pH-Triggered Assembling System Using Cooperative Binding between Cyclodextrin-Conjugated Poly(ϵ-lysine)s and Anionic Guest in Aqueous Media

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Various types of cyclodextrin-conjugated poly(ϵ-lysine)s (CDPLs) were prepared as polymeric hosts, and the inclusion properties with 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS) were investigated at a low concentration under various environmental stimuli. Fluorescence studies revealed that the ability of CDPLs for inclusion complexation was much stronger than that of the corresponding CDs due to their cooperative binding. This property can be controlled by the chemical composition of polymeric hosts such as changing the ring size and varying the degree of substitution of CDs. In addition, the inclusion property of the polymeric host can be modulated by environmental stimuli such as temperature, pH, and ionic strength of aqueous media. Such an interesting functionality results from the combination of the cooperative host−guest interactions and the ionic interaction between the negatively charged TNS moieties and the positive amino groups of poly(ϵ-lysine).

Introduction

Cyclodextrins (CDs), cyclic oligomers of α-1,4-linked glucopyranose units, are well-known for their abilities to include various kinds of guest molecules into their hydrophobic cavities.[1] A variety of low or high molecular weight molecules were found to be included partially or completely into their corresponding CD molecules by complex driving forces involving hydrophobic interaction, hydrogen bonding, and the release of high-energy water molecules from the CD cavity.[2]

Recently, to improve the molecular inclusion abilities of the native CDs, a great deal of effort has been concentrated on the design and synthesis of novel CD derivatives.[3−5] One way to improve or modulate the binding property of CDs is to introduce CDs to polymeric structures. The introduction of such polymeric structures makes it possible to gather CD molecules closer so that neighboring CD molecules participate in the process of inclusion complexation cooperatively and simultaneously. As a result, introducing CD molecules to polymeric systems with various structures, such as side/end groups, backbone components, or cross-linked type, can improve the ability for complexation by providing a high local concentration at binding sites.[6]

On the basis of these studies, in the course of our systematic studies, a novel polymeric host was prepared by conjugating CD molecules to biodegradable and cationic poly(ϵ-lysine) (PL).[7,8] The CD-conjugated PL (CDPL) was found to have many promising properties such as high water solubility, biodegradability, and functionality that would be useful for a broad range of applications. We have investigated the inclusion property of α- or *β*-CDPL with a model guest 3-(trimethylsilyl)propionic acid (TPA) at relatively high concentrations (over 1 wt %). An interesting complexation-induced phase separation was observed as a result of the dual complexation phenomena: cooperative interactions of TPA/CD inclusion and ionic interaction between the PL backbone and TPA. On the basis of these dual complex interactions, the supramolecular assembly could exhibit rapid responses to any small change of pH or temperature. However, we could not observe any significant change in both pH-sensitivity and thermosensitivity at relatively low concentrations (below 0.5 wt %).

In this study, α- and *β*-CDPLs with different degrees of substitution (DS) were synthesized and used as cationic polymeric hosts. The inclusion properties of α- or *β*-CDPLs were investigated with 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS) to understand the mechanism of intermolecular interactions at a low concentration (CDPL, 1×10^{-3} M). TNS was selected as a fluorescence probe due to the hydrophobic and ionic end-groups such as TPA, which would play a dominant role in inducing dual complexation phenomena. The fluorescence intensity of TNS was observed in the presence of the polymeric hosts under various environmental stimuli such as temperature, pH, and ionic strength.

Results and Discussion

TNS is a well-known fluorescence probe, which shows a strongly increased intensity with shift of the emission maximum in apolar solvents or in hydrophobic cavities of CD. Kondo et al. suggested that the fluorescence enhancement of TNS in the presence of CDs resulted from the inclusion complexation of TNS.[9] This property of TNS was exploited to evaluate the complexation abilities of α- or *β*-CDPL in comparison with those of α- or *β*-CD. In addition, the effect of chemical structures of host, the degree of substitution (DS) of CDs and ring size, and solution conditions such as ionic strength, surfactant effect, temperature, and pH on the fluorescence intensity of TNS changes in various CDPL solutions.

