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Myelin-like protrusions of giant phospholipid vesicles prepared by electroformation

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Abstract

Quasistable shapes of phospholipid bilayer vesicles obtained by formation in an alternating electric field [M.I. Angelova, S. Soleau, Ph. Méléard, J.F. Faucon, P. Bothorel, Prog. Colloid Polym. Sci. 89 (1992) 127; V. Heinrich, R.E. Waugh, Ann. Biomed. Eng. 24 (1996) 595] are observed. The vesicles appearing as composed of a mother sphere and a thin tubular myelin-like protrusion, are found to be a common phase in the spontaneous slow shape transformation that yields giant fluctuating phospholipid vesicles of different shapes. In the shape transformation, the myelin-like protrusion, which acts as a reservoir for the membrane area, is integrated into the mother vesicle.

Keywords: Electroformation; Giant phospholipid vesicles; Myelin-like protrusions

1. Introduction

Thin tubular structures were observed in phospholipid systems [1,2], in erythrocytes [3–6], cells [7–9] and surfactant systems [10–15]. Understanding the physical properties of thin tubular structures is of interest in medicine and technology. Therefore, the study of these structures has become a subject of increasing interest and is also the scope of this work.

It was previously observed that the giant phospholipid vesicles prepared by electroformation [16] are spherical and connected by thin tubular structures [1]. There are numerous studies of flaccid fluctuating vesicles (for a review, see Ref. [17]). The aim of this work is to investigate the linking process that yields the fluctuating vesicles out of the spherical vesicles connected by thin tubular structures. This transformation that involves shapes with myelin-like protrusion(s) has, to our knowledge, not yet been reported.

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2. Experiment

The phospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids, Inc. The fluorescent phospholipid probe [2-(12-(7-nitrobenz-2-oxa-(1,3-diazol-4-yl)amino) dodecanoyl-1-hexadecanoyl-sn-glycero-3-phosphocholine)] (NBD-PC) was purchased from Molecular Probes, Inc.. Giant phospholipid vesicles were made from POPC and also from the mixture of POPC and 1.5% NBD-PC by the modified method of electroformation [16], as described in Ref. [18]. All the described features regarding the shapes of the vesicles were the same in the system with, and in the system without, the fluorescent probe.

The experiment was performed at room temperature. In the procedure, 20 μl of phospholipid (or mixture of phospholipid and fluorescent probe), dissolved in 2:1 chloroform/methanol mixture, were applied to a pair of Pt electrodes. The solvent was allowed to evaporate in low vacuum for 2 h. The electrodes were placed 4 mm apart in the electroformation chamber that was filled with 2 ml of 0.2 M sucrose solution. An a.c. electric field (1 V/mm, frequency 10 Hz) was applied for 2 h. Then, the a.c. field was reduced to 0.75 V/mm, 5 Hz, applied for 15 min, to 0.5 V/mm, 2 Hz, applied for 15 min, and to 0.25 V/mm, 1 Hz, applied for 30 min.

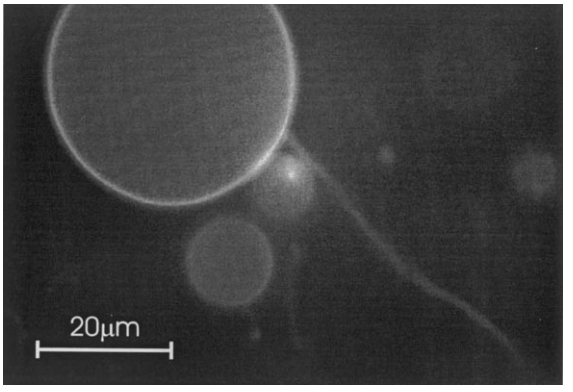

Fig. 1. Fluorescence microscope image of the vesicle made of POPC and 1.5% NBD-PC. The length of the myelin-like protrusion was several diameters of the spherical part. The fluorescence measurements were done using the inverted optical microscope (IMT-2, Olympus, Japan), using the reflected light fluorescence attachment IMT2-RFL. The dichroic mirror unit (IMT-DMB) allowed excitation from 380 to 490 nm and observation of the fluorescence at the wavelengths higher than 515 nm.

The contents of the chamber were poured out into a plastic beaker. The chamber was then filled with 2 ml of 0.2 M glucose solution that contained no phospholipid. This solution was also poured out, adding to the solution that was already in the plastic beaker. The contents of the plastic beaker were gently mixed by turning the beaker upside down.

Immediately after the preparation, the solution containing the vesicles was placed into the observation chamber made by a pair of cover glass and sealed by grease. The vesicles were observed by the phase contrast microscope and by the fluorescence microscope.

Immediately after being placed into the observation chamber, the vesicles appeared spherical and had different sizes. Neither the myelin-like protrusions nor the long-wavelength shape fluctuations were visible. The short-wavelength shape fluctuations were barely visible. After a certain period of time (about half an hour), long, thin myelin-like protrusions became visible under the fluorescence microscope (Fig. 1) and later also under the phase contrast microscope. Usually, when recognized, the myelin-like shapes appeared as very thin, long tubes connected to the vesicle surface at one end, while the movement of the myelin-like shapes indicated that they are otherwise free.

While one vesicle was chosen and followed for several hours, the sample was browsed from time to time in order to observe the changes in the population.

