PREPARATION OF LARGE UNILAMELLAR VESICLES

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1. Introduction

Single (unilamellar) and oligolamellar lipid bilayer vesicles of large diameter have great potential in membrane research. In particular, large unilamellar bilayer vesicles (diam. >50 μm) would permit the insertion of microelectrodes for the measurement of the electrical properties of lipid bilayer membranes of different compositions. Furthermore, vesicles large enough to be observed by light microscopy could be fused with cells with the aid of the electric field method [1–4]. Since vesicles (liposomes) can be loaded with macromolecules, this might be an elegant means of transferring proteins or plasmids into cells. Large vesicles with different lipid compositions and only one unit membrane would also be ideal objects for the identification of the mechanism of cell rotation in the presence of an alternating electrical field [5–7].

Several methods for preparation of liposomes have been reported [8–11], yielding either very small liposomes (20–200 nm) or mixtures of unilamellar and multilamellar liposomes (≤1 μm) [9–11]. Only the method in [9] allows the preparation of large oligolamellar liposomes on a large scale.

Here, we describe a simple procedure for obtaining a high yield of single bilayer vesicles, almost uncontaminated by multilamellar vesicles. With this method it is possible to produce large vesicles made up of various lipids. The method is based on the use of thin lipid films and relatively high temperature (i.e. 70°C, which is much higher than the phase transition temperature).

2. Materials and methods

The following lipids were used: Di-palmitoyl-phosphatidyl-choline (Fluka, Neu Ulm); di-oleoyl-phosphatidyl-choline (Sigma, München); di-myristoyl-phosphatidyl-choline (Fluka); di-myristoyl-phosphatidic acid, di-sodium salt (Fluka); di-palmitoyl-phosphatidic acid, di-sodium salt (Fluka); di-palmitoyl-phosphatidyl-glycerol, ammonium salt (Sigma); di-palmitoyl-phosphatidyl-serine (Serva, Heidelberg) and several polymerisable lipids [12–15] with phosphatidic acids and ammonium groups as hydrophilic moieties were also taken. These lipids are listed in [13,14].

Vesicle formation was investigated using a Leitz Orthoplan microscope equipped with a set-up for Normarski interference-contrast. For freeze-fracture and electron microscopy small amounts of the vesicle solution were rapidly frozen in melting nitrogen. Replicas were obtained using a Bio-Etch 2005 (Leybold-Haereus). Electron micrographs were taken with a JEM 100C (Jeol).

The equipment for dielectrophoresis is described in [1].

3. Results and discussion

Lipid (3 mg) is dissolved in 1 ml chloroform/methanol (10:1). The solution is then evaporated under vacuum (15 Torr, water-jet pump) in a horizontally rotating cylindrical reaction vessel. During this process the lipid separates out onto the vessel walls and forms a lipid film of homogeneous appearance. Distilled water or salt solution (≤1 mM KCl) (5–10 ml) were subsequently added. The lipid film is lyophilised for 1–4 h without agitation in a 70°C water bath. During this period the lipid film detaches from the vessel walls and forms lipid vesicles of <1 μm diam.

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Within 4 h the liposomes aggregate to a large sphere of ~5–10 mm diam. Gentle shaking of the vessel for ≲5 s results in disintegration of the lipid sphere by reforming vesicles which, however, have ~1–50 μm diam. Alternatively vesicle formation can also be achieved by application of a shear force (e.g., by sucking the sphere and solution through a pasteur pipette). The amount and correspondingly the size of the lipid vesicles depends on the incubation time, but also on the time of shaking the sample at 70°C.

A typical preparation is shown in fig.1. The vesicles were made up of di-palmitoyl-phosphatidyl-choline incubated for 4 h at 70°C. Freeze-fracture pictures (fig.2a,b) of these vesicles show that most of these vesicles are unilamellar (60%). Some of the vesicles exhibit ripples on the membrane. This does not indicate that they are multilamellar and can be explained as local curvatures in the bilayer membrane, similar to [16]. A few of the vesicles are partially or completely filled internally with smaller unilamellar vesicles.

