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Circular Dichroism Study of the Inclusion-Dissociation Behavior of Complexes between a Molecular Nanotube and Azobenzene Substituted Linear Polymers

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Abstract

We have investigated the inclusion properties of molecular nanotubes composed of crosslinked α-cyclodextrin. Induced circular dichroism was used to probe the formation and dissociation of complexes between the nanotubes and azobenzene modified linear polymers. The polymer was poly(ethylene glycol) (PEG), either with or without a hydrophobic alkyl chain.It was found that the inclusion complex between the nanotubes and polymers formed at room temperature, and that the polymers dissociated from the nanotubes with increasing temperature. Further, the polymer with hydrophobic alkyl chain was bound inside the nanotube more strongly and dissociated more abruptly with increasing temperature than its hydrophilic counterpart as expected theoretically.

Introduction

Supramolecular structures have recently attracted great interest [1, 2], in particular, nanotubes, which have fine capillaries with inner diameters on the order of nanometers in a wide variety of scientific fields. Harada *et al.* successfully synthesized a new series of nanotubes from α-cyclodextrin (α-CyD), a cyclic oligosaccharide containing six glucopyranose units [3]. This molecular nanotube was obtained by crosslinking the adjacent α-CyD units of a polyrotaxane, which are threaded on a polymer chain and enfolded by bulky ends. By removing the bulky ends of the polymer thread after crosslinking, the tube was unthreaded and acted as a host for reversible binding of small molecules, such as iodine and surfactant oligomers [3–5].

We have recently shown that the molecular nanotube forms an inclusion complex with a linear polymer chain of poly(ethylene glycol) [6, 7]. The polymer chain forming an inclusion complex with a molecular nanotube has an extended conformation with no degrees of freedom other than translational motion along its longitudinal axis because of the infinitesimal diameter of the nanotube. Thus, the inclusion of a polymer chain into a molecular nanotube is entropically unfavorable and promoted by attractive interaction between the chain and nanotube. Okumura et al. predicted based on the Flory-Huggins lattice model that polymer chains in nanotubes dissociate with increasing temperature owing to the large conformational entropy and that the inclusion-dissociation behavior becomes the first-order transition in a poor solvent [8]. This transition originates from the competition between two stable states: the aggregation of polymer chains outside nanotubes and the formation of inclusion complexes between nanotubes and polymer chains.

In this study, we investigated the inclusion-dissociation behavior between a molecular nanotube and a polymer with a hydrophobic chain by using induced circular dichroism (ICD), and estimated the thermodynamic parameters of inclusion complex formation. We used polymers modified with azobenzene at one end, which can be included into α-CyD, for probing the inclusion complex formation by the ICD method [9–11].

Experimental

Materials and apparatus

α-CyD, poly(ethylene glycol) methyl ether (m-PEG), 2,4-dinitrofluorobenzene (DNFB) and epichlorohydrin were commercially available. *p*-phenylazobenzoylchloride and poly(ethylene glycol) monooleyl ether ($n = 50$) ($C_{18}E_{50}$) were purchased from Tokyo Kasei Co., Ltd. α,ω-diaminopoly(ethylene glycol) (PEG-BA) ($M_w = 3350$) was obtained from Sigma Chemical Co. and used without further purification.

^{1}H NMR spectra were measured with the 500-MHz AMX500 (BRUKER). We used TSKgel G3000PW$_{XL}$ column (7.8 × 60 cm), G4000PW$_{XL}$ column (7.8 × 30 cm) and water as eluent in GPC. GPC signals were detected with

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a RI-8021 type refractive index detector (TOSOH Co.) and a OR-2090 type chiral detector (Japan Spectroscopic Co., Ltd.). UV-Vis absorption was measured with a U-110 type spectrometer (Hitachi Ltd.). The ICD spectra were obtained with a circular dichroism (CD) spectrometer (Jasco J-720) with a 10 mm thick, thermally controlled cell.

