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COLLOIDS AND SURFACES A

Shape transformations of giant unilamellar vesicles induced by ethanol and temperature variations

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Abstract

The shape transformations of giant unilamellar vesicles (GUVs) of dimyristoylphosphatidylcholine (DMPC) in water/ethanol solutions were investigated as a function of temperature (18–43°C) and ethanol content (0–40 vol%). GUVs were prepared by electroformation and their shapes were monitored by phase-contrast microscopy furnished with an image-recording set-up. Two systems were compared: (1) GUVs formed in presence of ethanol; and (2) GUVs preformed in distilled water with ethanol added subsequently. In the first case, GUVs in the gel state are typified by a multifaceted, irregular shape that transforms into a smooth, rounded shape with increase of temperature. This transformation is reversible and takes place at about or below 24°C. It coincides in temperature with the DMPC gel–liquid crystalline phase transition in the respective water/ethanol solutions. The effect of ethanol added to GUVs preformed in distilled water is strikingly different. The temperature of the multifaceted(folded)-to-smooth shape transformation first increases from 24 to 37°C with increase of ethanol content up to 10 vol%, then decreases back to 24°C upon further increase of ethanol up to 40 vol%. The reversal point of this biphasic effect at 10 vol% of ethanol coincides with that for formation of DMPC interdigitated phase. The shape transformations are reversible with a temperature hysteresis of 2–3°C. They are rather cooperative and take place in a narrow range of less than 0.2°C at temperatures that remain constant for times of up to 15 h.

Keywords: Interdigitation; Giant vesicles; DMPC; Ethanol; Shape transformations

1. Introduction

The influence of ethanol on the properties of phospholipid bilayers has been the subject of numerous studies. In saturated phosphatidylcholines, ethanol induces interdigitation of the lamellar gel phase at threshold concentrations that decrease with the lipid chain length [1]. The interdigitation is associated with a biphasic effect on the gel–liquid crystalline phase transition of the lipid multilayers. The temperature of this transition first decreases slightly, and then increases when the ethanol concentration exceeds the threshold concentration. For dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC), the interdigitation threshold concentrations are at 10.7 and 6.3 vol% of ethanol, respectively [2]. Higher ethanol contents of about 45–50 vol% are known to strongly facilitate the formation of black foam films from dispersions of DMPC, DPPC and phospholipid mixtures [3–5]. Although a characterization of these effects is also of interest, most of the experimental studies on

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bulk lipid/water/ethanol systems have been limited to ethanol concentrations around the interdigitation threshold. Only recently have X-ray and calorimetric measurements at high ethanol contents been carried out [6].

The giant unilamellar vesicles (GUVs) represent a convenient tool for studies of the influence of low-molecular-mass additives on the membrane properties. With sizes of the order of 50 μm, they are easily observed by phase-contrast microscopy. By using GUVs prepared from DMPC by electroformation, we have undertaken a detailed investigation of the effects of ethanol on vesicle shape and stability at temperatures below and above the chain-melting phase transition. In the present work we compare the properties of (1) GUVs prepared in the presence of ethanol and (2) GUVs preformed in distilled water with subsequent addition of ethanol, and observe a significant difference between the shape-transformation temperatures of these two systems.

2. Materials and methods

Giant unilamellar vesicles of 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC), Fluka #41803, were prepared by the liposome electroformation method [7–9]. The particular electroformation protocol established in this work was as follows. A 1 μl droplet of DMPC solution in diethyl ether/methanol 9:1, 0.25 mg/ml, was deposited on each of two parallel platinum wires (diameter 0.8 mm, distance between axes 3 mm) and dried under nitrogen for 30 min. An AC field (10 Hz, 1 Vpp volts peak-to-peak) was applied and distilled water or ethanol/water solution was added at 18°C (the main phase transition of DMPC in water is at 23.8°C). The temperature and AC field voltage were increased continuously for about 30 min up to 43°C and 7 Vpp, respectively. After 2 h, the AC frequency was lowered down to 0.5 Hz at 3 Vpp, and then electroformation was terminated by switching off the AC field, with a yield of several tens of GUV observable under the microscope. Their typical diameters were in the range 40–80 μm for vesicles prepared in water, and 5–30 μm for vesicles prepared in water/ethanol solutions.

Vesicle formation and shape changes were observed with a phase-contrast microscope (Zeiss, Axiovert 135), equipped with a Hamamatsu CCD camera (C5985) and connected to an image-recording system. Temperature was controlled with a Peltier microscope stage and measured with a thermocouple placed at about 100 μm from the observation locus.

3. Experimental results

Two kinds of GUV were compared in the present study: (1) GUVs prepared in water/ethanol solutions and (2) GUVs preformed in distilled water with subsequent addition of ethanol. The ethanol content was varied in the range 0–40 vol%. At higher ethanol contents (50 vol%), the lipid bilayers disappear (dissolve) upon separation from the electrode during the electroformation process. The temperature was varied in the range 18–43°C. In both systems, the lipid membranes in the presence of ethanol and below the "shape-melting" temperature become more sticky — they adhere to each other, to the glass walls, and, at higher ethanol contents, they may fold upon themselves thus creating stable foldings on the GUV surface. This effect agrees well with the observed enhancement of phosphatidylcholine vesicle aggregation in the presence of ethanol [10,11].