Effect of Conjugating CDs on the Fluorescence Intensity. As shown in Figure 2, the relative fluorescence intensity (I/I_0) of TNS at 445 nm was markedly enhanced over 100-fold by inclusion complexation with various CDPLs, while α- or *β*-CD/

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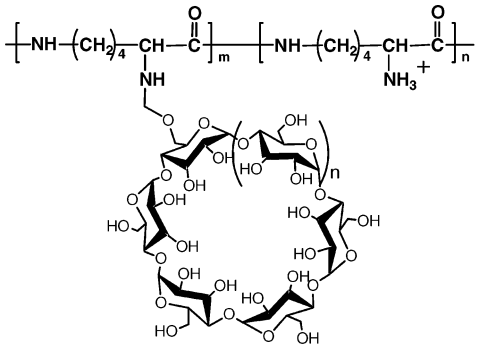
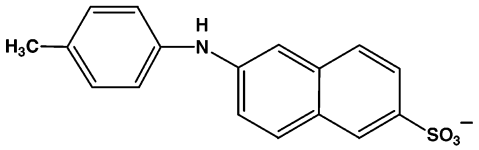
TABLE 1: Synthetic Results of Various α- and *β*-Cyclodextrin-Conjugated Poly(ε-lysine)s (α- and *β*-CDPLs)

code	feed ratio (ald-CD:Lys)	DS[a] (%)	CD/CDPL (wt %)	M_n[b] (g/mol)	yield (%)
α-CDPL-1	1.0:1	32.5	70.8	13 100	54.6
α-CDPL-2	2.0:1	44.3	76.8	16 500	45.3
β-CDPL-1	0.5:1	27.5	70.6	9 200	62.5
β-CDPL-2	1.0:1	33.2	74.3	15 000	66.1
β-CDPL-3	1.5:1	44.6	79.5	18 800	51.6
β-CDPL-4	2.0:1	48.3	81.0	20 100	49.4

[a] The degree of CD substitution per PL monomer unit. [b] The number-average molecular weight of CDPLs was determined by ^{1}H NMR and MALDI-TOF mass spectroscopies, respectively.



(a) Cyclodextrin-conjugated poly(ε-lysine) (CDPL) (n = 1: α-, n = 2: β-)



(b) 6-(*p*-Toluidino)-2-naphthalenesulphonate (TNS)

Figure 1. Chemical structures for CDPLs (a) and 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), a fluorescence probe (b).

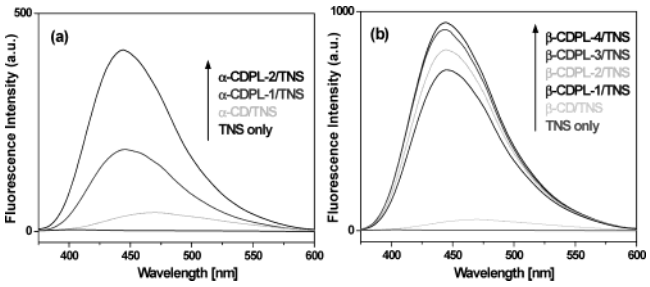


Figure 2. Fluorescence spectra of TNS in the presence of α-CDPLs at pH 7 (a) and *β*-CDPLs at pH 6 (b). The spectra of α-CD/TNS and *β*-CD/TNS were used as references. [TNS] = 5×10^{-5} M and [CD] = 1×10^{-3} M in 0.1 M phosphate buffer at 20 °C.

