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Preparation of Inclusion Complexes of Poly(ethylene glycol)-Bearing Artificial Lipids with α-Cyclodextrin and of a Poly(rotaxane) Based on the Complex

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ABSTRACT: We synthesized eight different ω-amino-terminated poly(ethylene glycol)-bearing double-chain or triple-chain artificial lipids (PEG-lipids) with the molecular weight (Mw) of the poly(ethylene glycol) (PEG) moiety being 700, 1,000 or 1,600. The mixing of the aqueous bilayers of these lipids with α-cyclodextrin gradually formed crystalline inclusion complexes that were characterized by ^{1}H NMR and FTIR spectroscopies, differential scanning calorimetry (DSC), and X-ray analysis. A large induced circular dichroism spectra was observed for an achiral bilayer of a chromophore-containing PEG-lipid during the initial stage of the complex formation process. The ^{1}H NMR spectra revealed that the stoichiometry number of the α-CyD/ethylene glycol unit in the inclusion complexes was 1.8 - 2.2, suggesting that only the poly(ethylene glycol) moiety in the lipids interacted with α-CyD. The bilayer of a triple-chain PEG-lipid with Mw=700 of the PEG moiety and of a phenyl-containing triple chain PEG-lipid with Mw =1,600 of the PEG moiety maintained the bilayer phase transition even after the complex formation with α-CyD. On the contrary, the phase transition was lost *via* the complex formation of the bilayers of the double-chain PEG-lipids with Mw =700, 1,000 or 1,600, as well as of triple-chain lipids with Mw = 1,000 or 1,600 of the PEG moiety. The FTIR spectral data for the complexes suggested that the difference in the phase transition behavior would come from the change in the molecular cross-sectional area (top view) of the double-chain and triple-chain in the lipids, as well as in the chain length of the PEG moiety. Lastly, we describe the synthesis of a poly(rotaxane) of α-CyD based on the inclusion complex.

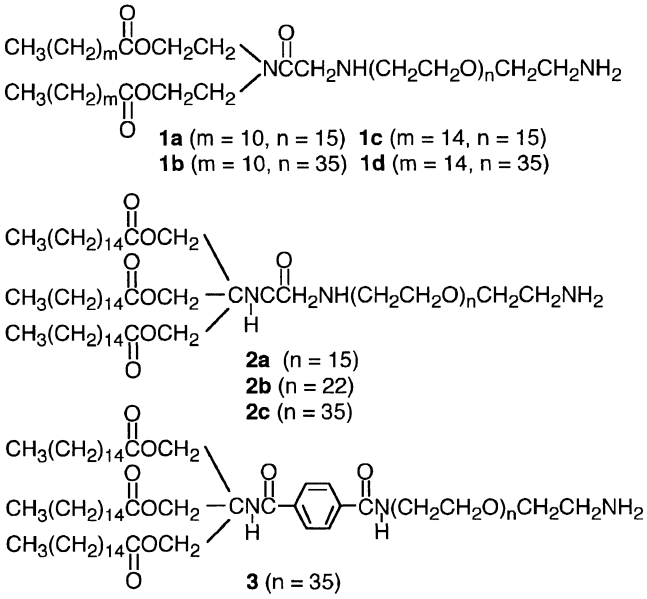
KEY WORDS Poly(ethylene glycol)lipid / α-Cyclodextrin / Inclusion Complex / Molecular Bilayer / Phase Transition / Poly(rotaxane) / Supramolecule /

The design, synthesis and functionalization of topological supermolecules such as catenanes, rotaxanes and knots are of great interest in recent years from both a fundamental and a practical point of view.[1] Rotaxanes[2] are compounds in which a ring is threaded by a chain having bulky terminal cap groups so that the chain cannot be extruded from the ring. The α-, β- and γ-cyclodextrins (CyDs) are cyclic oligosaccharides consisting of six, seven or eight glucose units, respectively, and are known to form inclusion complexes with a variety of molecules which fit into their hydrophobic cylindrical cavities.[3]

Since the first report[4] in 1990 of the synthesis of the crystalline inclusion complexes of α-cyclodextrins (α-CyD) with poly(ethylene glycol) in high yield, Harada and coworkers have developed a novel and unique CyD chemistry.[5] Yui and coworkers[6] prepared a thermally switchable β-CyD-based poly(rotaxane) of a triblock copolymer of poly(ethylene glycol) and poly(propylene glycol).

