Superstructure Formation of a Synthetic Lipid Bearing a Poly(ethylene glycol) Head Group with α-Cyclodextrin

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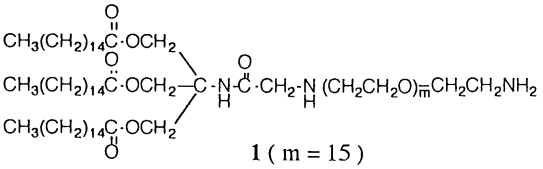
Mixing of an aqueous bilayer of an artificial nonionic lipid bearing a poly(ethylene glycol) as the hydrophilic head group with α-cyclodextrin(α-CD) was found to produce a stable crystalline inclusion complex which possessed the fundamental bilayer characteristic (phase transition) for the first time. ^{1}H-NMR spectra of the complex showed that 2.2 ± 0.1 ethylene glycol units in the lipid were captured in one α-CD molecule.

The chemistry of synthetic lipid bilayer membranes and the chemistry of host-guests have developed almost independently. The combination of these chemistries affords an exciting area in science; however studies published thus far are limited. Here, we describe one approach for such studies; *i.e.*, the formation of lipid supramolecular structures based on the combination of synthetic bilayer membrane systems and host-guest chemical systems. Cyclodextrins(CDs) are cyclic oligosaccharides consisting of six, seven or eight glucose units and they are well-known to form inclusion compounds with molecules which fit into their cavity.[1] Nonionic synthetic lipids bearing a poly(ethylene glycol) head groups have been reported to form aqueous bilayer membranes[2] as well as a variety of other lipids. Harada and co-workers recently described the complex formation of α-CD with poly(ethylene glycols),[3,4] and they also have succeeded in the synthesis of poly(rotaxanes) and nanotubes based on their complexation.[5,6] Li and McGown [7] reported the formation of molecular nanotubes from β- and γ-CDs linked by diphenylhexatrienes.

We would like to report here for the first time the crystalline complex formation of aqueous bilayers of a triple-chain lipid, **1**, bearing a poly(ethylene glycol) moiety with α-CD. By considering the molecular cross-sectional area of α-CD and lipids, the triple-chain lipid was designed and synthesized instead of the usual double-chain lipids.

The synthesis of the lipid is as follows. The reaction of N-chloroacetyl-O,O',O"-trihexadecanoyliminotrimethanol[8] (0.5 g) and α,ω-bis(amino)poly(ethylene glycol) (MW= 700) (1.5 g) in dry THF gave a waxy solid which was purified by columm chromatography to obtain lipid **1**. The product was analyzed by TLC, ^{1}H-NMR and elemental analysis.



1 (m = 15)

The addition of a one milliliter portion of an aqueous solution of bilayer **1** (10 mg/ml, pH 10) to an aqueous solution (10 ml) of α-CD (0.145 g/ml) gradually produced a precipitate. After 10 hrs of stirring, the crystalline precipitate was filtered by centrifugation, washed with pure water twice and dried at 50-60 °C under reduced pressure.

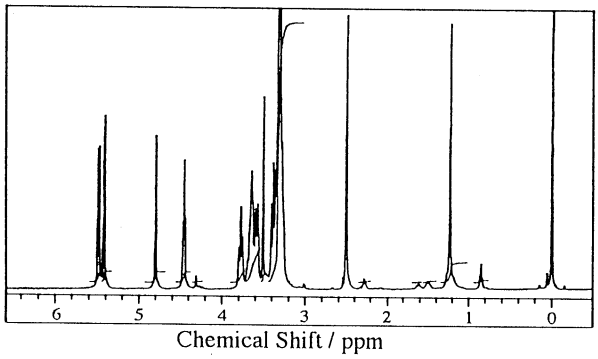


Figure 1. 400MHz ^{1}H-NMR spectrum of the **1** / α-CD complex. Solvent, DMSO-d6.

The product (yield, 50 mg) was insoluble in water, methanol, ethanol, acetone, THF, ethyl acetate, chloroform, benzene and hexane, but was soluble in dimethylformamide and dimethylsulfoxide (DMSO). The replacement of α-CD by β-CD did not produce a precipitate.

The depth of the α-CD cavity corresponds to the length of two ethylene glycol units.[5] The stoichiometry of the complex between poly(ethylene glycols) and α-CD is reported to be 2:1 (ethylene glycol unit : α-CD).[3] Figure 1 shows the ^{1}H-NMR spectrum[9] of the precipitate, which indicates the stoichiometry of the lipid/α-CD complex; here 7.5 ± 0.3 α-CD units were captured per one lipid molecule. Postulating that only the poly(ethylene glycol) moiety of the lipid interacts with α-CD, this number indicates that 2.2 ± 0.1 ethylene glycol units are captured in one α-CD molecule, which is close to the result for the complex of poly(ethylene glycols) with α-CD[3,4]. Using these results together with the results of Harada and co-workers[3-6], a model for the lipid superstructure is proposed as shown in Fig. 2.