The molecular nanotube

We synthesized molecular nanotubes according to Harada's method [3, 12, 13]. We first formed the inclusion complex of α-CyD (80 mL of α-CyD saturated solution) and PEG-BA (600 mg, 1.8×10^{-4} mol), and added DNFB (6.0 g, 3.1×10^{-2} mol) as a source of bulky ends to yield polyrotaxane. Then the polyrotaxane (2.0 g, 6.0×10^{-5} mol) was dissolved in a solution of 8% NaOH (200 mL) and treated with an excess of epichlorohydrin (34 mL, 4.4×10^{-1} mol), which linked the two adjacent α-CyDs. The solution was stirred for 36 hours at room temperature and then neutralized with HCl to stop the crosslinking reaction. The product was treated with 25% NaOH for 24 hours at room temperature to separate out dinitrobenzene and neutralized again with HCl. The final solution was dialyzed for three weeks using a dialysis tubing (purchased from BioDesign Inc., cutoff $M_w = 8000$) to remove impurities, such as dinitorophenyl groups, unreacted α-CyD and PEG-BA that were still left in solution. The product was confirmed by GPC using a chiral detector ($M_n = 18000$, $M_w/M_n = 1.4$). The length of the molecular nanotubes was estimated to be 25 nm on a highly oriented pyrolytic graphite (HOPG) substrate by scanning tunneling microscopy (STM) [14]. This length was consistent with the GPC results. ^{1}H NMR (DMSO-*d*6): δ 5.4–5.9 (broad, O^{2}H and O^{3}H of α-CyD), 5.0 (s, C^{1}H of α-CyD with bridge), 4.8 (s, C^{1}H of α-CyD), 4.6 (s, OH of bridge), 4.5 (s, O^{6}H of α-CyD), 3.2–4.0 (broad, C^{3}H, C^{6}H, C^{5}H, C^{2}H and C^{4}H of α-CyD, CH and CH$_2$ of bridge).

Polymer modified with azobenzene

We prepared hydrophilic and hydrophobic polymer chains: m-PEG ($M_w = 2000$) modified with azobenzene (PEG-Az) for the hydrophilic polymer and $C_{18}E_{50}$ modified with azobenzene ($C_{18}E_{50}$-Az) for the hydrophobic polymer. m-PEG (2.0 g, 1.0×10^{-3} mol) and triethylamine (200 mg, 2.0×10^{-3} mol) were mixed with molecular sieves 3A 1/16 in dichloromethane (4 mL) and cooled to 0 °C. Then *p*-phenylazobenzoylchloride (490 mg, 1.0×10^{-3} mol) was added and mixed at 0 °C for several days. When several drops of water was added, PEG-Az was obtained in dichloromethane separated from water. $C_{18}E_{50}$-Az was synthesized similarly from $C_{18}E_{50}$ (2.5 g). The modification of azobenzene was confirmed by ^{1}H NMR and UV-Vis absorption per weight. PEG-Az: IR (cm^{-1}): 2880 (–CH_2CH_2O– of PEG), 1277 (aromatic–COO–). ^{1}H NMR (chloroform-*d*): δ 8.2, 8.0, 7.5 (ddd, 2H, 4H, 3H of azobenzene), 4.5, 3.8 (two t, 4H of –COO–CH_2CH_2–), 3.6 (broad, 4H$\times$38 of –CH_2CH_2O–). $C_{18}E_{50}$-Az: IR (cm^{-1}): 2880 (–CH_2CH_2O– of PEG), 1277 (aromatic–COO–). ^{1}H NMR (chloroform-*d*): δ 8.2, 8.0, 7.5 (ddd, 2H, 4H, 3H of azobenzene), 4.5, 3.8 (two t, 4H of –COO–CH_2CH_2–), 3.6 (broad, 4H$\times$53 of –CH_2CH_2O–), 1.5, 3.4 (two t, 4H of alkyl–CH_2CH_2O) 1.3 (broad, 2H$\times$12 of alkyl chain), 0.9 (t, 3H of alkyl chain).