We have been interested in the design and construction of self-assembled lipid supramolecular structures based on the combination of the lipid chemistry and the host-guest chemistry.[7-8] In this paper, we describe the synthesis of eight different artificial poly(ethylene glycol)-bearing artificial lipids (**1a-d**, **2a-c** and **3**) and of the crystalline complexes of these lipids with α-CyD, as well as the characterization of the complexes using ^{1}H NMR, circular dichroism (CD) and FTIR spectroscopies, differential scanning calorimetry (DSC), and X-ray analysis. A chromophore is introduced in compound **3** in order to examine the inclusion complex formation process using CD. We also describe poly(rotaxane) based on the crystalline complex. A preliminary account of this study has been published elsewhere.[8]

Chart 1.



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EXPERIMENTAL

Materials

α-Cyclodextrin was purchased from Wako Pure Chemical Industries, Ltd. and used as received. α, ω-Diamino-poly(ethylene glycols) with the average molecular weight of 700, 1,000 or 1,600 were kindly provided by the NOF Corporation.

Synthesis of Poly(ethylene glycol)-bearing lipids (PEG-lipids)

*(N,N-Bis(2-dodecanoyloxyethyl)-(2-(ω-amino-polyethoxy)ethylamino)acetamide(**1c**).* The reaction of bis(hexadecanoyloxyethyl)amine · HCl (0.01mol) and chloroacetyl chloride (0.02 mol) in chloroform containing triethylamine (0.03 mol) gave N-chloroacetyl-O,O'-dihexadecanoyliminodiethanol[9] (yield, 70%), which was reacted with a 2.7eq amount of α,ω-diamino-poly(ethylene glycol) (Mw = 700) in 30 ml of ethanol for 2 days at 60 °C. After the removal of the solvent, cyclohexane (or ethylacetate) was added to the residue, followed by cooling at 5 – 10 ° C overnight to produce a white precipitate, which was then separated. The filtrate was concentrated to ca. 50 % by evaporation and then stored again at 5 – 10 ° C to separate the precipitate (if any). The above procedure was repeated until no precipitate appeared in the solution. The evaporation of the solvent gave **1c** as a white waxy solid. The products were analyzed using TLC, IR, ^{1}H-NMR and elemental analysis. Compounds **1a**, **1b** and **1d** were synthesized by the similar procedure. **1a:**IR (neat); 3300 (NH), 2920 and 2850 (CH), 1730 (C=O, ester), and 1640 (C=O, amide) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, TMS); δ 0.88 (t, 6H, CH_3), 1.25 (m, 32H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.77 (m, 4H, -$\underline{CH_2}CH_2COO$-), 2.30 (m, 4H, -$\underline{CH_2}COO$-), 3.62 (m, 66H, OCH_2 (PEG), $COCH_2N$, and $COOCH_2\underline{CH_2}N$), 4.22 (m, 4H, -$COO\underline{CH_2}$-). *Elemental Analysis*. Found: C = 57.54 %; H = 9.87 %; N = 3.59 %. Calcd for $C_{62}H_{123}N_3O_{20}$ + 3.5 H_2O: C = 57.56 %; H = 10.13 %; N = 3.25 %. **1b:**IR (neat); 3350 (NH), 2920 and 2850 (CH), 1730 (C=O, ester), and 1640 (C=O, amide) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, TMS); δ 0.88 (t, 6H, CH_3), 1.25 (m, 32H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.77 (m, 4H, -$\underline{CH_2}CH_2COO$-), 2.30 (m, 4H, -$\underline{CH_2}COO$-), 3.62 (m, 146H, OCH_2 (PEG), $COCH_2N$, and $COOCH_2\underline{CH_2}N$), 4.22 (m, 4H, -$COO\underline{CH_2}$-). *Elemental Analysis*. Found: C = 57.32 %; H = 9.70 %; N = 2.40 %. Calcd for $C_{102}H_{203}N_3O_{40}$ + 2 H_2O: C = 57.04 %; H = 9.71 %; N = 1.96 %. **1c**: IR (neat); 3300 (NH), 2920 and 2850 (CH), 1730 (C=O, ester), and 1640 (C=O, amide) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, TMS); δ 0.88 (t, 6H, CH_3), 1.25 (m, 48H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.77 (m, 4H, -$\underline{CH_2}CH_2COO$-), 2.30 (m, 4H, -$\underline{CH_2}COO$-), 3.62 (m, 66H, OCH_2 (PEG), $COCH_2N$, and $COOCH_2\underline{CH_2}N$), 4.22 (m, 4H, -$COO\underline{CH_2}$-). *Elemental Analysis*. Found: C = 59.88 %; H = 10.37 %; N = 3.32 %. Calcd for $C_{70}H_{139}N_3O_{20}$ + 3.5 H_2O: C = 59.80 %; H = 10.47 %; N = 2.99 %. **1d:** IR (neat); 3500 (NH), 2920 and 2850 (CH), 1730 (C=O, ester), and 1640 (C=O, amide) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, TMS); δ 0.88 (t, 6H, CH_3), 1.25 (m, 48H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.77 (m, 4H, -$\underline{CH_2}CH_2COO$-), 2.30 (m, 4H, -$\underline{CH_2}COO$-), 3.62 (m, 146H, OCH_2 (PEG), $COCH_2N$, and $COOCH_2\underline{CH_2}N$), 4.22 (m, 4H, -$COO\underline{CH_2}$-). *Elemental Analysis*. Found: C = 60.35 %; H = 10.04 %; N = 1.88 %. Calcd for $C_{110}H_{219}N_3O_{40}$: C = 59.41 %; H = 9.93 %; N = 1.89 %.