If the bilayer membrane properties are maintained in such complexes, novel possibilities in molecular membrane based-supramolecular science would arise. The phase transition between the crystalline and liquid crystalline phases is one of the fundamental characteristics of lipid bilayer membranes. The existence of a phase transition in the complex studied here was examined by DSC. Lipid **1** in water exhibited a phase transition at 47.5 °C (transition enthalpy(ΔH), 66.5 kJmol^{-1}). The lipid **1** /α-CD complex was found to possess a phase transition at 48.9 °C (ΔH, 28.0 kJmol^{-1}) in water but the transition temperature shifted to 35 °C in air (ΔH, 23.4 kJmol^{-1}, Fig. 3a). The shift to a lower temperature in air is not unusual.[10] The wavenumber of the asymmetric and symmetric methylene stretching vibrations in the FT-IR spectra of the lipid **1**/α-CD complex changed drastically

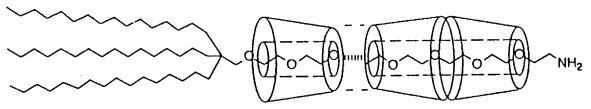


Figure 2. A proposed model for the **1** / α-CD complex.

near 35 °C (Fig. 3b). These results together with the DSC data indicate that the significant wavenumber shift is associated with the phase transition of the complex. The change in ν_{as} (CH_2) and ν_s (CH_2) from 2918 cm^{-1} to 2925 cm^{-1} and from 2850 cm^{-1} to 2854 cm^{-1}, respectively, is assigned to the *trans-gauche* conformational change of the long alkyl chain, which leads to the phase change in the bilayers. These wavenumber changes were almost identical with those of **1** itself.[11] Such behavior is typically observed for both biological[12] and synthetic[13] lipid bilayer membranes. It is evident that the complex retains its fundamental molecular conformation and vibrational modes of the lipid bilayer membranes at temperatures below and above the phase transition.

In conclusion, mixing of an aqueous bilayer of the synthetic lipid bearing the poly(ethylene glycol) moiety and α-CD forms a crystalline complex which retains the fundamental characteristics of the lipid bilayers. An intense effort is currently underway in our laboratory to fully define the lipid bilayer-based structural characteristics of this complex below and above the phase transition and to synthesize poly(rotaxanes) of **1**/α-CD and analogous complexes. The present study affords the opportunity to design self-assembled superstructures based on the combination of lipid chemistry and host-guest chemistry.

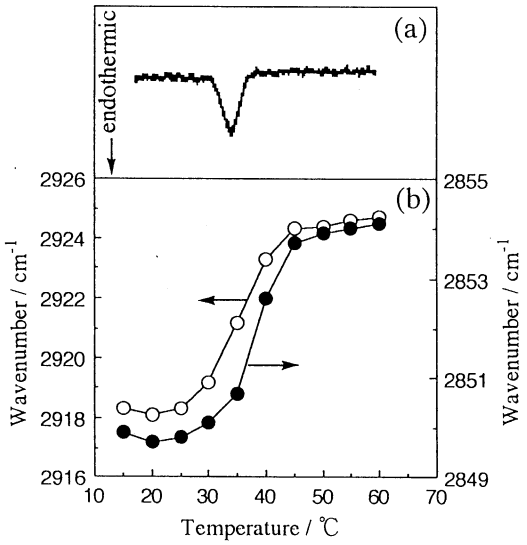


Figure 3. (a) DSC thermogram of the **1** / α-CD complex in air. (b) Plots of wavenumbers of the asymmetric (-○ -) and symmetric (-●-) CH_2 stretching from FT-IR spectra of the **1** / α-CD complex as a function of temperature.

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References and notes

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9 ^{1}H-NMR(DMSO-d_6) of **1** / α-CD complex : δ 0.85 (t, 9H, CH_3 of **1**), δ 1.23 (s, 72H, CH_2 of **1**), δ 1.50 (m, 6H, CH_2 of 1), δ 2.27 (m, 6H, $COCH_2$ of **1**), δ 3.27 (m, 6H×7.5, C(4)H of α-CD), δ 3.37 (t, 6H×7.5, C(2)H of α-CD), δ 3.51 (s, 64H, -OCH_2 of 1), δ 3.62 (m, 6H×7.5, C(5)H of C(3)H of α-CD), δ 4.31 (s, 6H, $COOCH_2$ of **1**), δ 4.45 (t, 6H×7.5, C(6)OH of α-CD), δ 4.80 (d, 6H×7.5, C(1)H of α-CD), δ 5.41 (d, 6H×7.5, C(3)OH of α-CD), δ 5.48 (d, 6H×7.5, C(2)OH of α-CD).
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11 Wavenumbers of lipid **1** are : ν_{as} (CH_2) ; 2916.3 - 2916.9 cm^{-1} (15-30 °C) and 2922.5 - 2922.7 cm^{-1} (40-45 °C), ν_s (CH_2) ; 2849.1 - 2849.5 cm^{-1} (15-30 °C) and 2853.0 - 2853.4 cm^{-1} (40-50 °C).
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