TNS marked much smaller intensity changes of about 10-fold enhancement at 465 nm. Here, I and I_0 were the observed fluorescence intensities in the presence and absence of the host, respectively. This resulted from the immobilization of TNS in the case of CDPL/TNS solutions. Conjugating CD to the polymeric chain enhanced the stabilization of the CD/TNS complex, which may contribute to an extreme increase in the fluorescence intensity. In addition, the electrostatic interaction between the protonated amino group of CDPL (α-CDPL, pK_a 7.23; *β*-CDPL, pK_a 6.95) and the sulfonate anion of TNS (pK_a 5.49) stabilized the complex systems of α-CDPL at pH 7 and *β*-CDPL at pH 6, respectively. As a consequence of these cooperative interactions, the TNS molecule can be immobilized to the polymeric host, so that the relaxation and the vibrational

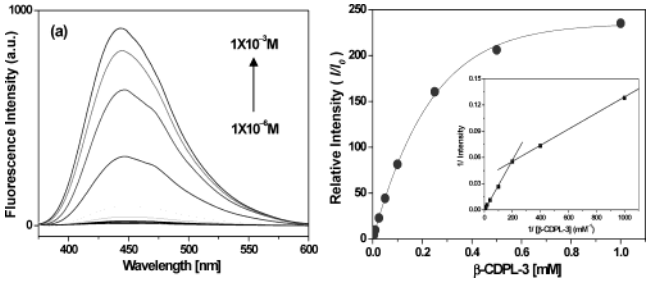


Figure 3. Effect of *β*-CDPL-3 concentration on the fluorescence intensity of TNS at pH 6 (a) and the curve-fitting data at λ = 465 nm (b). [TNS] = 5×10^{-5} M and [CD] = 1×10^{-3} M in 0.1 M phosphate buffer at 20 °C. Inset: double reciprocal plot for the same data. The solid curve represents the best fit of the data to the algorithm arising from the equilibria shown in eq 2.

activation of the excited TNS molecules on the CDPL are significantly increased.[10,11]

Effect of Ring Size on the Fluorescence Intensity. To clarify the size effect of the CD cavities, the fluorescence spectra of TNS with α- and *β*-CDPLs were compared. A difference in the fluorescence intensity between α-CDPL/TNS and *β*-CDPL/TNS systems results from the different inclusion capacities of their corresponding CDs. Kondo et al. reported that α-CD (diameter 0.47 nm) could form only a 1:1 complex with TNS, whereas both 1:1 and 2:1 complexes could be possible in the *β*-CD/TNS (diameter 0.60 nm) system because the relatively small α-CD cavity could not possess the bulky naphthalene moiety.[9] The protection of the methyl substituent, located in the para position on the toluidin moiety, from the aqueous solvent by *β*-CD provides the added stabilization required for 2:1 complex formation. As shown in Figure 2, the relative fluorescence intensity of TNS with α-CDPL-2 (DS 44.3%) or *β*-CDPL-3 (DS 44.6%) was more than 100-fold higher than that of the corresponding CDs. It means that the 2:1 complex of *β*-CDPL/TNS is much more stable than the 1:1 complex of α-CDPL/TNS, indicating that both toluidin and naphthalene groups can interact with neighboring *β*-CDs at the same time.

Effect of DS on the Fluorescence Intensity. α- and *β*-CDPLs with varying DS of CDs in the range of 27% to 48% were prepared as polymeric hosts (Table 1). There is an optimum distance of adjacent CDs for stabilizing CD dimmer/guest complexes that depends on the nature of the guest species and the linking of two *β*-CDs can lead to significant cooperativity in guest binding.[12] In our system, both α- and *β*-CDPLs showed a significant increase in the fluorescence intensity on increasing the DS of CDs on the copolymers. As shown in Figure 2b, *β*-CDPL-3/TNS and *β*-CDPL-4/TNS systems retain much stronger inclusion complex properties than *β*-CDPL-1/TNS due to the dense localization of CD molecules to the polymeric structure. However, there was a small change in the fluorescence intensity between *β*-CDPL-3/TNS (44.6%) and *β*-CDPL-4/TNS (48.3%) because the DS did not show further increasing.