*N-(Tris(hexadecanoyloxymethyl)methyl)-(2-(ω-amino-polyethoxy)ethylamino)acetamide (**2a-c**).* The synthesis of **2a** was briefly described elsewhere.[8] *N*-(Tris(hexadecanoyloxymethyl)-chloroacetamide[10] (0.5g) was reacted with 1.5eq of α,ω-diamino-poly(ethylene glycol) in dry THF at 60℃. The reaction was continued until a TLC spot of *N*-(tris(hexadecanoyloxymethyl)-chloroacetamide disappeared. After solvent evaporation, ethylacetate (10 ml) was added to the residue, followed by cooling at 5–10 °C. The produced precipitate was separated and the solvent was concentrated to about 50% by evaporation followed by cooling at 5 °C. This procedure, that is, precipitates separation and solvent concentration followed by cooling at 5 °C, was continued until no precipitate was produced. After the removal of the solvent, the obtained waxy solid was purified by column chromatography (silica gel) to obtain **2a-c** as white waxy solids. The Beilstein test was negative. **2a:** IR (neat); 2920 and 2850 (CH), 1740 (C=O, ester), and 1650 (C=O, amide) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, TMS); d 0.85 (t, 9H, CH_3), 1.23 (m, 72H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.74 (m, 6H, -$\underline{CH_2}CH_2COO$-), 2.31 (m, 6H, -$\underline{CH_2}COO$-), 3.60 (m, 62H, OCH_2 (PEG), and $COCH_2N$), 4.42 (m, 6H, -$COO\underline{CH_2}$-). *Elemental Analysis*. Found: C = 65.24 %; H = 10.59 %; N = 2.44 %. Calcd for $C_{86}H_{169}N_3O_{22}$: C = 64.67 %; H = 10.66 %; N = 2.63 %. **2b:** IR (neat); 2920 and 2850 (CH), 1740 (C=O, ester), and 1650 (C=O, amide) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, TMS); δ 0.85 (t, 9H, CH_3), 1.23 (m, 72H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.74 (m, 6H, -$\underline{CH_2}CH_2COO$-), 2.31 (m, 6H, -$\underline{CH_2}COO$-), 3.60 (m, 90H, OCH_2 (PEG), and $COCH_2N$), 4.42 (m, 6H, -$COO\underline{CH_2}$-). *Elemental Analysis*. Found: C = 60.19 %; H = 10.04 %; N = 2.54 %. Calcd for $C_{100}H_{197}N_3O_{29}$ + 5 H_2O: C = 60.18 %; H = 10.45 %; N = 2.11 %. **2c:** IR (neat); 3500 (NH), 2920 and 2850 (CH), 1740 (C=O, ester), and 1650 (C=O, amide) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, TMS); δ 0.85 (t, 9H, CH_3), 1.23 (m, 72H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.74 (m, 6H, -$\underline{CH_2}CH_2COO$-), 2.31 (m, 6H, -$\underline{CH_2}COO$-), 3.60 (m, 142H, OCH_2 (PEG), and $COCH_2N$), 4.42 (m, 6H, -$COO\underline{CH_2}$-). *Elemental Analysis*. Found: C = 58.66 %; H = 9.65 %; N = 1.59 %. Calcd for $C_{126}H_{249}N_3O_{42}$ + 5 H_2O: C = 58.92 %; H = 10.16 %; N = 1.64 %.