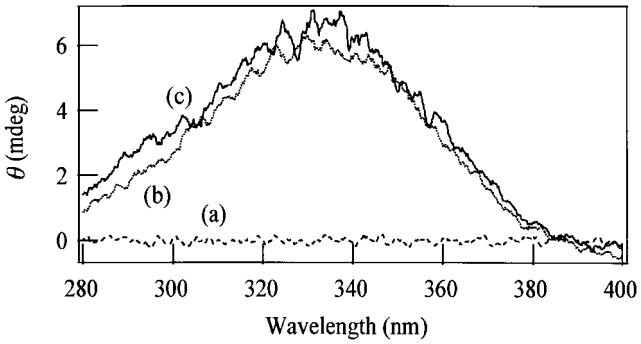

Figure 1. The CD spectra of aqueous solution of (a) $C_{18}E_{50}$-Az alone, (b) PEG-Az (60 μM) and the nanotube (30 μM), and (c) $C_{18}E_{50}$-Az (60 μM) and the nanotube (30 μM).

Results and discussion

Stoichiometry of inclusion complex formation

Figure 1 shows the CD spectra of (a) $C_{18}E_{50}$-Az alone, (b) the mixture of PEG-Az (60 μM) and the nanotube (30 μM), and (c) the mixture of $C_{18}E_{50}$-Az (60 μM) and the nanotube (30 μM) in aqueous solution. PEG-Az, $C_{18}E_{50}$-Az, and the nanotube by itself showed no CD signal, whereas mixtures of the polymer and nanotube exhibited positive CD bands at wavelengths of around 340 nm. Thus, we confirmed the inclusion complex formation between PEG-Az or $C_{18}E_{50}$-Az and the molecular nanotube by ICD.

Figure 2 shows the dependence of ellipticity θ at 334 nm for the polymer solution (PEG-Az or $C_{18}E_{50}$-Az) on the nanotube concentration [nanotube] at room temperature. The ellipticity increased, levelling off as more nanotubes were added to the polymer solutions. The saturation concentration of ca. 20 μM indicates that the molecular nanotube forms the inclusion complex with the polymer (PEG-Az or $C_{18}E_{50}$-Az) in about 1:3 stoichiometry. Since the molecular nanotube, using PEG-BA ($M_w = 3350$) as a template, is longer than PEG-Az and $C_{18}E_{50}$-Az, a nanotube can form an entire inclusion complex with a polymer chain, as well as partial complexes with two other chains in the residual cavity on either side as schematized in Figure 3. If the most hydrophobic part, azobenzene, is included, preferably in the manner shown in Figure 3, this model well explains the 1:3 stoichiometry and why the stoichiometry was independent of the length difference between PEG-Az and $C_{18}E_{50}$-Az.

Temperature dependence of inclusion complex formation

Figure 4 shows the temperature dependence of θ for (a) the mixture of PEG-Az (60 μM) and the nanotube (30 μM) and (b) the mixture of $C_{18}E_{50}$-Az (60 μM) and the nanotube (30 μM). It is seen that θ decreases with temperature for both mixtures. Since the ICD strength is proportional to the number of azobenzene units included into the nanotube, the nanotube dissociates polymers from the capillary

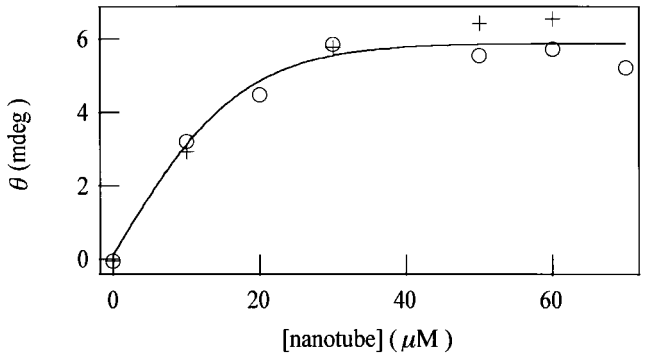

Figure 2. Ellipticity θ at 334 nm and room temperature as a function of nanotube concentration [nanotube] for the PEG-Az (+) and $C_{18}E_{50}$-Az (○) solutions.

