Surface effects in preparation of cell-size liposomes

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Received 12 July 1984

Effects of surface type and area were shown to be important in the yield of cell-size liposomes, but not in determining their size. The liposomes were prepared by dissolving lipids in a chloroform-methanol solution and then evaporating the solvent under nitrogen in the presence of glass beads. After evaporation of the solvent, which was rapid due to the increased surface area, the dried lipids were then swollen in water at high temperatures (higher than the phase transition of the lipids), which led to formation of giant liposomes. The number of liposomes prepared in the presence of pyrex glass beads, which increase more than 100-times the surface area of lipid-glass contact, is more than 5-times larger than in the control experiments without glass beads. The yield of liposomes in the presence of another type of glass bead was almost the same as in the control experiments. These effects may be due to long- and short-range intermolecular interactions in the glass/water/lipid system.

Cell-size liposome *Lipid-surface contact* *Lipid bilayer membrane*

1. INTRODUCTION

Liposomes have great potential in membrane research, biotechnology and medicine. A number of methods have been developed for their preparation (review [1]). Recently, a simple procedure for preparation of single bilayer vesicles (liposomes) of cell size (several microns) has been suggested [2]. The method is based on the formation of thin lipid films on glass vessel walls with subsequent swelling at relatively high temperatures (higher than the lipid phase transition temperature).

It seems in this case that the specific intermolecular interactions in the glass/lipid and glass/water/lipid systems are of crucial importance for the rate and yield of liposomes. Here, by changing the surface area (more precisely, the number of lipid molecules, or lipid bilayers, per unit glass surface area) and the type of surface area, we can alter the liposome formation process.

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Two basic steps are involved in preparation of cell-size liposomes by this procedure:

(i) Drying of the lipids, dissolved in a mixture of polar and non-polar solvent. During the evaporation of the solvent, the lipids should adsorb onto the glass surface and form a multilayer structure. The type of this structure will depend on the glass/lipid and lipid/lipid interactions. It may be expected that near the glass surface the lipid layer will have different properties than the lipids far from the glass surface. Two possible cases may occur: (a) the glass/lipid interactions favor the formation of liquid-crystalline or solid layers up to a certain distance from the glass surface and (b) the opposite case. As a result, we may have a large yield of liposomes if in the first case, the amount of lipids per unit glass surface area is reduced to a number of bilayers corresponding to such a structured region. In the second case we can expect that the yield of liposomes will be low, at least if the formation of liposomes is a glass surface mediated process.

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

(ii) Swelling of the lipid in water. This step needs a rather high temperature in order to overcome the attraction between glass/lipid surfaces and lipid bilayers themselves. It may be hypothesized that only the lipids which are arranged in bilayers, i.e., eventually the lipids in the region near the glass surface will form liposomes.

Here, we describe a simple procedure, based on the use of glass beads to change the area and type of surface, which leads to a rapid, and high yield of cell-size liposomes.

2. MATERIALS AND METHODS

L-α-Phosphatidylcholine from frozen egg yolk (Sigma, St. Louis) and glass beads from Corning Glass Works (Corning, New York) (average diameter 5 mm) and Touzart & Matingnon (Paris) (average diameter 0.4 mm) were used. Liposome formation was observed under a phase-contrast microscope. The distribution of vesicle size was measured by counting the vesicles microscopically.

The lipid is dissolved in chloroform/methanol (9:1) solvent and the glass beads are added to the solution; the solution just covers the glass beads. Then, the solvent is evaporated under vacuum (15 torr, water-jet pump) in nitrogen atmosphere in a stationary flask. The drying procedure is fast because of the large glass surface area; it usually takes from 10 to 60 min depending upon the temperature. Double-distilled water is subsequently added and the lipid swells for 1–4 h in a 70°C water bath. During this period the lipid forms liposomes.

3. RESULTS AND DISCUSSION

Lipid (1 mg) in chloroform/methanol solution was added to Pyrex glass beads (with surface area corresponding to 21 lipid bilayers on the glass surface, if the area per lipid molecule is 0.42 nm^2), to Touzart & Matingnon glass beads (4 bilayers on the glass surface) and without glass beads. The evaporation temperature of the solvent was 20°C and the swelling temperature 70°C. The swelling was carried out for 3 h. An additional experiment was made with 5 mg lipid with the larger beads (105 bilayers).