*Tris(hexadecanoyloxymethyl)-(4-(N-(2-(ω-amino-polyethoxy)ethyl)carbamoyl)phenyl)carbonylaminomethane(**3**).* To a solution containing triethylamine (0.5 g, 5.0 mmol) and α,ω-diamino-poly(ethylene glycol) (Mw = 1,600, 12.0 g, 7.5 mmol) in dry THF was added dropwise a solution of tris(hexadecanoyloxymethyl)-((4-(chlorocarbonyl)phenyl)carbonylamino)methane (3.0 g, 3.0 mmol) in dry THF at 0 °C over 60 min, followed by stirring at room temperature overnight. The generated triethylammonium chloride was filtered off, and then the solvent of the filtrate was evaporated to give a crude precipitate, to which acetonitrile was added to separate the insoluble α,ω-diamino-poly(ethylene glycol). The filtrate was cooled at 4 °C overnight. The generated precipitate was collected by centrifugation at –5 °C and then air-dried to yield **3** as a waxy solid (1.2 g, 16 %). **3**: IR (neat); 3500 (NH), 2920 and 2850 (CH), 1735 (C=O, ester), and 1650 (C=O, amide) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, TMS); δ

0.85 (t, 9H, CH_3), 1.23 (m, 72H, -CH_2-), 1.61 (m, 4H, CH_2N(PEG)), 1.74 (m, 6H, -CH_2CH_2COO-), 2.33 (m, 6H, -CH_2COO-), 3.64 (m, 140H, OCH_2 (PEG)), 4.57 (m, 6H, -$COOCH_2$-), 7.93 (dd, 4H, Ar). *Elemental Analysis*. Found: C = 61.31 %; H = 9.37 %; N = 1.41 %. Calcd for $C_{132}H_{251}N_3O_{43}$: C = 61.73 %; H = 9.85 %; N = 1.64 %.

Preparation of Inclusion Complexes

A typical procedure is as follows. The addition of a one-milliliter portion of an aqueous bilayer of a synthesized PEG-lipid to an aqueous solution (10 ml) of α-CyD (0.145 g/ml) gradually produced a precipitate. After 10 hrs of stirring, the produced precipitate was collected, washed with a small amount of water and then dried at 55-60 °C under reduced pressure to give a poly(pseudo-rotaxane).

Preparation of Poly(rotaxane) (**4**)

A suspension of poly(pseudo-rotaxane) **3** (40 mg, 2.3 μmol) containing sodium 2,4,6-trinitrobenzenesulfonate (125 mg, 390 μmol) and α-CyD (saturated) in a 10 mM borate buffer (pH 9, 10 mL) was stirred for ten days at room temperature. The precipitate in the solution was collected by centrifugation, and then washed with water, followed by air-drying under reduced pressure at 50 °C to give poly(rotaxane) (**4**). Anal: ^{1}H NMR (400 MHz, DMSO-d_6, TMS); δ 0.84 (t, 9H, CH_3), 1.22 (m, 72H, -CH_2-), 1.49 (m, 4H, CH_2N(PEG)), 1.74 (m, 6H, -CH_2CH_2COO-), 2.25 (m, 6H, -CH_2COO-), 3.2-3.9 (m, 392H, OCH_2 (PEG), C(2)H, C(3)H, C(4)H, C(5)H, C(6)H_2 (CyD)), 4.50 (m, 48H, , -$COOCH_2$-, C(6)OH (CyD)), 4.79 (d, 42H, C(1)H), 5.45 (d, 42H, C(2)OH), 5.53 (d, 42H, C(3)OH), 7.93 (m, 4H, COC_6H_4CO), 8.54 (s, 2H, $NHC_6H_2(NO_2)_3$).

Differential Scanning Calorimetry (DSC)

DSC (Shimadzu DSC-50) was applied to the PEG-lipids and their complexes with α-CyD. The amount of each sample was 2.0 mg. The measurement was conducted at the scan rate of 2 °C/min in air or in the presence of water.

FTIR measurement

Cast films of the PEG-lipids from chloroform were prepared on CaF_2 plates. The KBr method was applied for the lipid/α-CyD complexes. Each sample was assembled in a temperature-controlled flow-through cell (Harrick Scientific Corporation). Temperatures were maintained at a constant value within ±0.1°C (Neslab Instruments, Inc., Circulator RTE-100).

X-Ray Study

X-ray diffraction diagrams of lipid/α-CyD complexes were obtained using a Rigaku Rad-C.

Circular Dichroism

Sonication of the artificial lipid **3** in water (Mill-Q water, pH 10) gave a transparent solution to which given amounts of α-CyD were added to measure the circular dichroism spectra (JASCO, J-720).

RESULTS AND DISCUSSION

Bilayer Formation and Induced Circular Dichroism

Aqueous solutions of compound **3** prepared by sonication formed a bilayer structure (Figure 1) as well as other poly(ethylene glycol)-bearing artificial lipids[11]. Chiral synthetic bilayers that contain aromatic chromophores were reported to show remarkably enhanced circular dichroism.[12] A large induced circular dichroism was detected for the system of an aromatic chromophore bound to the bilayer of a chiral lipid.[13] We now describe the large induced circular dichroism for the complex of the chromophore-containing lipid **3** with α-CyD.

