Spontaneous Formation of Giant Liposomes from Neutral Phospholipids

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Giant liposomes were formed spontaneously from neutral phospholipids in an aqueous solution containing $MgCl_2$. Based on both dark-field microscopy and transmission electron microscopy, we concluded that these giant liposomes are unilamellar.

It has been well established that phospholipids are the main constituent of biomembranes. Currently, the most popular method for preparing a unilamellar bilayer membrane with a closed vesicular structure is to use sonication.[1] With this method, small spherical vesicles on the order of several ten's of nanometers in size are obtained, which indicates that the curvature of the membrane is much greater than that of a natural biomembrane. To prepare giant liposomes on the order of several micrometers in size, several techniques have been reported, including reversed phase evaporation,[2] solvent vaporization,[3] preparation with detergent,[4] fusion with Ca^{2+} for negatively charged lipid,[5] swelling with water of almost zero ionic strength,[6,7] swelling of charged lipid with an aqueous solution,[8] and combinations of the above techniques. To prepare or reconstitute liposomes with various enzymes and/or chemical species that are unstable with an organic solvent, it is desirable to establish a method which does not require an organic solvent. It would also be desirable to prepare liposomes with neutral phospholipids in the presence of a small amount of electrolytes. None of the above-mentioned techniques satisfies these needs. This letter describes a novel method for preparing giant liposomes of neutral phospholipids in an aqueous electrolyte solution without the use of an organic solvent.

Neutral phospholipids were obtained from Sigma, and included soybean phosphatidylcholine (soybean PC) and dioleoylphosphatidyl-choline (DOPC). These lipids were dissolved in pure methanol or in a 1 : 1 (v : v) chloroform-methanol mixture. Next, 0.1 μmol of dry lipid film was prepared by evaporation of the organic solvent in a 10-mm ϕ test tube under a nitrogen stream, and then stored for at least 2 h under aspiration. The resulting lipid film was swollen with 100 μl of TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH adjusted to 8.0 by HCl) or aqueous solution containing $MgCl_2$, where the final concentration of phospholipid was 1 mM. As a control experiment, we also prepared giant unilamellar vesicles by the ether vaporization method.[3] Diethylether solution of 2 mM DOPC was injected at a rate of ca. 0.2 ml/min into a buffer solution (same as above) at 55 °C using a glass syringe. The ether was removed under reduced pressure for one hour.

A dark-field microscope (Optiphoto 2, Nikon, Japan) with 100-W high-pressure Hg lamp as a light source was used to observe the liposomes. The images were recorded with a SIT camera (Hamamatsu Photonics, Japan) and further processed with an image processor (Argus 50, Hamamatsu Photonics, Japan). Transmission electron microscopic observation was performed by negative staining using a JEM1200EX (JEOL) operated at an acceleration voltage of 100 kV. Carbon-coated 400 Cu mesh grids (EM Shizai) were rendered hydrophilic by glow discharge. The grid was floated on a droplet of sample solution (20 μl) for 2 min, drained on filter paper, and finally stained for 30 s with an aqueous solution of 1% uranyl acetate. All of the experiments were performed at room temperature.

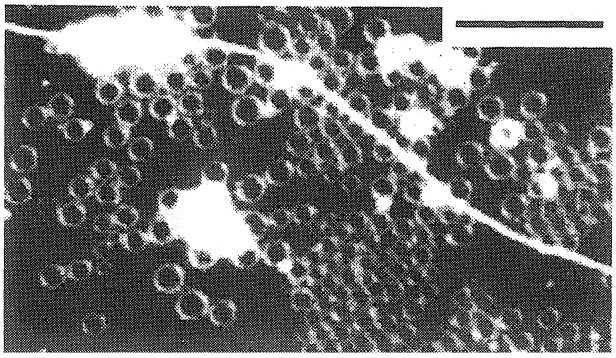

Figure 1. Dark-field micrograph of DOPC liposomes in an aqueous solution of $MgCl_2$. The white (bright) region is due to the aggregate of the lipid. The length of horizontal bar is 10 μm.

Figure 1 shows a dark-field microscopic image of a giant liposome obtained by swelling the dry lipid film in the presence of 1 mM MgCl2. Giant liposomes are formed regardless of whether the solution is distilled water or TBE buffer, with no significant differences in the shape or size of the liposomes. Myelin, tubular, and "pearling"[9] structures were observed simultaneously in addition to spherical liposomes at low concentrations of MgCl2 (< 10 mM, 1 M = 1 mol dm^{-3}), as shown in Figure 1. At an intermediate concentration of MgCl2, spherical giant liposomes and pearling structures were observed. At a high concentration (≈ 100 mM), almost no giant liposomes were observed. Therefore, the most suitable concentration for the formation of giant spherical liposomes was 10 mM. The liposomes were stable at room temperature for at least several days. Essentially the same results were obtained with soybean PC.

Figure 2 shows the light-intensity distribution along a cross-section of a liposome. The intensity profile for vesicles prepared by the present method indicates two sharp maxima at the periphery and almost zero intensity in the central region (a). Essentially the same intensity profile was obtained for the giant unilamellar vesicles prepared by the ether vaporization method (b). Thus, it is likely that the giant liposomes prepared by the present method have a unilamellar structure. Figure 2 also shows the light-intensity profile for a multilamellar vesicle (c), which is markedly different from that for a unilamellar vesicle.