The solution of the large unilamellar liposomes can

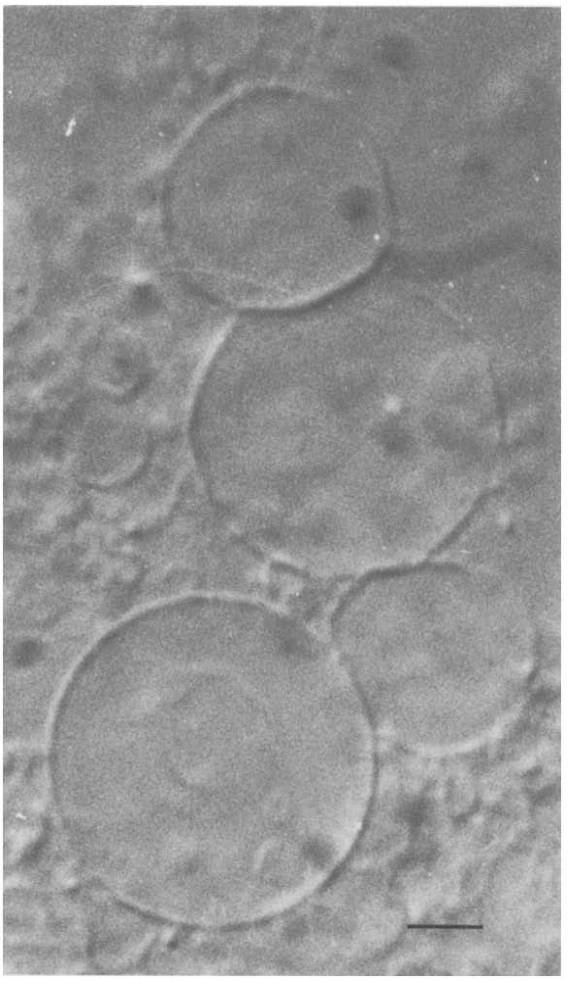
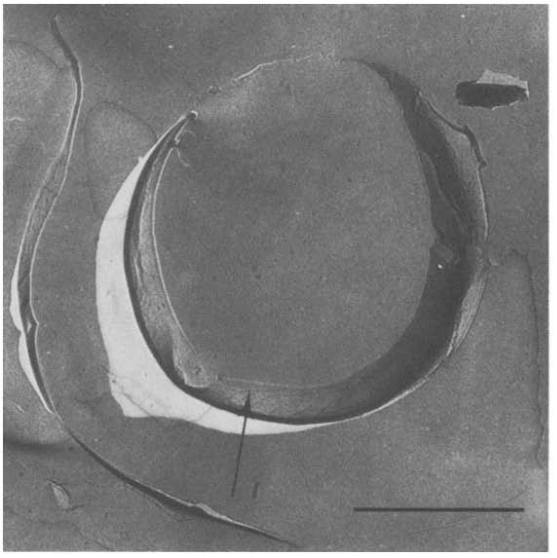


Fig.1. Light micrograph (Normarski interference contrast) of a sample of di-palmitoyl-phosphatidyl-lecithin vesicles prepared by incubation at 70°C. Bar represents 10 μm.

a



b

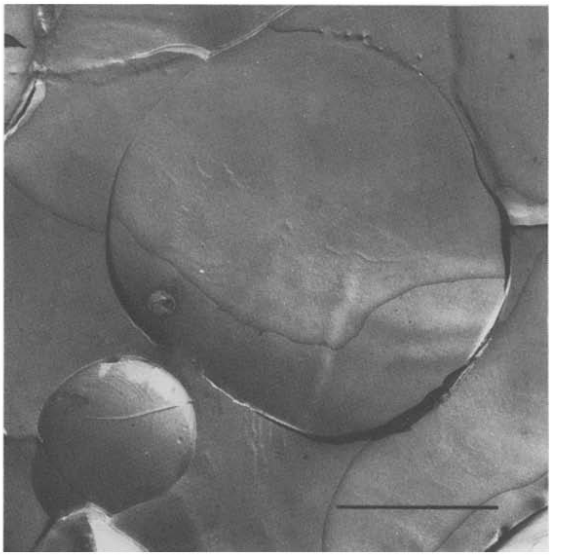


Fig.2. (a) Freeze-fracture electron micrograph of di-palmitoyl-phosphatidyl-lecithin vesicles. Arrow indicates ripples at the outer membrane plane. Bar represents 2 μm. (b) Freeze-fracture electron micrograph of di-palmitoyl-phosphatidyl-lecithin vesicles with a cleavage perpendicular to the membrane of the larger vesicle. The smaller vesicle exhibits ripples of the membrane. Bar represents 760 nm.

be concentrated by a process known as dielectrophoresis [1,4,17–19]. In the case of positive dielectrophoresis the particles migrate to the highest field strength. Larger particles are collected more readily because the dielectrophoretic force is proportional to the volume [17–19]. The slightly inhomogeneous field strength required for dielectrophoresis can easily be attained between 2 cyclindrical and parallel electrodes connected to a frequency generator [1].

The vesicles prepared in dilute electrolyte solution must be transferred to a non-electrolyte solution (mannitol or sucrose) to avoid thermal effects and turbulence which occur after application of electric fields in the presence of ions [4]. The electrodes (100 μm distance, electrode diam. 200 μm) are dipped into the solution and an electric field is applied (2 V, 1 MHz). Within 30 min the gap is filled with liposomes. By withdrawing the 2 electrodes and the volume of fluid trapped between them these liposomes can be isolated.

The foregoing procedure may be a useful tool in future for electrical measurements, but also for fusion of liposomes by means of the electric field fusion technique.

The formation of lipid vesicles partially compartmentalized by smaller vesicles may also give an interesting model system for the origin of cells during evolution. The step by which a cell surrounded by a semipermeable membrane envelope has been developed during evolution is still not understood even though many hypotheses have been put forward (reviewed in [20,21]).

The conditions used here for the preparation of vesicles of the same size as normal living cells may have been well established at the beginning for evolution.

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