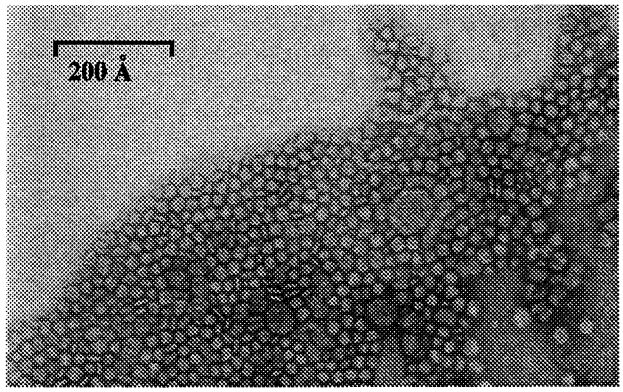

Figure 1. Electron micrograph of an aqueous solution of **3** stained with uranyl acetate.

Since the addition of α-CyD to the aqueous bilayers of **3** did not produce a precipitate within a few hrs, we could measure CD spectra of the solution. Figure 2 shows the result. It is evident that the addition of α-CyD induces a circular dichroism for the achiral bilayer of **3** in the 207 nm region which corresponds to the absorption band (π-π* transition) of the phenyl moiety in **3** and the [θ] values reached 4×10^5 deg cm^2 dmol^{-1} at the higher concentration of α-CyD. Mason's g-value[14] was calculated to be 5×10^{-3}, which indicates a fixed mutual orientation between the achiral lipid bilayer of **3** and α-CyD.

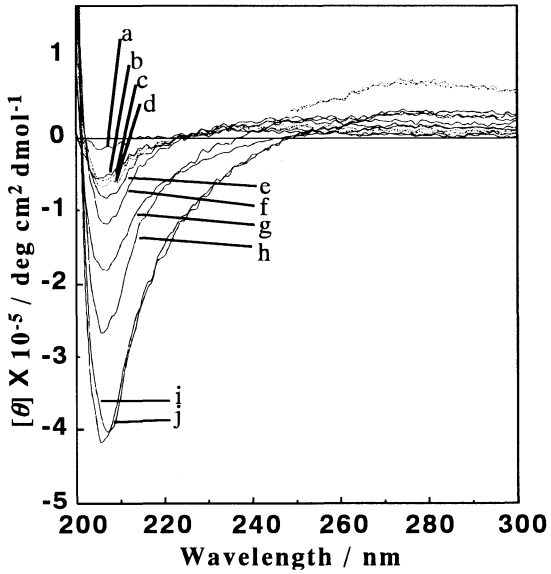

Figure 2. Induced circular dichroism spectra of an aqueous bilayer of **3** (1.0×10^{-4} M) in the presence of α-CyD at 25 °C. The concentrations of α-CyD are: (a) 0.001 M, (b) 0.01 M, (c) 0.02 M, (d) 0.03 M, (e) 0.04 M, (f) 0.06 M, (g) 0.08 M, (h) 0.01 M, (i) 0.12 M, and (j) 0.13 M.

Formation of Inclusion Complexes

The addition of α-CyD to the aqueous bilayers of the PEG-lipids synthesized in this study gradually produced precipitates that were insoluble in water, methanol, ethanol, acetone, chloroform, ethylacetate, benzene and hexane but were soluble in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Harada and coworkers[4-5] found that α-CyD and poly (ethylene glycols) formed inclusion complexes, whose stoichiometry is 2:1 (ethylene glycol unit: α-CyD). We reported that for the complex of an aqueous bilayer **2a** and α-CyD, the stoichiometry was 2.2±0.1, postulating that only the poly(ethylene glycol) moiety in the lipid interacts with α-CyD.[7] We found here that aqueous bilayers of **1a-d**, **2b-c** and **3** also interacted with α-CyD to produce crystalline complexes. The ^{1}H NMR spectra of the complexes reveal that the stoichiometry between the PEG moiety in the lipids and α-CyD is 1.8 - 2.2, which are close to those for the complexes of poly(ethylene glycols) with α-CyD[4]. Therefore, the obtained results suggest that only the poly(ethylene glycol) moiety in the PEG-lipids interacts with α-CyD in the bilayer aggregates to form the inclusion complexes.