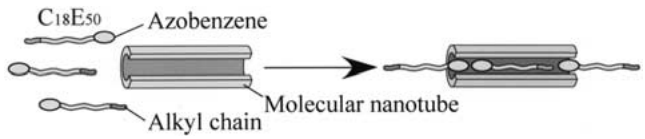

Figure 3. Schema of the inclusion complex formation between the molecular nanotube and $C_{18}E_{50}$-Az.

as temperature increases. Further, it is found that hydrophilic PEG-Az chains are dissociated gradually from the nanotube with increasing temperature, while most of hydrophobic $C_{18}E_{50}$-Az chains are dissociated abruptly around 70 °C. This indicates that the inclusion-dissociation behavior becomes critically dependent on temperature changes when using a poor solvent for the polymer chains, as predicted by theory [8]. The solution of the hydrophobic chain and nanotube has two competing stable states: (i) inclusion complex formation between the molecular nanotube and polymer chain and (ii) aggregation of polymer chains with their hydrophobic segments outside the nanotube.

The nanotube can be regarded as having three binding sites for the polymer. For the sake of simplicity, we assume that each binding site includes a polymer chain independently. The equilibrium constant K is then given by:

$$\frac{1}{3}\mathrm{T} + \mathrm{P} = \mathrm{T}_{\frac{1}{3}}\mathrm{P} \qquad K = \frac{[\mathrm{T}_{\frac{1}{3}}\mathrm{P}]}{[\mathrm{T}]^{\frac{1}{3}}[\mathrm{P}]} \tag{1}$$

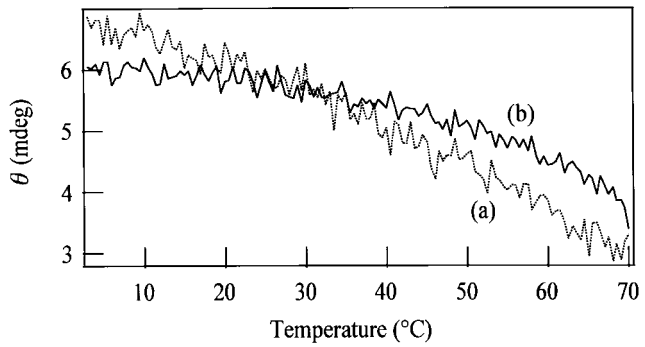

Figure 4. The temperature dependence of θ for the mixtures (a) PEG-Az (60 μM) and the nanotube (30 μM) and (b) $C_{18}E_{50}$-Az (60 μM) and the nanotube (30 μM).

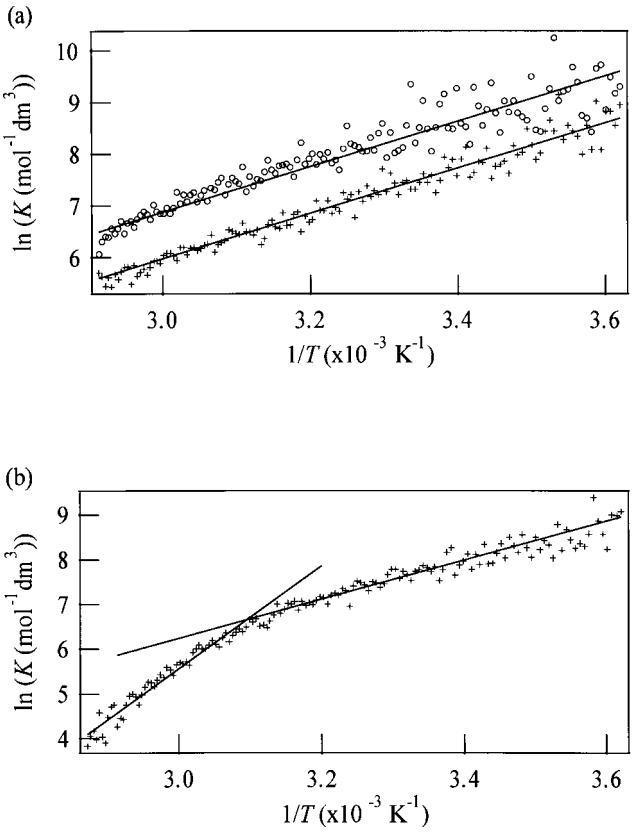

Figure 5. The Arrhenius plots for inclusion complexation between (a) PEG-Az (60 μM) and the nanotube (30 μM) (+), $C_{18}E_{50}$-Az (60 μM) and the nanotube (30 μM) (○); (b) $C_{18}E_{50}$-Az (60 μM) and the nanotube (20 μM).

