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Two-Step Phase Transition in Self-Assembled Glycolipid Nanotube

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Recently, chiral glycolipids with unsaturated hydrocarbon chains proved to self-assemble in water to form nanotubes. We investigated the phase transition behavior of a glycolipid in tube-forming process by using ultra sensitive differential scanning calorimetry (DSC) and circular dichroism (CD). Our experimental results showed that the tube-forming process occurred in several hours and the phase transition from vesicles (liquid crystalline phase) to tubes (gel phase) followed two steps with decreasing temperature. We also found that the glycolipid nanotubes appearing in low temperature region were in a glassy state ascribable to reducing freedom of molecular motion of the glycolipid.

Key Words: lipid, nanotube, DSC, phase transition

INTRODUCTION

A variety of chiral amiphiles spontaneously form various nanostructures such as tubes, fibers and helical ribbons [1-3]. Chiral molecules self-assembly induce bilayer membranes of the amphiphilic molecules to form helical structures and eventually to result into tubular structure. The tubular structure possess hollow cylindrical nanospace with typical diameters of sub-μm scale. Facile synthesis of the nanotube structures is also attracting great interests for applications such as molecular electronics, bioscience and so on. For such applications, tubular structure and tube-forming process have been studied theoretically and experimentally. Helfrich and Prost explained theoretically the stability on lipid nanotubes in view of an intrinsic bending force due to chirality [4]. They thought that chiral membranes in a tilted phase formed a hollow cylindrical structure because of chiral packing of lipid molecules. This concept has been generalized and developed by several researchers [5-10]. Further, Ou-Yang and Liu obtained the general free energy for spherical vesicles, helical structures and tubes [6].

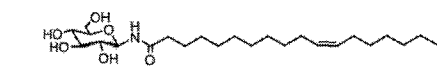
On the other hand, many experiments on the structure and forming process of phospholipid nantotubes have been reported by using optical microscopy, X-ray diffraction, circular dichroism (CD) and calorimetry [11-17]. These reports showed that the tube-forming process between spherical vesicles and nanotubes was driven by an intralamellar first-order phase transition, $L_\alpha - L_{\beta'}$, with conformational changes of alkyl chains.

Recently, chiral glycolipid molecules were also observed to form tubular structure [18]. The typical diameter of the glycolipid nanotube, unlike the phospholipid one, is of 10-15 nm, and the length is typically μm to mm scale. These glycolipids also self-assemble to form nanofibers, helical nanoribbons, nanotubes and so on [19]. In this paper, we report the study of phase transition between tubes and vesicles in dilute dispersion of the chiral glycolipid **1** [20,21] by using ultra sensitive differential scanning calorimetry (DSC) and CD.

EXPERIMENT

Sample-We have synthesized newly chiral glycolipid *N*-(11-*cis*-octadecenoyl)-β-D-glucopyranosylamine **1** (Fig. 1), and this chiral glycolipid also formed a nanotube structure [20]. The synthesis of the glycolipid **1** is described elsewhere [21], and the method for the lipid nanotube (LNT) formation is as follows. First, 1 mg of the glycolipid **1** dissolved in methanol (2-3ml) was evaporated to form lipid films, and then 20 ml water was added (1.1 μM) and sonicated. This lipid dispersion in water was heated and kept at 105°C to be well-dispersed in water. After keeping at this temperature, this lipid dispersion was slowly cooled to room temperature. This method converted the glycolipid **1** to nanotubes (Fig.1). The outer diameter of the LNTs was 200-500 nm and inner were 20-50 nm.

1



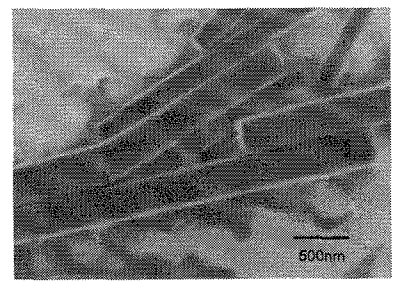


Fig.1 The chemical structure of the glycolipid **1** and FE-SEM image of the self-assembled nanotubes from the glycolipid **1**.