Phase transition

The phase transition between the crystal phase and the liquid crystal phase is one of the most fundamental characteristics of both biological and artificial lipid bilayers.[15] We describe here the phase transition behavior of double-chain or triple-chain PEG-lipids and their complexes with α-CyD using DSC and FTIR spectroscopies. Typical DSC thermograms are shown in Figure 3. The phase transition temperatures for eight PEG-lipids and their complexes with α-CyD are summarized in Table I. We have obtained the following interesting features.

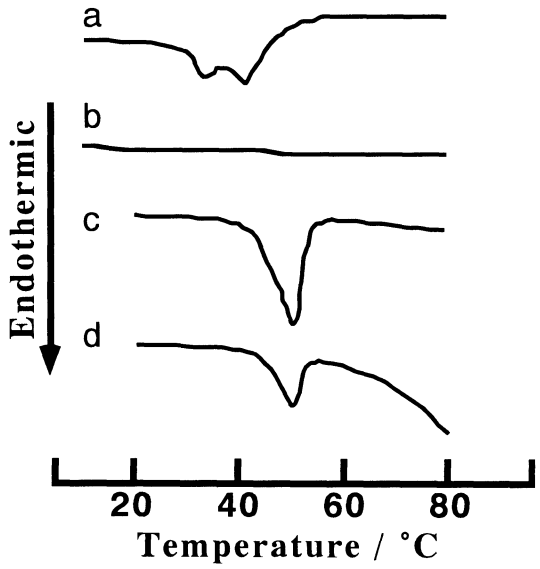

Figure 3. DSC thermograms in the presence of water: (a) a cast film of **1c** in water, (b) a powder of **1c**/ α-CyD complex in water, (c) a cast film of **2a** in water, and (d) a powder of **2a**/ α-CyD complex in water.

Table I. Phase transition temperatures of PEG-lipids and their complexes with α-CyD

Compounds	Tc/°C in air (△H/kcal mol^{-1})	Tc/°C in the presence of water (△H/kcal mol^{-1})
1a	nd	nd
1a/α-CyD complex	nd	nd
1b	nd	nd
1b/α-CyD complex	nd	nd
1c	30-35	34.0, 42.0 (5.2)
1c/α-CyD complex	nd	nd
1d	nm	40 (1.6)
1d/α-CyD complex	nm	nd
2a	35.7 (17.9)	47.5 (15.9)
2a/α-CyD complex	34.9 (5.6)	48.9 (6.7)
2b	38.1(19.7)	43.7 (10.1)
2b/α-CyD complex	nd	nd
2c	33.40 (broad)	41.2 (10.2)
2c/α-CyD complex	nd	nd
3	25, 33, 40.2 (32.5)	27.0, 34.5, 39.0 (16.7)
3/α-CyD complex	nd	45.4 (11.9)

nd : not detected, nm : not measured.

(a) The complex of the triple-chain lipid **2a** with α-CyD possesses a phase transition both in air and in the presence of water. This is in sharp contrast with the double-chain lipid **1c** / α-CyD complex, where no phase transition is observed.
(b) Triple-chain lipids **2b** and **2c**, in which the PEG chain length is longer than that in **2a** lose the phase transition both in air and in the presence of water *via* the complex formation with α-CyD.
(c) Despite the longer PEG-chain length, the complex of lipid **3** with α-CyD possesses the phase transition in the presence of water.

The obtained result indicates that the molecular cross sectional area of the lipids as well as the PEG-chain length are important factors for the existence of the phase transition of the PEG-lipid/α-CyD complexes.

We measured the FTIR spectra to shed light on the mechanism of the phase transition. The phase transition dependent FTIR spectra for aqueous bilayers of biological and synthetic lipids and for cast films of poly (ion complexed) lipids have already been reported.[16] Figures 4 and 5 show the temperature dependence of the frequency for the symmetric and asymmetric stretching bands of the methylene chains in **1c** and **1c**/α-CyD and in **2a**, **2b** and their complexes with α-CyD, respectively. As can be seen in the figures, the frequency of the CH_2 asymmetric stretching band in cast films of **1c** and **2a** shows breaks near 35 °C and 40 °C, respectively, which are close to the phase transition temperatures of **1c** and **2a**, respectively. The observed behavior is ascribable to the typical bilayer phase transition from the crystalline phase to the liquid crystalline phase derived from the *trans-gauche* conformational change in the alkyl chains.[15-16]