Binding Ability of CDPL with TNS. As shown in Figure 3a, the fluorescence intensity of TNS was enhanced at the emission maximum with increasing *β*-CDPL concentration. This concentration dependence of CDPLs to the intensity change of TNS can be analyzed by the Benesi−Hildebrand plot.[13] K_1 and K_2 represent the stepwise association constants.

$$K_1 = [\text{CDPL-TNS}]/[\text{CDPL}][\text{TNS}] \quad (1)$$

$$K_2 = [(\text{CDPL})_2\text{TNS}]/[\text{CDPL-TNS}][\text{CDPL}] \quad (2)$$

As discussed above, α-CDPL/TNS systems form 1:1 inclusion complexes, and they could be illustrated as a single straight

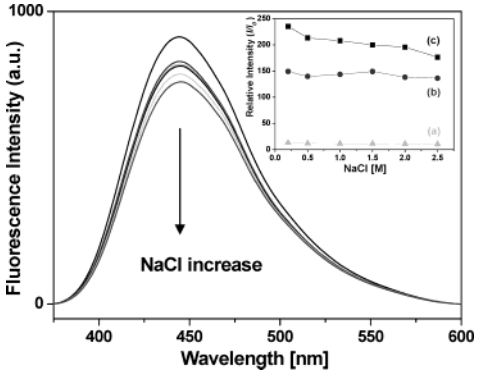


Figure 4. Effect of ionic strength on the fluorescence intensity of TNS in the presence of β-CDPL-3 at pH 6. Inset: curve-fitting data of β-CD/TNS ($\lambda = 465$ nm) at pH 6 (a), β-CDPL-3/TNS ($\lambda = 445$ nm) at pH 10 (b), and β-CDPL-3/TNS ($\lambda = 445$ nm) at pH 6 (c). [TNS] $= 5 \times 10^{-5}$ M and [CD] $= 1 \times 10^{-3}$ M in 0.1 M phosphate buffer at 20 °C.

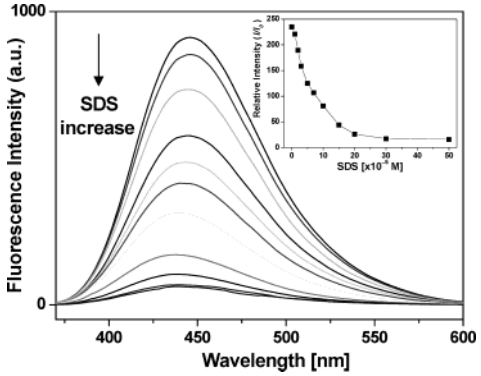


Figure 5. Effect of SDS concentration on the fluorescence intensity of TNS in the presence of β-CDPL-3 at pH 6 and the curve-fitting data at $\lambda = 445$ nm (inset). [TNS] $= 5 \times 10^{-5}$ M and [CD] $= 1 \times 10^{-3}$ M in 0.1 M phosphate buffer at 20 °C.

line in their double reciprocal plots (data not shown).[6] The association constant (K) of α-CDPL/TNS could be calculated from eq 1, whereas the stepwise associative eqs 1 and 2 were applied to obtain that of the β-CDPL/TNS system. Clearly the plot in Figure 3b is not well-described as a single straight line but is best described by two linear segments. At low concentrations ($\leq 5 \times 10^{-5}$ M), β-CDPL-3/TNS formed a 1:1 complex with an association constant (K_1) of 92.5 mM^{-1} ($r^2 = 0.999$). Upon increasing the concentration of β-CDPL-3 in TNS solutions above 5×10^{-5} M, another linear portion of the line with a steep slope was observed. In this case, the additional step could be adopted from eq 2, considered as the 2:1 inclusion complexation. From the slope and intercept, the association constant (K_2) for β-CDPL-3/TNS was calculated as 258.4 mM^{-1} ($r^2 = 0.998$). This result indicates that the 2:1 complex of β-CDPL/TNS is kinetically stable and geometrically well-defined, whereas the 1:1 complex of β-CDPL/TNS is structurally less preferable owing to incomplete inclusion. In this system, the first binding of TNS to β-CD could occur with the toluidin moiety because the toluidinyl group is more hydrophobic than the naphthalenesulfonate group, and cooperative second binding occurs simultaneously from the tapered rim of the β-CD to the deprotonated sulfonate group.[14]