3.1. Shape transformations in GUVs prepared in water/ethanol solutions

The solid line in Fig. 1 shows the temperatures at which GUVs start to grow upon increasing the temperature in the respective ethanol solutions. This line practically coincides with the phase line of the chain-melting transition as determined by differential scanning calorimetry (DSC) for DMPC multilamellar dispersions in water/ethanol solutions. At the same temperatures, vesicle shape change takes place. From an irregular (multifaceted) shape at low temperatures similar to that in Fig. 2(c)–(d), the GUVs transform into smooth, rounded vesicles like those in Fig. 2(a). The "shape melting" of GUVs is reversible with a small tem-

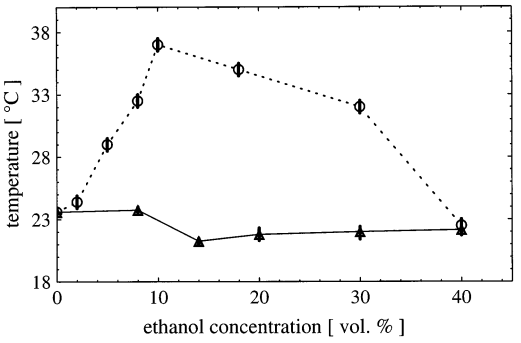

Fig. 1. The solid line (triangles) shows the shape-melting temperatures of GUV prepared in water/ethanol solutions. GUV start to grow at the same temperatures during their electroformation. The dotted line (circles) shows the shape-melting temperatures of GUV prepared in distilled water with ethanol added subsequently.

perature hysteresis, as shown by sequences of up to four successive heating–cooling cycles.

3.2. *Effects of ethanol addition to GUVs preformed in distilled water*

Irregular-to-smooth shape transformations also take place in GUVs preformed in distilled water with subsequently added ethanol, but at significantly higher temperatures. In these experiments, GUVs preformed at 43°C (Fig. 2(a)) were cooled down to 18°C at 0.2°C min^{-1}. Cooling through the DMPC main phase transition does not affect the spherical shape of these vesicles, if hydrodynamic or mechanical stresses are avoided, but lets them dissociate from each other and from the electrode surface where they are formed (Fig. 2(b)). Then ethanol solution was added slowly (over about an hour) with use of a microscrew-driven microsyringe so as to avoid mechanical perturbations of the suspension. After reaching the desired ethanol concentration, the system was left to equilibrate at 18°C for 1 h prior to the measurements. On the basis of phase-contrast observations, we believe that ethanol equilibrates between the external volume and the vesicle interior within 5 to 6 min. Adding ethanol at 18°C transforms the smooth, spherical GUV shapes mostly into multifaceted, irregular shapes in the range below 10 vol% of ethanol (Fig. 2(c)), and mostly into membrane shapes folded upon themselves at ethanol amounts above 15 vol% (Fig. 2(d)). The "shape-melting" line for these GUVs is given as the dotted line in Fig. 1. Addition of up to 10 vol% ethanol results in a steep, close-to-linear increase of the shape-melting temperature from 24°C up to 36–37°C. Further increase of ethanol up to 40 vol% results in a decrease of this temperature back to the initial temperature of 24°C. It is noteworthy that the reversal point of this biphasic effect coincides with the threshold ethanol concentration of 10.7 vol% required for DMPC gel phase interdigitation [2]. The shape-melting transformations are reversible with temperature at a scan rate of 0.2°C min^{-1}. Their temperature remains unaltered for times of up to 15 h. They are rather cooperative and take place in a narrow temperature range of less than 0.2°C.

The position of the dotted line in Fig. 1 was confirmed by the following set of experiments. GUVs preformed at 43°C were cooled down to 30°C or 26°C, without crossing the DMPC main transition temperature, and equilibrated at the respective temperature. Ethanol was added until a smooth-to-irregular shape change took place. At both temperatures noted above, the shape transformations took place upon crossing the dotted line in Fig. 1. It thus turns out that the position of the dotted phase line in Fig. 1 can be determined either by increasing the temperature at constant ethanol content, or by increasing the ethanol content at constant temperature.