To obtain further information regarding the unilamellar structure of the liposomes formed by the present method, we performed electron microscopic measurements (Figure 3). The electron micrograph shows a unilamellar structure for spherical vesicles similar in size to those observed by dark-field microscopy.

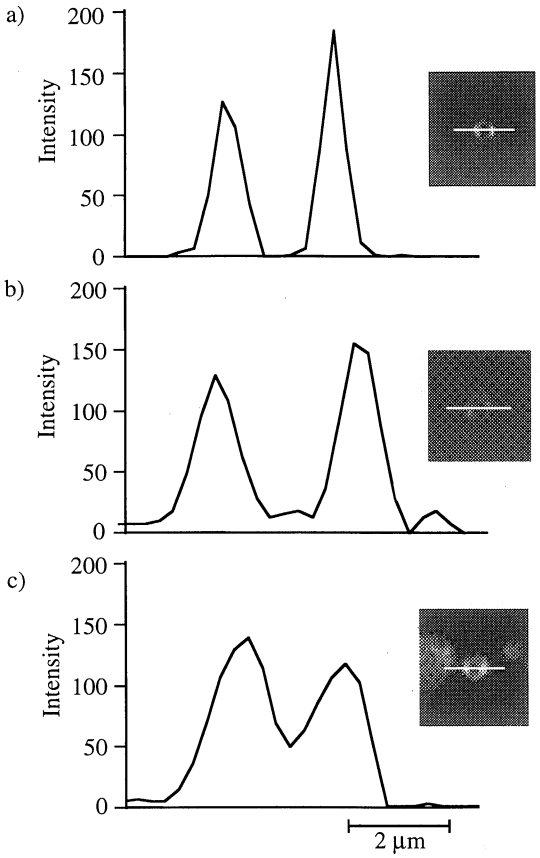

Figure 2. Light-intensity distribution along a cross-section of DOPC liposomes in TBE buffer. a) 10 mM $MgCl_2$ solution, b) ether vaporization method, c) without $MgCl_2$. The vertical scale is arbitrary.

These results suggest that large unilamellar vesicles are formed spontaneously from neutral phospholipids in the presence of magnesium ion. This leads us to speculate on the mechanism of the formation of these unilamellar giant vesicles. It is well known[1] that magnesium ions have a high affinity for the negatively charged head-group in lecithin. This implies that lecithin behaves as a positively charged lipid in the presence of magnesium ion. It has also been reported that charged lipids or a mixture of neutral lecithin with charged lipids spontaneously form unilamellar giant vesicles.[6,10] Therefore, we should address why charged lipids can form giant unilamellar vesicles. In an aqueous environment, lecithin can form a multiple-bilayer, or multilamellar, structure. To generate large liposomes, the interfacial distance between the bilayers should be expanded to on the order of a micrometer. In our method, giant liposomes are prepared even in TBE buffer solution, where the Debye length is calculated to be ca. 0.4 nm. The Coulombic repulsion between the charged bilayers is not sufficient to increase the interlayer spacing to on the order of a micrometer. The effect of the counter ion, Cl^- in our case, may also contribute to increasing the interlayer spacing. With regard to the interlayer space between positively charged bilayers, a large number of chloride ions are condensed to fulfill the condition of electroneutrality. An increase in chloride ions in the interlayer space induces an increase in osmotic pressure.

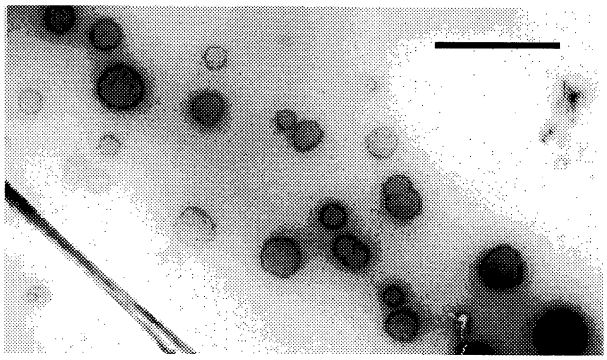

Figure 3. Transmission electron micrograph of liposomes. The length of bar is 2 μm.

Since osmotic pressure has a very long-ranging effect, the interlayer spacing is greatly expanded; i.e., to the cell-size scale. Further physico-chemical study is necessary to confirm this hypothesis.

Miyata and Hotani observed changes in the morphology of giant liposomes accompanied by the polymerization of actin filament within the intervesicular space.[11] They prepared giant liposomes which incorporated actin by the natural swelling of PC in the presence of Ca^{2+} or Mg^{2+}. Recently, it has also been reported that long DNA molecules on the order of 100 kbp are entrapped within a giant liposome of PC,[12] which has been formed by natural swelling in the presence of Mg^{2+}. The present results may provide a physico-chemical background for these previous reports. Since Mg^{2+} ion is very common in the intervesicular space in living cells, the present method should be very useful for future studies on the reconstruction of "artificial cells".

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