Effect of Ionic Strength on the Fluorescence Intensity. As shown in Figure 4, the effect of ionic strength upon the fluorescence spectra of TNS was investigated by adding sodium chloride (NaCl) in 0.1 M phosphate buffer solutions. Due to the known propensity of host−guest moieties to express either their hydrophilic or hydrophobic nature in response to an environmental change, a complex formation is also expected to depend on the ionic strength of a solvating solution. In our system, increased ionic strength of the solution might result in the increased screening effects which would reduce the electrostatic interactions between the amino side groups of PL and the naphthalene sulfonate groups of TNS, and thus the assembly formed at high ionic strength is expected to adopt a decreased association.

The β-CD/TNS solution showed relatively small fluorescence intensity and did not lead to any significant difference, indicating no relation with ionic interaction (Figure 4a). However, the complexation ability of β-CDPL-3/TNS at pH 6 was stronger at low salt concentration, which reveals that the ionic strength affects the complexation phenomenon of the β-CDPL (Figure 4c). The decreased electrostatic interactions between TNS and the host with increasing ionic strength would thus contribute to the observed decrease in binding of TNS to β-CDPL-3. In the case of β-CDPL-3/TNS at pH 10, no significant change was observed in the fluorescence intensity, where amino side groups of PL (pK_a 9) might be deprotonated (Figure 4b). For the α-CDPL/TNS series, the same tendency was observed (data not shown).

Inhibition Test with SDS. It has been demonstrated that the addition of CDs to an aqueous solution of surfactant dramatically affects the physicochemical properties of the solution.[15,16] Sodium dodecyl sulfate (SDS) has been used as an interchangeable surfactant because it is known to form both 1:1 and 1:2 complexes with CD more strongly than with TNS.[17] As shown in Figure 5, the fluorescence intensity of β-CDPL-3/TNS significantly decreased at pH 6 as the SDS concentration was increased, whereas β-CD/TNS showed a slight change. This might result from the ability of CD to screen the hydrophobic moieties of surfactant molecules from contact with the surrounding aqueous media by the formation of an inclusion complex, in which the hydrophobic chain of the surfactant is inserted into the CD cavity. In addition, SDS might interfere with an ionic interaction between TNS and β-CDPL. In the case of the α-CDPL/TNS solutions, similar results were obtained (data not shown).

Effect of Temperature on the Fluorescence Intensity. In the previous study, the supramolecular assembly of the β-CDPL/TPA system showed rapid response to a small change of temperature on the basis of host−guest association and dissociation at relatively high concentration (over 1 wt %).[8] However, there was no significant change in thermosensitivity of the β-CDPL/TPA system at relatively low concentrations (below 0.5 wt %).

In this study, we designed the solution at a low concentration ([CDPL] $= 1 \times 10^{-3}$ M) to evaluate the thermal property of the supramolecular assembly in diluted condition. As shown in Figure 6, the fluorescence intensity of TNS with β-CDPL-3 decreased in a straight line with an increase in the temperature at a low concentration. These data suggest that there is a shift in the equilibrium toward the uncomplexed form with an increase in the temperature. From this result, it is suggested that no significant cooperative effect with temperature changes occurs at relatively low concentrations because the supramolecular assembly between CDPLs and guest moieties cannot gather closer so that neighboring CD molecules cannot participate in the supramolecular complexation process cooperatively and simultaneously. Therefore, they did not show gelation but gave clear evidence of aggregation in the low-temperature regime by highly generated hydrogen bonding.