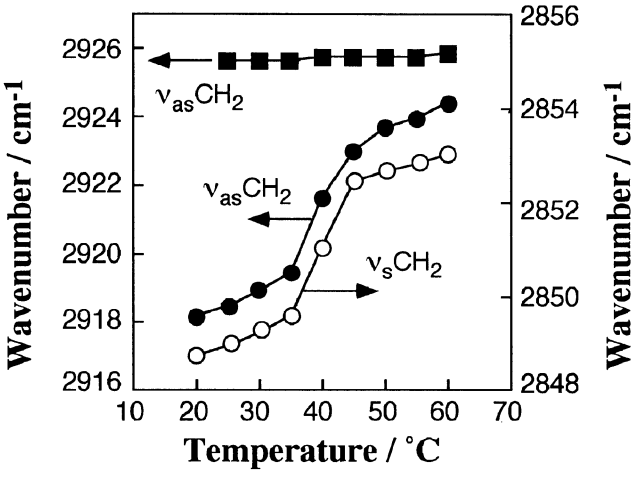

Figure 4. Temperature dependence of the frequencies of the CH_2 stretching vibration for a cast film of **1c** (open and closed circles) and for a powder of **1c**/α-CyD complex (squares).

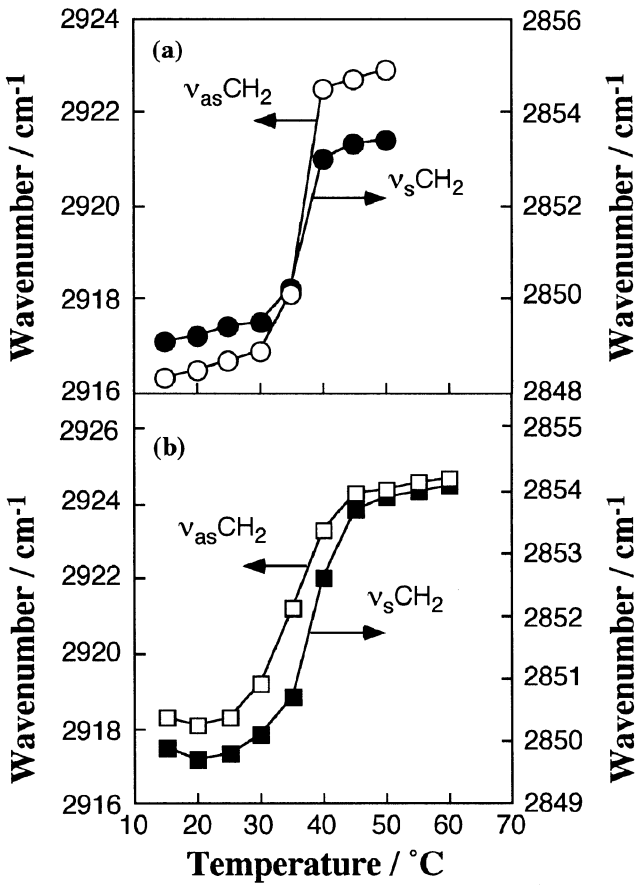

Figure 5. Temperature dependence of the frequencies of the CH_2 stretching vibration for a cast film of **2a** (a) and for a powder of **2a**/α-CyD complex (b).

On the contrary, the frequency of the CH_2 stretching band for the **1c**/α-CyD complex exhibited no temperature dependence. The observed frequency of 2925.5 cm^{-1} for the complex indicates that the long methylene chains form a *gauche* conformation at all the measured temperatures. The difference in the phase transition temperature behavior between the double-chain and triple-chain lipids would be derived from the difference in the molecular cross-sectional area of these lipids. Although the molecular cross-sectional area (top view) of α-CyD (0.95 nm^2/molecule) is somewhat larger than that of **2a** (0.8 nm^2/molecule), the observed almost identical phase transition temperatures between **2a** and **2a**/α-CyD indicate that the complex formation does not significantly cause the change in the structure of the long alkyl chains in the bilayer.

Structure of the Complex

In order to determine the structure of the PEG-lipid/α-CyD complex, we measured the X-ray diffraction diagrams for the powders of **2a-c**/α-CyD and the result is shown in Figure 6. As can be seen in the figure, the complexes of three different PEG-lipids with α-CyD have almost identical X-ray diagrams that are similar to those of the complex of α-CyD with valeric acid or octanol, indicating that the PEG-lipid/α-CyD complexes are crystalline with an extended column structure in the PEG/α-CyD parts in the complexes.[17] A possible structure for the complex of **2a** with α-CyD is shown in Figure 7.

