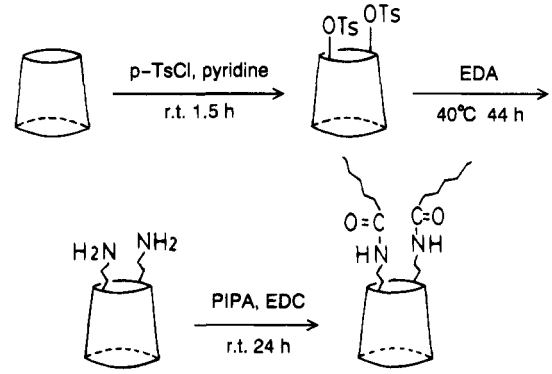


**Figure 1.** Scheme of preparation of β-CD-EDA-PIPA.

The tosylated β-cyclodextrin (1.25 g) was reacted with ethylenediamine (20 mL) at 40 °C for 44 h. After evaporation of ethylenediamine, the oily reaction mixture was put into cold acetone (30 mL). The precipitate was filtrated and dissolved in MeOH−water (3:1; 20 mL). The solution was poured into cold acetone, and after filtration, the precipitate was dried *in vacuo* (β-CD-EDA, 300 mg). $^1$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 ($\nu_{NH}$), 1060 ($\nu_{CN}$). Anal. Calcd for β-CD-(EDA)$_5$: C, 46.43; H, 7.44; N, 10.42. Found: C, 46.57; H, 7.47; N, 9.96.

The (aminoethyl)amino-β-cyclodextrin (97 mg) was incubated with poly(*N*-isopropylacrylamide) carrying a carboxyl end group (1.21 g) at pH 5.5 in the presence of [1-ethyl-3-(3-dimethylamino)propyl]carbodiimide hydrochloride (EDC; 86 mg) for 24 h. The PIPA-carrying cyclodextrin was repeatedly ultrafiltrated with H$_2$O (Amicon Model 8020, membrane, YM-3; exclusion limit, 3000) to remove unreacted β-CD−(EDA)$_5$ ($M$ = 1345.44) and other small impurities. The filtrate was lyophilized, giving a slightly yellow powder which was dispersed in THF. The β-CD modified with PIPA chains was obtained by precipitation from THF−*n*-hexane (2:1) (β-CD−EDA−PIPA, 35 mg). $^1$H NMR (D$_2$O): δ 5.48 (7H, C(1)-*H*), 2.27 (9H, −C*H*$_2$CO-EDA-β-CD), 2.90 (−C*H*(CH$_3$)$_2$), 2.57 (−C*H*$_2$S−), 2.01 ()C*H*CONH−), 1.57 ()CHC*H*$_2$CH(), 1.14 (−C*H*$_3$). IR (KBr, cm$^{-1}$): 2050 ($\nu_{RNCO}$), 1660 ($\nu_{CO}$), 1620 ($\delta_{NH}$), 1280, 1260 ($\nu_{CN}$). Anal. Calcd for β-CD-(EDA-PIPA)$_4$·136H$_2$O: C, 53.88; H, 9.75; N, 9.88. Found: C, 53.67; H, 9.00; N, 10.68.

*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.

*Fluorescence Measurements.* The fluorescence of an ANS solution in the presence of cyclodextrins was observed by using a fluorescence spectrophotometer (FP-777, Japan Spectroscopic Co.). The observation cell was thermostated by using a Neslab waterbath (RTE-8). The excitation wavelength was 350 nm, while the emission wavelength was from 370 to 650 nm. The spectral bandpasses were 1.5 nm.

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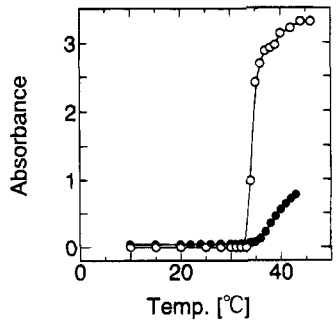


**Figure 2.** Turbidity of PIPA and β-CD-EDA-PIPA solutions at various temperatures. Wavelength: 500 nm. ○, PIPA. ●, β-CD-EDA-PIPA. Concentration, 5 mg/mL.