Effect of pH on the Fluorescence Intensity. A change in the fluorescence intensities of TNS with various host solutions

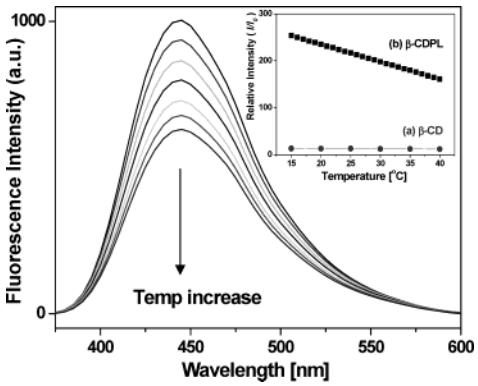


Figure 6. Effect of temperature on the fluorescence intensity of TNS in the presence of β-CDPL-3, and curve-fitting data of β-CD (a) and β-CDPL-3 (b) at each maximum point (inset). [TNS] $= 5 \times 10^{-5}$ M and [CD] $= 1 \times 10^{-3}$ M in 0.1 M phosphate buffer at pH 6.

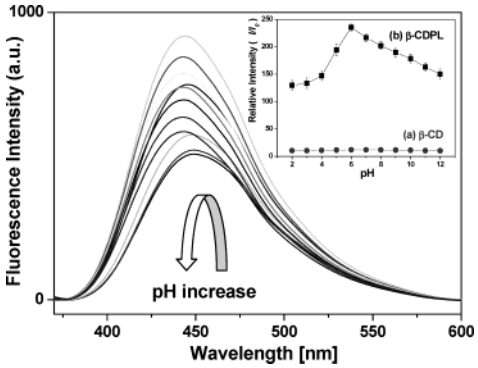


Figure 7. Effects of pH on the fluorescence intensity of TNS in the presence of β-CDPL-3, and curve-fitting data of β-CD (a) and β-CDPL-3 (b) at each maximum point (inset). [TNS] $= 5 \times 10^{-5}$ M and [CD] $= 1 \times 10^{-3}$ M in 0.1 M phosphate buffer at 20 °C.

was observed as a function of the pH. The fluorescence intensity of the β-CD/TNS system was approximately constant irrespective of a variation in pH (Figure 7a). On the other hand, β-CDPL/TNS solutions showed a clear tendency of fluorescence enhancement with varied pH in aqueous solution, and the maximum was found to be at pH 6 (Figure 7b). The significant increase in fluorescence intensities may be due to the enhanced intermolecular interaction by ionic association between anionic TNS and the β-CDPL containing protonated amino side groups.[18,19] In acidic conditions, repulsive interactions between cationic amino side groups along the PL chains work against the aggregative interactions, preventing them from intermolecular aggregation. In neutral conditions, attractive ionic interaction between cationic groups on PL chain and anionic TNS that is included into the CD cavity of another PL chain is dominant. However, in alkaline conditions, only repulsive interaction exists between anionic TNS molecules included into the cavities of polymeric hosts due to the deprotonation of amino groups in the PL main chain. There exists no intermolecularly aggregative interaction, resulting in relatively low fluorescence intensity. As a result, it is confirmed that the pH of the CDPL/TNS aqueous media can be one of the important factors that can modulate the interaction between PL and TNS. It coincides well with the CDPL/TPA system of our previous paper.[8]

Conclusions

The effects of α- and β-CDPLs on the fluorescence spectra of TNS were investigated under various environmental conditions. It is well-known that α-CD and β-CD form 1:1 and 2:1 inclusion complexes with TNS, respectively. The same species were formed by interaction of TNS with CDPLs, but much stronger enhancement of fluorescence intensity was observed. This can be explained in full by the electrostatic interactions between the protonated amino group of CDPL and the sulfonate anion of TNS, which stabilizes the complex system. In addition, the enhanced ability for inclusion complexation of TNS with high DS of CDPLs was accomplished due to the cooperative process of CD molecules densely localized along the polymer chains. Because the ionizable α-amino groups of PL and sulfonate groups of TNS play an important role for controlling the intermolecular aggregations, complexation behavior and stability were influenced in response to pH changes as well as salt and surfactant concentrations in aqueous media. Significantly increased fluorescence intensities were observed in the β-CDPL/TNS system with low concentrations of salt and surfactant at pH 6. On the other hand, any profound phenomenon was not observed with temperature changes in CDPL/TNS systems at a low concentration. Thus, the responsive properties of this system around physiological pH could be utilized in various biomedical and bioengineering fields, such as pulsatile drug delivery, targeting, embolization, sensors, and bioseparation.