DSC-We investigated the phase transition of the nanotube from **1** in dilute dispersion by using ultra sensitive DSC. This measurement was carried out on a VP-DSC instrument (MicroCal), scan runs were performed in temperature range of 5-105°C. To observe the tube-forming process, we measured heating and cooling scan in 1.1 μM lipid dispersion pretreated with the method of forming LNT. Especially, the sample was held at 105°C with 1 h before cooling scan for the purpose of in-situ measurement of tube-forming. The scan runs of water were measured as references before ones of samples, and the thermograms were obtaiened by subtracting reference scan from sample scan.

CD-Circular dichroism (CD) signal gives information about chiral order in molecules. LNT has intermolecular chiral packing and should yield large CD signal [22]. Thus, we also investigated the phase transition in melting LNT by CD spectrometric measurement (JASCO J-820) with changing temperature.

RESULTS

DSC thermograms of heating scan at the rate of 30°C/h and cooling scan at 10°C/h are shown in Fig.2. In the DSC curves, heating scan in glycolipid **1** dispersion exhibited an endotherm at 62°C. This transition corresponds to the change from crystalline lipids to well-dispersed vesicles or other aggregated structure, which could not be observed using optical microscopy. Cooing process of the glycolipid **1** dispersion exhibited multiple exothermic peaks, suggesting the presence of metastable and stable phases. Incidentally, these exothermic peaks cannot be observed at higher scan rate such as 60°C/h. So, the phase transition in cooling process was found to be slow process in time scale of several hours. In addition, it seemed that the cooling process, that is, the tube-forming process is different from heating process of glycolipid **1** dispersion and the tube forms through two-step phase transition.

In Fig.3, the thermogram of heating scan on 1.1 μM LNT in water (melting LNT process) shows that major endotherm shifted to higher temperature position at 75°C (enthalpy of 114 kJ/mol), and glass transition occurred at 68.5°C. However, since the glass transition temperature was quit dependent on a scan rate, this transition was also decided to be slow process. Although this glass transition was not exhibited in glycolipid **1** dispersion at any scan rate, it is thought that freedom of molecular motion was restricted in the stable LNT phase at lower temperature. Actually, we confirmed that the LNT had the rigid body with the persistent length of more than 10 μm by using the optical microscope. This result also indicated that the alkyl chains of the glycolipids were in the glassy state.

While, the temperature dependence of the CD spectra in 1.1 μM LNT dispersion at the wavelength of 209 nm, which is the wavelength of the negative peak [21] of the glycolipid **1** assignable to amide group or cis-double bond at room temperature, is shown in Fig.4. Heating rate was 30°C/h so as to compare the DSC result. This result indicates that the LNT fabricated from **1** in water gradually changed CD activity, over heating, until the 74°C, and sharply loosed from the point of 75°C. The glass transition behavior is good agreement with the DSC result of the LNT melting.

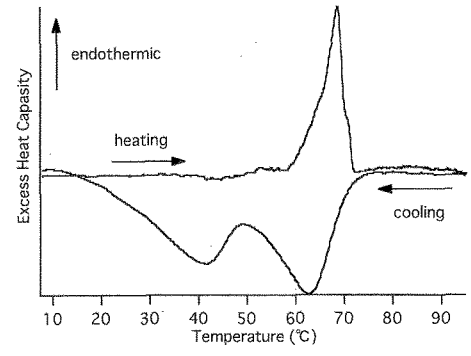


Fig.2 DSC thermograms of 1.1 μM glycolipid **1** water dispersion pretreated with the method of forming LNT. Heating scan rate was 30°C/h. Cooling scan rate was 10°C/h, and sample was held at 105°C with 1 h in this scan.