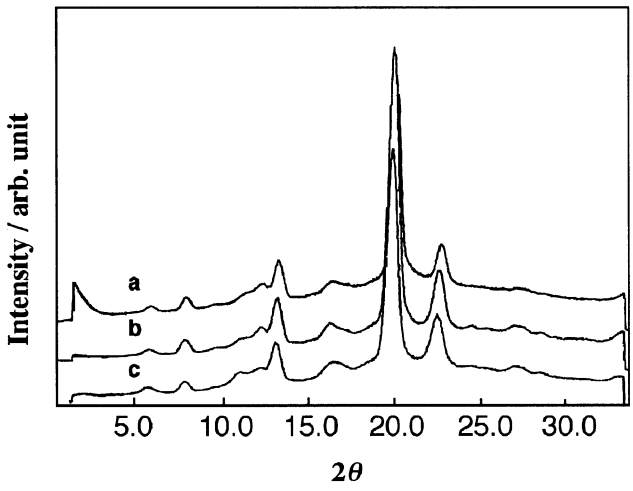

Figure 6. X-ray diffraction diagrams of **2a**/α-CyD (a), **2b**/α-CyD (b), and **2c**/α-CyD (c).

Poly(rotaxane)

The reaction of **3** with a capping reagent, sodium 2,4,6-trinitrobenzenesulfonate (TNBS) was conducted in the presence of α-CyD (saturated) in water, where lipid **3** forms the inclusion complex with α-CyD. As is described in the Experimental section, the chemical shift of the protons of the aromatic group in **4** in DMSO-d_6 appears at 8.54 ppm, which shifted to the higher magnetic field by 0.31 ppm compared to that of TNBS in the same solvent. The ^1H NMR spectrum of **4** revealed that ca. 10 molecules of α-CyD were captured per one lipid molecule. A proposed model for the poly(rotaxane) is shown in Figure 8.

CONCLUDING REMARKS

We prepared eight PEG-lipids that form a bilayer structure in aqueous solutions. It was found that i) α-CyD strongly interacts with the PEG-moiety of aqueous achiral bilayers to induce circular dichroism during the initial stage

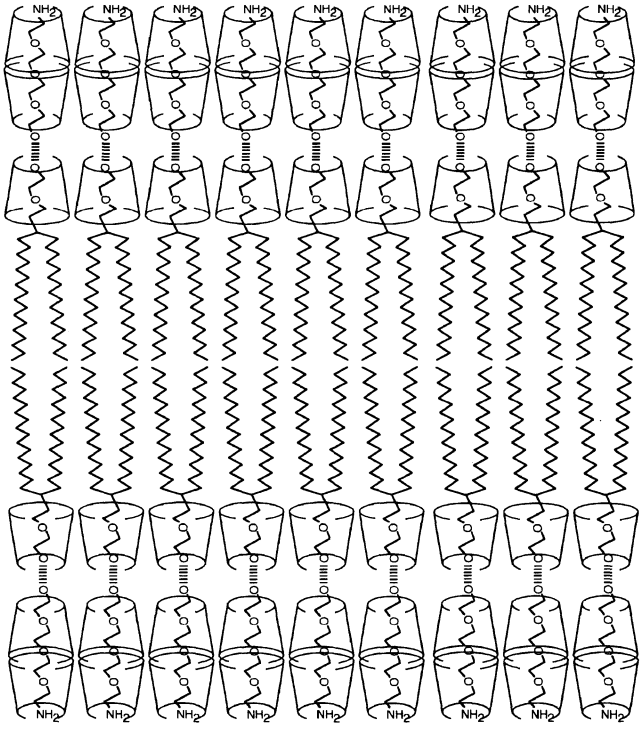

Figure 7. A possible structure for **2a**/α-CyD.

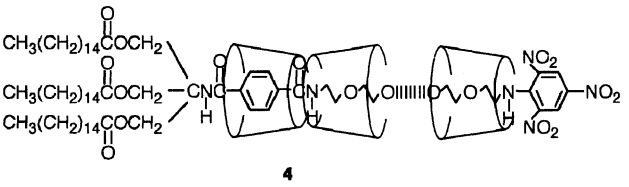

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Figure 8. A proposed model for the poly(rotaxane)(**4**).

and then gradually produces crystalline inclusion complexes with an extended column structure, and ii) the **2a**/ α-CyD and **3**/ α-CyD complexes possess the bilayer phase transition in air as well as in the presence of water though they were insoluble in water. It is noteworthy that the packing mode and orientation of the long alkyl chains between the aqueous bilayer state and the crystalline complex (solid state) are not much different, since the phase transition temperatures of both states are close and the frequency mode in the temperature dependent FTIR is essentially the same. The finding is important for the design and construction of a variety of biomembrane-mimetic supramolecular aggregates with the phase transition. We prepared, for the first time, a poly(rotaxane) of α-CyD based on the lipid bilayer structure. Further studies including the relation between the structure and function of such super aggregates are in progress.

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