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Elastic precursor of the transformation from glycolipid nanotube to vesicle

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Abstract

Using a combination of manipulation with optical tweezers and digital video microscopy, the flexural rigidity of single glycolipid 'nano' tubes has been measured below the transition temperature at which the lipid tubules are transformed into vesicles. Consequently, we have found a clear reduction in the rigidity before the transition as temperature is increasing. Further experiments using infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) have suggested a microscopic change of the tube walls, synchronizing with the precursory softening of the nanotubes.

1. Introduction

One of the most promising supramolecular structures is the lipid tubule, a spontaneous aggregate of chiral and amphiphilic molecules [1–3]. The structure is a hollow cylinder made of a lipid bilayer, and the typical diameter is of the sub-μm to μm scale. Recently, it has also been found that a variety of glycolipids form fine tubules with diameters of tens of nanometres [4]. These lipid tubules, unlike carbon nanotubes, have the unique characteristic that both sides of the lipid-bilayer walls, internal and external, are hydrophilic. Therefore, the quasi-one-dimensional nanopore of the glycolipid tubule has the potential for chemical reactions, the transfer of biomolecules and so on. Partly motivated by these fascinating possibilities, the spontaneous formation of lipid tubules has been studied both experimentally [5–12] and theoretically [13–15], and it is now possible to tune their morphology [16–18].

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Cellular microtubules, on the other hand, have similarities to lipid nanotubes in that the diameter is of the same order and that the inner space is hydrophilic, since they are made of proteins. The microtubules play the role of the cytoskeleton or flagellum, suggesting another application for both microtubules and lipid nanotubes: they are regarded as elastic nanorods, and are good candidates for the components of micro- or nanostructured materials. Accordingly, it is important to study fabrication mechanics, i.e. the mechanical properties of the single nanotubes, as well as to investigate the self-assembly mechanism.

The stiffness of the microtubule and cytoskeleton has been measured using various techniques [19–25]; for example, recent results using atomic force microscopy (AFM) have shown that the rigidity of microtubules decreases with increasing temperature [25], corresponding to a certain change in the protein wall. By contrast, however, there have been few experiments on the elasticity of the lipid nanotubes except for the preliminary result by some of the present authors [26]. We then performed rigidity measurements using optical tweezers, precisely varying the temperature below the tube–vesicle transformation. Further experiments, using Fourier-transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC), were also carried out from a perspective of microscopic structure. Here we will report the new finding that the single glycolipid nanotubes exhibit a precursory reduction of their rigidity before transforming to vesicles.

2. Material

The lipid tubules used here consist of a renewable-resource-based synthetic glycolipid (cardanyl-β-D-glucopyranoside). We prepared the tubules through self-assembly of the lipids under mild conditions as follows: the powdery sample was dissolved in water by vortexing at 100 °C for 30 min (about 3×10^{-4} wt%). Then 1 week of incubation at room temperature was sufficient to obtain supramolecular assemblies which maintained the nanotube structure for years. It was found from proton NMR analysis (JEOL LA 600 NMR spectrometer, 600 MHz, CDCl3) of the tubes that the lipid contains different unsaturations of the alkyl chains: saturated (7%), monoene (80%), diene (12%) and triene (1%). We have found from the transmission electron microscopy (TEM) images that the outer and inner diameters of the tubules are 50 and 10 nm, respectively, with narrow dispersion (i.e they are nanotubes) [26].

The structural transition temperature was determined by the dependence on incubation temperature of the ability to form a tube and the tube to vesicle transition. We counted, using differential interference microscopy, the number of nanotubes in a fixed volume after 1 week of incubation keeping the temperature between 24 and 42 °C, as shown in figure 1. This indicates that the transformation temperature is located between 36 and 38 °C, which is consistent with the observation of the transformation from nanotubes to vesicles when raising the temperature of the nanotube dispersion.

3. Methods

3.1. Rigidity measurement

To measure the flexural rigidity of the single lipid nanotubes, we used the optical tweezers system (Sigma Koki LMS-46755). The process consisted of three steps.

First, we placed a drop of the aqueous dispersion of the lipid nanotube on a glass slide, then enclosed it with a coverslip and resin. The concentration used was the same as that in the sample preparation. We have confirmed that nanotubes are completely isolated and form no bundles in the density [26]. The sample was somewhat pressed between the coverslip and the slide.