The results have shown that in the first case a lot of cell-size liposomes of average diameter 4–5 μm (see fig.1, where the distribution of liposome size is shown) were formed. In the second case (Touzart & Matingnon glass beads) the number of liposomes was almost the same as without beads, i.e., about 5-times smaller than in the case of Corning glass beads. Increasing the initial concentration of lipid to 5 mg led to a slight increase in the number of liposomes.

Several other experiments were made at different temperatures of evaporation of the solvent and swelling of the lipids. In addition, the role of gentle shaking after swelling was investigated with glass beads and in control experiments. The basic results can be summarized as follows:

(i) The type of glass surface is of basic importance for the yield of liposomes. The Corning Pyrex glass can increase the yield several times. The Touzart & Matingnon glass surface had minimal, if any, effect on the liposome yield. At the time of the experiment we had no glass beads from Corning Glass Works and Touzart & Matingnon of exactly the same size. Since the diameter of the liposomes is of the order of micrometers, while the glass beads have diameters in the millimeter range, however, the effect of cur-

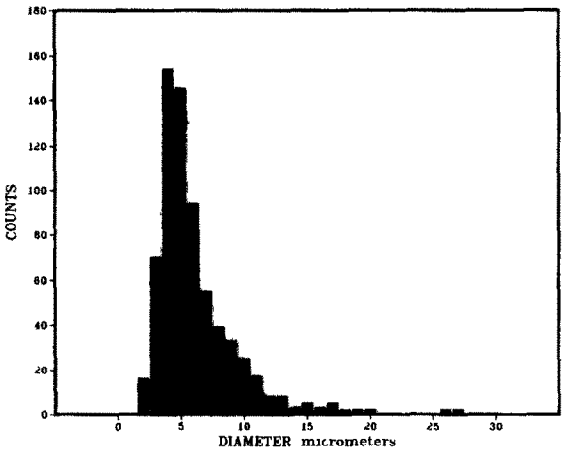

Fig.1. Distribution of size of liposomes, prepared in the presence of pyrex glass beads (average diameter 5 mm). The amount of lipid was 1 mg; the evaporation temperature of the solvent 20°C, lipid swelling temperature in bidistilled water 70°C and swelling time 3 h.

vature should be of no importance. In both cases the glass bead surfaces can be considered flat with respect to the liposomes.

(ii) The increase of the surface area leads to faster evaporation of the solvent and more importantly to a higher liposome yield.

(iii) The shape of the distribution curve does not depend significantly on the surface type and area and temperature of solvent evaporation. It seems to depend primarily on the type of lipid used. However, the absolute value of the number of liposomes per unit volume depends on the type of glass surface, i.e., the glass surface may change the scale but not the character of distribution curves (number of liposomes *vs* liposome size).

(iv) While the formation of cell-size liposomes without glass beads depends on gentle shaking after swelling, with glass beads the liposomes were formed without shaking.

(v) Liposomes were not formed in the presence of Touzart & Matingnon beads at 45°C, but they were formed at this temperature without beads. This probably means that the glass surface mediated interbilayer and glass/lipid interactions are stronger than for the glass surface of the vessel wall at that particular temperature.

These results have shown the importance of the glass surface effects in liposome preparation. These effects should be taken into consideration when using the recently developed methods [2,3] of preparation of cell-size liposomes and any other type of method involving surface/lipid interactions. It should be pointed out that at this time we do not know the mechanism of cell-size liposome formation. In order to understand this mechanism we need to know the physicochemical properties of the glass (chemical composition, surface potential, etc.) and the lipids. Theoretical calculations on the rate of liposome formation, based on recent theories of kinetics of membrane adhesion, instability and fusion are currently in progress [4–7], as well as experiments at different conditions, with the basic goal being to clarify the role of the surface interactions on liposome formation.

As pointed out in [2] the formation of cell-size liposomes in strictly defined experimental conditions may help the understanding of origin of cells during evolution. Surface effects may have played an important role in formation of vesicular structures in the ancient ocean, in particular on different rocks and sands exposed to water and high temperatures.

ACKNOWLEDGEMENTS

This work was supported by a grant no. INT 8209490 from the National Science Foundation of the USA. Interesting discussions with Dr U. Zimmermann, Dr D. Papahadjopoulos, Dr F. Szoka, Dr J. McGrath, D.V. Zhelev, N. Stoicheva, Dr I. Tsoneva and R. Mutafchieva are highly appreciated.

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