$$\frac{\theta}{\theta_{\max}}[\mathrm{P}]_0 = [\mathrm{T}_{\frac{1}{3}}\mathrm{P}], \tag{2}$$

where T and P represent the nanotube and polymer, respectively, $[P]_0$ is the total concentration of the polymer, $\theta_{\max}$ the maximum value of θ. Further, the concentration of the nanotube with free binding sites can be written as follows:

$$[\mathrm{T}] = [\mathrm{T}]_0 - [\mathrm{T}_{\frac{1}{3}}\mathrm{P}]/3, \tag{3}$$

where $[T]_0$ is the total nanotube concentration. From these relations, we can obtain the Arrhenius plots for the inclusion complex formation between PEG-Az (60 μM) and the nanotube (30 μM) (+) and between $C_{18}E_{50}$-Az (60 μM) and the nanotube (30 μM) (○), shown in Figure 5a. Incidentally, the error of K values is somewhat higher around room temperature because most of nanotube forms the inclusion complex at room temperature. Since all the points lie along nearly straight lines, the activation enthalpy ΔH can be derived from the following equation:

$$\ln K = -\frac{\Delta H}{k_{\mathrm{B}}T} + \ln \chi. \tag{4}$$

Thus, ΔH is calculated to be -36 ± 2 kJ mol^{-1}, for PEG-Az and $C_{18}E_{50}$-Az. Because the alkyl chain of $C_{18}E_{50}$-Az is not observed to have any effect, ΔH is ascribed to the dissociation of the capping polymers included partially in the two ends of the nanotube. Thus, ΔH corresponds mainly to

the inclusion energy of azobenzene. This is consistent with the enthalpy change for the inclusion complexation between α-CyD and azobenzene derivatives (20–40 kJ mol^{-1}) [15].

Figure 5b shows the Arrhenius plot for the inclusion complex formation between $C_{18}E_{50}$-Az (60 μM) and the nanotube (20 μM). An inflection point is clearly observed at ca. 50 °C in the plot. Since the slope in the lower temperature regime is nearly equal to that in Figure 5a, ΔH_{low} is ascribed to the dissociation of the capping polymers included partially in the two ends of the nanotube. On the other hand, ΔH_{high} is calculated to be -95 ± 4 kJ mol^{-1} from the slope in the high temperature regime. Assuming that the inclusion energy of the hydrophilic part of $C_{18}E_{50}$-Az is negligible, the difference $\Delta H_{high} - \Delta H_{low} \cong -59$ kJ mol^{-1} corresponds to the inclusion energy of the hydrophobic alkyl chain. Hence, ΔH per CH_2 for inclusion complexation with the molecular nanotube is estimated to be -3.3 kJ mol^{-1} (-59 kJ mol^{-1}/18 CH_2), which is close to the value of -4.4 kJ mol^{-1} reported previously based on isothermal titration calorimetry [5].

Conclusion

The inclusion-dissociation behavior between molecular nanotubes and the polymers PEG-Az or $C_{18}E_{50}$-Az were investigated by induced circular dichroism. An inclusion complex formed between the nanotubes and polymers at room temperature in 1:3 stoichiometry. While the dissociation of the PEG-Az chains from the nanotubes with increasing temperature was gradual, $C_{18}E_{50}$-Az was bound inside the nanotube more strongly by its hydrophobic alkyl chains and dissociated more abruptly with increasing temperature as expected theoretically. The activation enthalpies of the bonds between the nanotube and azobenzene and between the nanotube and CH_2 were determined.

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