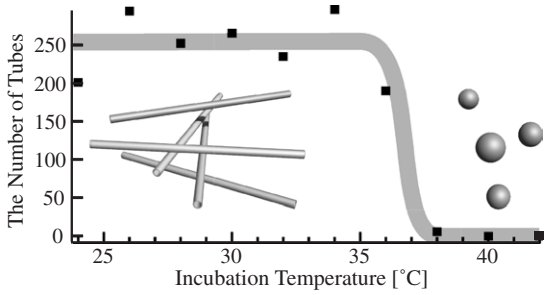

Figure 1. Incubation temperature versus the number of tubes which were counted and averaged in a specified area after 1 week of incubation, using differential interference microscopy. The grey line is to guide the eye.

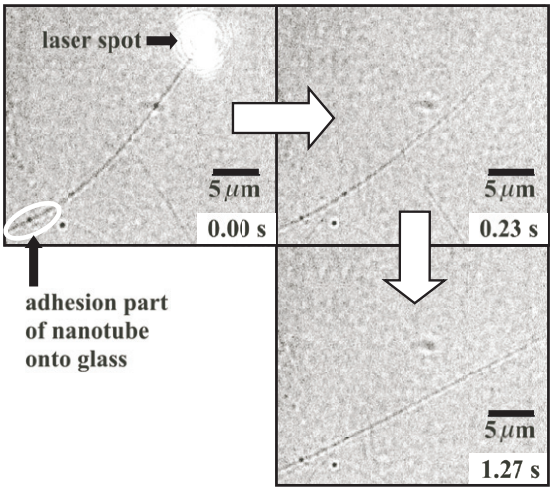

Figure 2. Pictures from bow shape to the initial straight form of a single nanotube. The temperature was fixed at 27 °C.

As a result, some nanotubes adhered firmly to the glass surface, especially at their ends, and it became easy to find nanotubes fixed at only one end. Next, a nanotube that was free at one end was bent similarly to microtubules [23, 24] as follows: using optical tweezers controllable with a spatial resolution of 0.1 μm, we nudged the free end in the direction perpendicular to the long axis of the tube so that the nanotube could be bent to an adequate angle, a few tens of degrees. Finally, the laser beam was switched off after the bending process, and correspondingly the bow-shaped nanotube started relaxing to its initial straight form (see figure 2). The relaxation process was recorded as a movie at a rate of 30 frames s^{-1}.

In figure 3, a typical result of the shape relaxation is shown as the time dependence of $y(l, t)$, where y is the ordinate perpendicular to the initial straight line, and l is the contour length from the adhesion point to the trapped point; $l = 23$ μm in the present case. The solid line in figure 3 represents the curve fitted by the following single exponential:

$$y(l, t) = y(l, 0) \exp(-t/\tau) \tag{1}$$

$$\tau = \frac{\pi \eta L^4}{60K \ln(L/2d)} \left\{ \left(\frac{l}{L}\right)^5 - 10\left(\frac{l}{L}\right)^3 + 20\left(\frac{l}{L}\right)^2 \right\}, \tag{2}$$

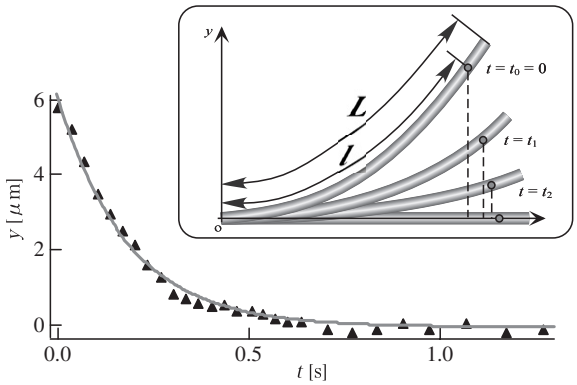

Figure 3. The relaxation data analysed on figure 2. We plot by the triangles a time series of ordinates defined in the schematic of the inset. The the solid line represents the best fitting result using equations (1) and (2). In this case, evaluating τ and K gives 0.23 (s) and 1.3 (10^{-22} N m^2) when $l = 22.6$ μm, $L = 26$ μm and $d = 50$ nm.

where τ is the relaxation time, η is the viscosity of surrounding water, K is the flexural rigidity, L is the contour length between the fixed point and the free end, and d is the outer diameter of the present nanotube. The above equations come from the balance condition between the elastic and hydrodynamic force [24].