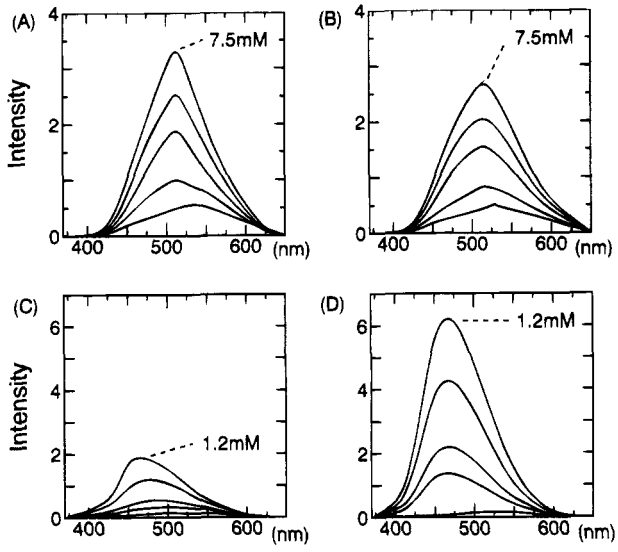


**Figure 3.** Fluorescence spectra of ANS in the presence of various concentrations of β-CD and PIPA. (A and B) [ANS] = 0.1 mM, [β-CD] = 0, 1, 3, 5, 7.5 mM. (A) 20 °C; (B) 41 °C. (C and D) [ANS] = 0.01 mM, [PIPA] = 0, 0.2, 0.4, 0.8, 1.2 mM. (C) 20.5 °C; (D) 41.5 °C.

## Results and Discussion

**A. Turbidity of PIPA-Containing Solutions.** Turbidity of solutions of both PIPA and CD modified with PIPA chains was largely increased above 33 °C (PIPA) and 35 °C (β-CD–EDA–PIPA) (Figure 2). This turbidity change is corresponding to the coil–globule transition of PIPA chains.[6–11] The observed transition temperature is slightly higher than that reported (32 °C)[12] because the molecular weight of PIPA chains 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.[11]

**B. Fluorescence of ANS in PIPA-Containing Solutions.** Next we examined fluorescence spectra of ANS below and above the transition temperature from the coil to globule of PIPA chains. It is well-known that the fluorescence of ANS is largely affected by the polarity of the microenvironment.[13] By the presence of CD, the fluorescence (emission 500 nm) was largely increased (Figure 3A,B), and the fluorescence intensity at 20 °C was larger than that at 41 °C.

Assuming that ANS makes a 1:1 inclusion complex with CD (eq 1), the association constant of the ANS–CD complex, $K$, could be estimated from the double reciprocal plot of [β-CD] vs increase in the relative fluorescence intensity ($\Delta F$) of ANS using eq 2 (Figure 4 and Table 1).

$$\mathrm{ANS} + \mathrm{CD} \overset{K}{\rightleftharpoons} \mathrm{ANS{\cdot}CD} \qquad (1)$$

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

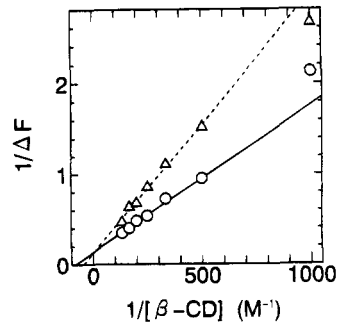


**Figure 4.** Double reciprocal plot of the concentration of CD and the increase in fluorescence intensity at 20 °C (○) and 41 °C (△). [ANS] = 0.1 mM.

**Table 1. Association Constants for Various Systems**

| system | temp (°C) | $K$ ($M^{-1}$) |
|---|---|---|
| ANS + β-CD | 11.0 | 84 ± 12 |
| | 20.0 | 83 ± 10 |
| | 31.0 | 79 ± 10 |
| | 41.0 | 44 ± 10 |
| | 51.0 | 32 ± 8 |
| | 62.0 | 55 ± 15 |
| ANS + PIPA | 12.0 | *a* |
| | 20.5 | *a* |
| | 30.5 | *a* |
| | 41.5 | 67 ± 10 |
| | 50.0 | 90 ± 5 |
| | 60.0 | 80 ± 7 |
| ANS + β-CD-EDA-PIPA β-CD[b] | 11.0 | 9000 ± 210 |
| | 19.5 | 9800 ± 670 |
| | 30.0 | 7900 ± 330 |
| | 40.0 | 630 ± 50 |
| | 53.0 | 730 ± 70 |
| | 60.0 | 450 ± 30 |
| PIPA[b] | 11.0 | 1200 ± 30 |
| | 19.5 | 900 ± 60 |
| | 30.0 | 870 ± 60 |
| | 40.0 | 510 ± 190 |
| | 53.0 | 480 ± 90 |
| | 60.0 | 760 ± 80 |

[a] Too small to evaluate. [b] After peak separation.