4. Discussion

The present data demonstrate that the influence of ethanol on the GUV shape transformations depends strongly on whether ethanol has been added prior to or after GUV electroformation. In the first case, the shape melting coincides in temperature with the gel–liquid crystalline phase transition in DMPC multilamellar dispersions prepared at the respective water/ethanol ratios (solid line in Fig. 1), while in the second case it takes place at significantly higher temperatures (dotted line in Fig. 1). Since GUVs preformed in water with subsequently added ethanol represent an initially

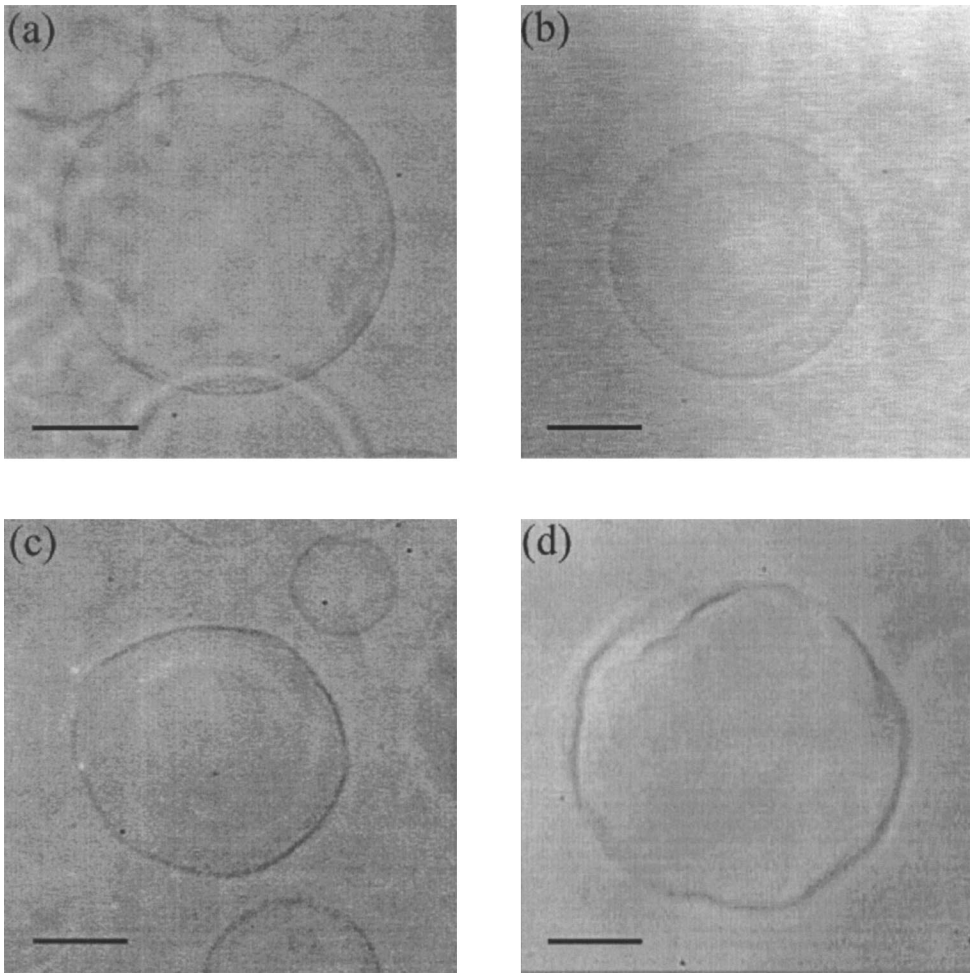

Fig. 2. (a) DMPC vesicles prepared in distilled water at 43°C, this picture also represents the equilibrium "molten" shapes of GUV prepared in water/ethanol solutions; (b) DMPC vesicles in distilled water cooled to 18°C; (c) DMPC vesicles prepared in distilled water with 8 vol% of ethanol added subsequently at 18°C; (d) DMPC vesicles prepared in distilled water with 18 vol% of ethanol added subsequently at 18°C. Scale bar is equal to 20 μm.

non-equilibrium system, one may expect that its properties will gradually relax and approach the properties of GUV suspensions prepared in water/ethanol solutions. To check for such possibility, we carried out measurements for times of up to 15 h after the addition of ethanol, but observed no shifts in the position of the dotted line towards the direction of the solid line in Fig. 1. Since we expect that ethanol equilibrates between the external volume and GUV interiors on the scale of minutes, it thus appears that the observed difference in shape-melting temperatures is not a direct consequence of a non-equilibrium ethanol distribution in the GUV suspension.

Because of the very low bulk lipid concentrations (less than 1 μg per ml), it is not possible to determine directly by calorimetry the position of the gel–liquid crystalline phase transition in GUV suspensions preformed in distilled water with subsequently added ethanol. It is therefore unclear whether the position of the dotted line in Fig. 1 also corresponds to chain melting in these GUVs (experiments to resolve this problem are currently in progress). If we assume that GUVs electro-

formed in distilled water consist of several concentric bilayers separated by equilibrium aqueous layers, addition of ethanol might then be expected to modify both the interbilayer spacings and the surface areas of the lipid molecules. In turn, this would result in certain area–volume disproportionations that would not be present in GUVs formed in water/ethanol solutions. Such disproportionations would have rather long relaxation times and might serve as a possible cause for the observed different behaviour of the two kinds of GUV suspensions.

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