We take the parameter d equal to 50 nm, a typical value for the nanotube [4], in the rigidity analyses, since the variation of tube diameter is irrelevant to the rigidity evaluation based on equations (1) and (2); even doubling or reduction by half of the diameter changes the stiffness by about 10%. The contour length L, on the other hand, was evaluated within an accuracy of 0.5 μm for each temperature, because the time series of pictures in the movie of structural relaxation determined definitely the locations of both the fixed point, working as a fulcrum, and the other free end. It was also confirmed that the contour length was invariant with change in temperature.

The rigidity K was obtained from the fitting result in the temperature range of 20–30 °C. We raised the temperature of the aqueous dispersion of the nanotubes from the lower end to the other, keeping the rate at 0.1 °C min^{-1} within an accuracy of 0.1 °C throughout. The measuring time at each temperature was so short that the temperature change during each movie acquisition was negligible.

3.2. FT-IR

We investigated the temperature dependence of FT-IR spectra for the aqueous dispersion of nanotubes, using a spectrometer (SHIMAZU FTIR-8700) with an ATR-type cell (DuraSampl IR II). The temperature was controlled within 0.1 °C and varied in the same range as that for the elasticity measurements. The measurements were isothermally performed at every 1.0 degree in the temperature range, and the heating rate was 0.1 °C min^{-1}, equal to that in the rigidity measurements. To get a sufficient signal intensity and wavenumber resolution, we raised the sample concentration by osmotic compression to 10 times as high as that used in the rigidity measurements and obtained a resolution of 3.85 cm^{-1}. Even after the condensation, optical microscopy confirmed that the nanotubes were still present.

3.3. DSC

We also performed thermoanalysis using a differential scanning calorimeter (Seiko Instruments DSC6100). The temperature was changed in the range of 20–40 °C at a heating rate of

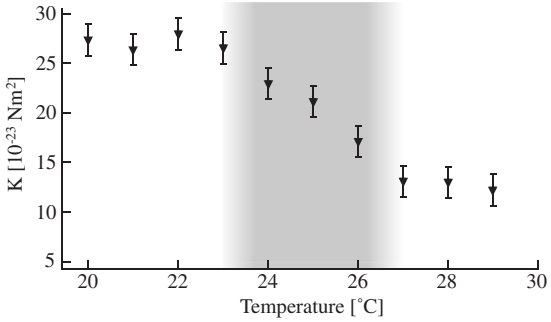

Figure 4. Temperature dependence of the flexural rigidity K. The triangles represent the average, and the error bars were estimated from measurement variations and the resolution of the movies. The filled zone marks the temperature range where stiffness is decreasing.

1.0 °C min^{-1}. In order to obtain a sufficient DSC signal intensity, the concentration of the lipid solution was required to be more than 5 wt%, which is too high for nanotubes to keep their supramolecular structure. This measurement, therefore, was performed for simple solution of the lipid and does not reflect the supramolecular structure of the lipid nanotubes but the nature of the lipid molecules themselves.

4. Results and discussions

4.1. Rigidity

Figure 4 shows the temperature dependence of the flexural rigidity, indicating that the stiffness drops off in the range 23–27 °C with increasing temperature, and the ratio of the minimum to maximum is about half, though we observed no sign of the macroscopic transformation. The results for different nanotubes were reproducible within the error bars. This supports the assumption that the friction due to the glass slides, which should vary considerably as a result of the tube arrangement on the prepared slide, is much less than the viscosity of water. It should also be noted that the above variations of both the tube diameter d and tube length L lead to deviations within the error bars.

The FT-IR and DSC measurements provided meaningful results for the investigation of whether this precursory softening before the tube–vesicle transformation is a generic phenomenon reflecting a change of microscopic structure.

4.2. FT-IR

Figure 5 represents the IR spectra upon raising the temperature. As is clearly shown in the figure, the absorption band around 1460 cm^{-1} changes its shape from twin peaks to a single one between the spectra at 26 and 27 °C where the reduction of elasticity also occurs as mentioned above. Up to now it has been found that this absorption band arises as a result of the scissoring motion of the CH_2 unit in hydrocarbon chains and is sensitive to the molecular in-plane packing of lipid bilayers [27]. The twin- to single-peak change has also been reported for the lamellar structure of other lipids when temperature increased [28]; in this case, while the twin-peak spectrum is due to the tight packing of lipids in the crystal phase, the single-peak is associated with the gel phase. The previous reports suggest that the present result is ascribable to a microscopic change in the tube walls inducing softening of the nanotube.