Thermodynamic parameters in the free CD system were estimated as −6.6 ± 0.2 kJ·mol$^{-1}$ ($\Delta H$) and 13 ± 0.4 J·mol$^{-1}$·K$^{-1}$ ($\Delta S$), which are in accordance with the thermodynamic behavior for the inclusion of organic compounds into the CD cavity (−6.9 kJ·mol$^{-1}$ and 16 J·mol$^{-1}$·K$^{-1}$ for the ANS-β-CD system).[14]

In the presence of free PIPA, the fluorescence intensity was small at 20.5 °C, whereas at 41.5 °C, the fluorescence intensity increased due to a hydrophobic microenvironment around ANS (corresponding to aggregated globules of PIPA chains) (Figure 3C,D). Being different from the free CD system, the maximum of the emission was 470 nm (blue-shifted from the peak of free CD system by 30 nm). However, the concentration of the PIPA solution examined was low because of its low solubility in water, and the double reciprocal plot was largely dispersed. In other words, the $K$ values for PIPA below the transition temperature were very small. Therefore, the $K$ values of PIPA below the transition temperature could not be evaluated unequivocally.

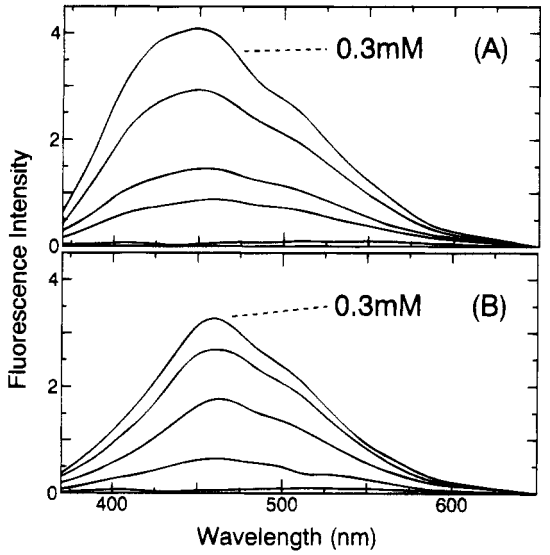


**Figure 5.** Fluorescence spectra of ANS in the presence of various concentrations of β-CD-EDA-PIPA. (A) 19.5 °C; (B) 40 °C. [β-CD-EDA-PIPA] = 0, 0.05, 0.1, 0.2, 0.3 mM. [ANS] = 0.01 mM.

By the addition of β-CD–EDA–PIPA, the fluorescence was large at both 19.5 and 40 °C (Figure 5). At both temperatures, there were two emission peaks around 450 and 500 nm. The former (which is blue-shifted from that of the free PIPA solutions by 20 nm) might correspond to the hydrophobic microenvironment in PIPA chains and the latter to the CD cavity. After a peak separation, $K$ values for the PIPA chains and the β-CD cavities for β-CD-EDA-PIPA were estimated from the fluorescence intensity of each peak.

The fluorescence peak of ANS in the β-CD–EDA–PIPA system at 450 nm at 19.5 °C corresponding to PIPA chains is in contrast to the weak fluorescence intensity in the free PIPA system at the same temperature. In addition, the fluorescence intensity of β-CD–EDA–PIPA at 40 °C was *smaller* than that at 19.5 °C, which is similar to the free CD–ANS system. Thermodynamic parameters for the inclusion of ANS into the β-CD cavity in the β-CD–EDA–PIPA system below transition temperature were estimated as $-0.56 \pm 0.04$ kJ·mol$^{-1}$ ($\Delta H$) and $74 \pm 5$ J·mol$^{-1}$·K$^{-1}$ ($\Delta S$), while those above the transition were estimated as $-1.8 \pm 0.5$ kJ·mol$^{-1}$ and $48 \pm 1$ J·mol$^{-1}$·K$^{-1}$.

These results suggest that the PIPA chains attached to the CD molecule have a more hydrophobic microenvironment than free PIPA chains even below the transition temperature from coils to globules, because of the large local concentration of PIPA chains in the vicinity of the CD moiety, which extraordinarily induces the increase in fluorescence intensity.

It is noteworthy that the association constants, $K$'s, of 1:1 complexations for both CD moiety–ANS and PIPA moiety–ANS systems were much larger than those of free CD–ANS and free PIPA–ANS systems, because of the cooperativity of the CD cavity and surrounding PIPA chains to bind ANS.

By the increase in temperature, the PIPA chains are largely dehydrated to increase its hydrophobicity and disturb the inclusion of ANS into the cavity of the CD molecule to which the PIPA chains are anchored. Consequently, the fluorescence intensity and the association constant, $K$, at 40 °C were smaller than those at 19.5 °C. Though the tendency was similar, the decrease in the $K$ value corresponding to the CD moiety ($K$(19.5 °C)/$K$(40 °C) = 15.5) was much more significant than that in the free CD system ($K$(20 °C)/$K$(41 °C) = 1.9).

Such a system would be useful to prepare the temperature-sensitive receptor model system.

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