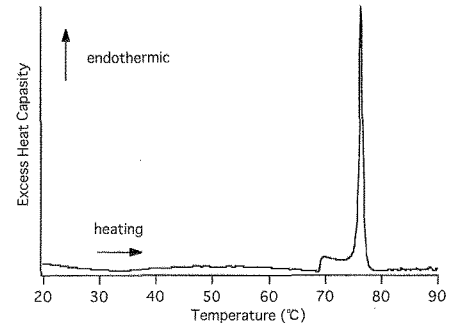


Fig.3 DSC heating thermogram of 1.1 μM LNT fabricating from glycolipids **1** in water. Heating scan rate was 30°C/h.

DISCUSSION

We have studied the phase transition behavior of the glycolipid nanotube from **1** by DSC and CD. As a result, we found two-step phase transition, whereas similar two-step phase transition for this system was also reported by using elasticity measurement and FT-IR [23]. Taking our results into consideration, we propose the following model for the phase transition through a series of tube-forming process (Fig.5). At first, the procedure of heating and keeping the glycolipid **1** dispersion in water at 105°C for 1 h gives well-dispersed vesicles or some aggregated structure in water [6]. Thereafter, as this dispersion is cooled slowly, the vesicle bilayer or aggregation of the lipids gradually looses fluidity of the alkyl chains and becomes unstable lamellar structure. However, due to large part of hydrophobic groups faced to water, this structure changes to a metastable structure, for example rippled phase, so that the hydrophobic effect should weaken. Finally, the rippled lamellar sheet or helical ribbon further develops and stacks to form tube structure in $L_{\beta'}$, that is, gel phase. Rippled phase has been studied in glycolipids and phospholipids [24-30] and it is known that there is validity in rippled metastable phase owing to canceling instability from hydrophobic effect in lamellar sheet [9]. Following the tube-forming process, as LNT dispersion is heated, first-order phase transition in $L_{\beta'}$–L_{α}, occurs and tubes melt to form aggregated vesicles [31]. This phase transition contains glass transition at 68.5°C and conformational changes of alkyl chains at 75°C. Since the glass transition at 68.5°C

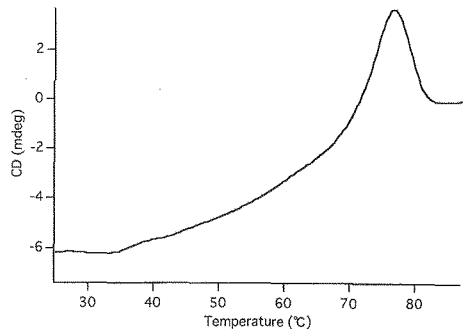


Fig.4 Temperature dependence of CD spectra in 1.1 μM glycolipid 1 solution at wavelength of 209 nm. Heating rate was 30°C /h.

distinguished from main endotherm at 75°C in Fig.3, freedom of molecular motion freezes up [32] on the LNT at low temperature of 5°C. The phase transition in the melting of the LNT agrees with the results of CD signal. Fig.4 showed that CD activity is gradually changing till the melting point of LNT in correspondence with glass transition, then disappeared at the point of main endothermic event. It is suggested that there are glassy LNTs in cooled condition at lower temperature.

In conclusion, it is suggested that the glycolipid nanotubes from **1** formed through two-step phase transition indicating the existence of metastable phase, for example rippled phase. The glycolipid nanotubes cooled in water were in a glassy state. It is important to control the metastable phase to guarantee the quality and length of LNT for the various applications of the LNT.

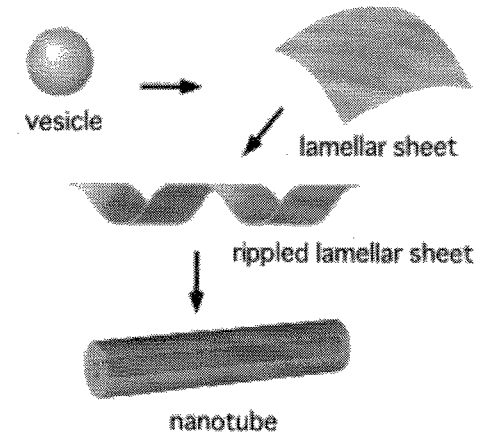


Fig.5 Schematic illustration, that is presumable model based on this research, for the phase transition in a series of tube-forming process of **1**.

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