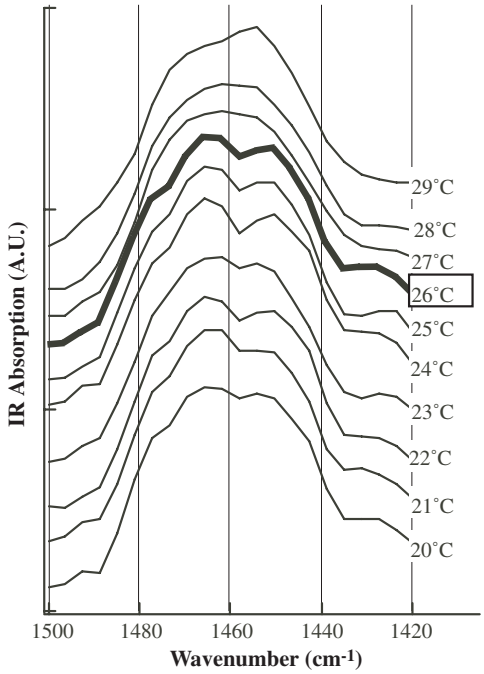

Figure 5. Temperature dependence of FT-IR spectra. For visual clarity, each spectrum is vertically shifted at even intervals of temperature. The spectrum at 26 °C is emphasized by a thicker line, because the peak shape changes around here.

4.3. DSC

It is found from figure 6 that there is an endothermic peak around 25.5 °C where the tube stiffness has started to decrease as shown in figure 4. This result indicates that nanotube softening and the change in the molecular packing in the tube walls are based on a thermodynamic phenomenon. Also, the DSC peak is broad which is similar to the gradual softening in figure 4. Although the present DSC result is obtained from condensed solutions of the glycolipids without forming nanotubes as described in section 3.3, the blunt thermo-anomaly below the tubule–vesicle transformation temperature has also been reported in a similar way for other lipid tubules with large diameters [29].

5. Concluding remarks

We have thus unveiled the key temperature located at around 26 °C where the reduction in stiffness (see figure 4), the change in the IR spectrum in the 1460 cm^{-1} band (see figure 5) and the endothermic peak (see figure 6) have been observed synchronously for the glycolipid nanotubes. Combining all the results suggests that the softening of the lipid nanotubes is linked with the microscopic change of constituent lipids. It is then reasonable to say that the gentle decrease in the elasticity seen in figure 4 is due to the broad endothermic peak in figure 5. The present unsharpness may be explained by considering that the sample is a mixture of different types of alkyl chains as mentioned above. That is, although each pure lipid has a fast equilibration time, the changes are smeared and appear to be gradual due to the averaging of mixed lipids.

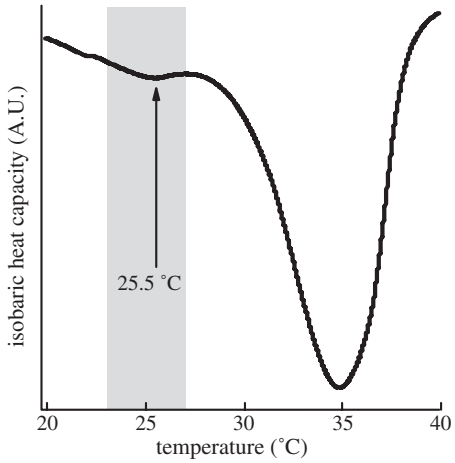

Figure 6. DSC result for the lipid solution, which indicates some thermodynamic change in the single lipid molecules around 25.5 °C. The filled zone marks the temperature range where the stiffness is decreasing (see figure 4).

To summarize, we investigated the temperature dependence of nanotubes made of the cardanol glycolipid below the tube–vesicle transformation temperature. Elasticity measurements have revealed a precursory phenomenon of supramolecular structure transformation in the lipid nanotube system. That is, a decrease of the stiffness with increasing temperature while the lipids maintain the tubule structure. The results of FT-IR and DSC further suggest that the softening is associated with a microscopic change in the lipid wall which we are unable to detect using optical microscopy.

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