Experimental Section

Materials. α- and β-CDs were purchased from Wako (Tokyo, Japan) and purified by recrystallization in distilled water, followed by drying in vacuo at 60 °C. Poly(ϵ-lysine) (MW 3860) was kindly supplied by Chisso Co. Ltd. (Tokyo, Japan) and freeze dried before use. 6-(*p*-Toluidino)-2-naphthalenesulfonate (TNS) and sodium cyanoborohydride were purchased from Aldrich (Milwaukee, WI). Dimethyl sulfoxide (DMSO, Wako) was dried over CaH_2 and distilled. The other synthetic reagents were used as received without further purification.

Synthesis of α- and β-CDPLs. Detailed synthetic methods and results for α- and β-CDPLs were introduced in the previous reports.[7,8] Briefly, PL (0.27 mmol) was allowed to react with a predetermined amount of mono-6-aldehyde-CD in 0.2 M acetate buffer (pH 4.4) at 25 °C. After being stirred for 1 h, 2 equiv of sodium cyanoborohydride was added to the resulting solutions. The mixture was stirred for 72 h, and then neutralized with 2 M sodium hydrate, followed by dialysis against water and freeze-drying. The chemical composition of CDPLs was confirmed by ^{1}H NMR and FT-IR spectrometries. The representative chemical structure is depicted in Figure 1.

^{1}H NMR (D_2O, α-CDPL): δ 4.96 (s, 6 H, H-1, CD), 4.30−3.35 (2m, CD protons), 3.35−2.6 (3m, α and ϵ protons, PL), 2.00−1.00 (3m, β, γ, and δ protons, PL).

^{1}H NMR (D_2O, β-CDPL): δ 4.96 (s, 7 H, H-1, CD), 4.00−3.35 (2m, CD protons), 3.35−2.9 (3m, α and ϵ protons, PL), 1.78−1.00 (3m, β, δ, and γ protons, PL).

FT-IR (KBr, α-CDPL): 3413 (s, OH), 2929 (s, C−H), 1637 (s, C═O), 1559 (m, N−H), 1458 (m, C−H), 1154 (m), 1077 (m), 1031 cm^{-1} (m, C−O).

FT-IR (KBr, β-CDPL): 3413 (s, OH), 2929 (s, C−H), 1637 (s, C═O), 1559 (m, N−H), 1458 (m, C−H), 1200−800 cm^{-1} (m, C−C and C−O).

Characterization. The chemical compositions and CD contents of CDPLs were characterized by means of ^{1}H NMR (750 MHz FT-NMR spectrometer, Varian, Unity plus, CA) and FT-IR spectrometers (VALOR-III, Jasco, Tokyo, Japan). The molecular weight of CDPL was determined by MALDI-TOF mass spectroscopy, using Voyager-DERP (PE Biosystem, Forster city, CA) in linear mode. Samples for mass spectrum

were prepared by casting the matrix compound, α-cyano-4-hydroxycinnamic acid (α-CHCA), with CDPLs in water or CH_3CN/H_2O (1:1) onto the slide, followed by evaporating the solvents. Ionization was accelerated with 20 kV in the positive ion mode.

The fluorescence intensity of the TNS solution (5×10^{-5} M, 0.1 M phosphate buffer) in the presence of CDs or CDPLs was observed by using a fluorescence spectrophotometer (FP-6500, Jasco, Tokyo, Japan). The observation cell was thermostated by a temperature controller (ETC-505T, Jasco, Tokyo, Japan). The excitation wavelength was 366 nm, while the emission wavelength was from 370 to 600 nm. The spectral band-pass was 3 nm. The fluorescence spectra of TNS were measured in the presence of various hosts in 0.1 M phosphate buffer at 20 °C. The concentration of α- or β-CDPL was calculated as CD units in the copolymers.

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