A typical time course of the shape transformation can be seen in Fig. 2. The sequence started from a spherical mother vesicle with a long, thin myelin-like protrusion that appeared as a cylinder (A). By the time the myelin-like protrusion thickened and shortened (B, C), the undulations of the cylinder became noticeable and more pronounced. The mother vesicle remained more or less spherical. In the shortened myelin-like protrusion, the necks seemed to persist while exhibiting oscillations in their width. The final phases of the pro-

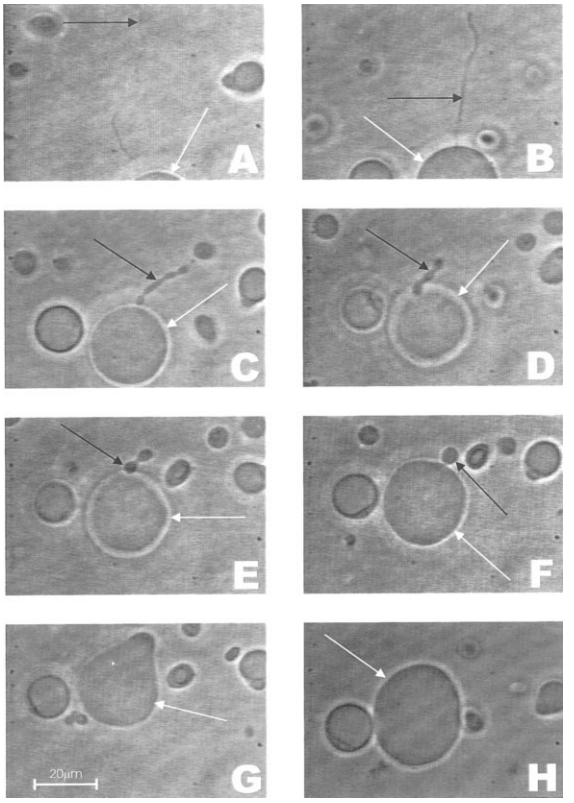

Fig. 2. Shape transformation of the giant phospholipid vesicle (made of POPC and 1.5% NBD-PC) with time. The times after the preparation of the vesicles are A: 3 h, B: 3 h 20 min, C: 4 h, D: 4 h 2 min, E: 4 h 4 min 30 s, F: 4 h 8 min 15 s, G: 4 h 14 min 25 s, H: 4 h 14 min 30 s. The black arrows indicate the protrusion, while the white arrows indicate the mother vesicle. The vesicles were observed under the inverted Zeiss IM 35 microscope with the phase-contrast optics.

cess look like stepwise integration of the beads into the mother vesicle (D, E, F). Finally, the neck of the only remaining daughter vesicle opened (G), yielding a globular vesicle (H). Before opening, the neck widened and shrunk several times. The subsequent transformation of the vesicle into the pear shape and further into the prolate shape was completed in seconds.

The long-wavelength fluctuations of the mother vesicle increased with shortening of the myelin-like protrusion and became vigorous when the myelin-like protrusion was completely incorporated into the membrane of the mother vesicle.

3. Discussion

It was previously indicated [1,15] that after the preparation by electroformation, the giant vesicles were connected by a network of thin myelin-like structures that were difficult to visualize and were also very fragile. Accordingly, it can be speculated that the myelin-like protrusions observed in our experiments are remnants of the myelin-like structure network created during the vesicle formation phase in the electroformation chamber, which is partially torn when the vesicles are flushed from the chamber. In the sample, we also observed a small amount of closed vesicles with a very low volume-to-area ratio that may have developed from the detached myelin-like structures (not shown).

As the vesicles were flushed into the observation chamber by the solution that contained no phospholipid molecules, the inequality of the chemical potential of the phospholipid molecules in the vesicle membrane and in the solution may cause a slow but continuous loss of the phospholipid molecules from the outer membrane layer into the solution and consequently a slow shape transformation. The transformation may also be promoted by the drag of the lipid from the outer solution of the vesicles by the glass walls of the chamber, by slight evaporation of the liquid caused by imperfect sealing of the chamber by the grease, by chemical modification of the lipids and by the phospholipid flip-flop [19,20].

It was established [21] that the difference between the outer membrane layer area and the inner membrane layer area, $\triangle A$, is a geometrical parameter that characterizes the shape, while the corresponding area difference of the unstressed membrane layers $\triangle A_0$, which is determined by the number of the molecules constituting the outer and the inner layer, respectively, importantly influences the equilibrium shape. The shapes that have evaginations have a large area difference, $\triangle A$, while the shapes that have invaginations have a small area difference, $\triangle A$. It can be seen in Figs. 1 and 2 that after the formation the observed vesicles have a large area difference, $\triangle A$, due to a long, thin evagination, which indicates that the area difference of the unstressed

layers, $\triangle A_0$, is large as well. In theoretical studies, until recently, attention has been focused mostly on the shapes with smaller values of $\triangle A$ and $\triangle A_0$, e.g. the shapes that generally appear globular [21–25]. Shapes with a large area difference were studied within the tether pulling experiment [26], but these shapes are subject to an external force acting upon the membrane. A theoretical description of the shapes reported in this work would be an interesting task in future work.

In conclusion, we have observed that when prepared by electroformation, the vesicles usually exhibit long, thin myelin-like protrusions that could be stable for many hours. The large fluctuating globular vesicles are formed by the integration of the myelin-like protrusion into the mother vesicle.

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