FACILITATED FUSION OF LIPOSOMES WITH GLYCEROL MONOLEATE PLANAR BILAYER

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1. Introduction

The fusion of liposomes or vesicles with planar bilayer membranes may serve as a model for exocytosis [1–6]. The use of voltage-dependent anionic channels [2,4] or amphotericin B [1] as conductance probe allows one to distinguish between true fusion and exchange phenomena, which could occur by adherence of the liposomes to the planar lipid film. Before the elegant demonstration of Ca^{2+}-stimulated fusion of liposomes with bilayers containing a Ca^{2+}-binding protein [2], large amounts of divalent cations [3,4] or the addition of fusogenic agents [1] were usually required to facilitate the fusion process. We report here that fusion of liposomes occurs in the absence of Ca^{2+} when the black lipid film is formed of glycerol monoleate.

2. Materials and methods

Bilayer membranes of glycerol monoleate (Sigma, St Louis MO) obtained from neither *n*-decane or squalene solutions (Merck, Darmstadt), which were passed through alumina (grade III), were formed as in [7,8] on an aperture (1.2 mm diam.) in a Teflon cell with two separated aqueous phases (2 ml each). The solvent squalene was used in some experiments to obtain solvent-free bilayers [9,10]. The formation of the black lipid membranes was observed under reflected light with a low power microscope. The membrane conductance was measured [11] with a Keithley electrometer (model 602C) using an imposed potential of either 20 mV (amphotericin B experiments) or 40 mV (valinomycin experiments). Multilamellar liposomes formed of egg yolk phosphatidylcholine (EYPC), phosphatidylserine (PS) and, as required, cholesterol, valinomycin (all reagents from Sigma) and amphotericin B (a gift from Dr G. Laurent, Bordet Institute, Brussels) were prepared from a chloroform solution, the solvent being evaporated under a stream of N_2 and the lipid film dried overnight under vacuum. The liposomes were obtained by mechanical agitation, followed by 4 periods (1 min each) of ultrasonication (Branson Sonifier B12; 75 W) to reduce the size of the multilamellar liposomes [12]. The liposomes were added with a micropipette to the aqueous phase and maintained in a disperse state by gentle stirring with a teflon-coated magnetic flea. The aqueous solutions added on each side of the black lipid membrane were first passed through an 0.22 μm Millipore filter. All results are expressed as the mean ± SEM, together with the number of individual measurements (n).

3. Results and discussion

In a first series of experiments, multilamellar liposomes formed of EYPC, cholesterol and PS (molar ratio 7/2/1, respectively) and containing amphotericin B (3 mol/1000 mol lipid) were added to a buffered solution of Tris–HCl (20 mM, pH 7.4) containing NaCl (120 mM) to yield of final concentration of 2.5 μg lipid/ml. The addition of the liposomes increased the membrane conductance from a basal value 2.22 ± 0.24 nS . cm^{-2} (n = 15) to a maximal value of 2.65 ± 0.28 μS . cm^{-2} (n = 5), before the membrane broke. The increase in membrane conductance was observed after a 30–60 s lag period. Comparable results were obtained whether the black lipid membranes were formed from glycerol monoleate

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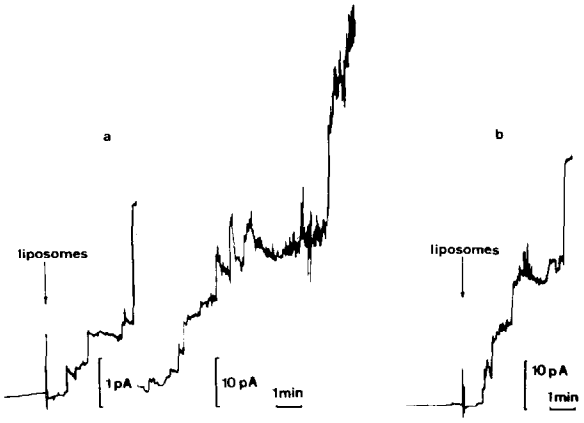

Fig.1. Changes in conductance of glycerol monoleate planar bilayers provoked by the addition of liposomes (2.5 μg lipids/ml) formed of EYPC, cholesterol and PS and containing amphotericin B. The lipid bilayer was formed from either a *n*-decane (a) or squalene (b) solution. Note the change in the scale of the ordinates in (a).

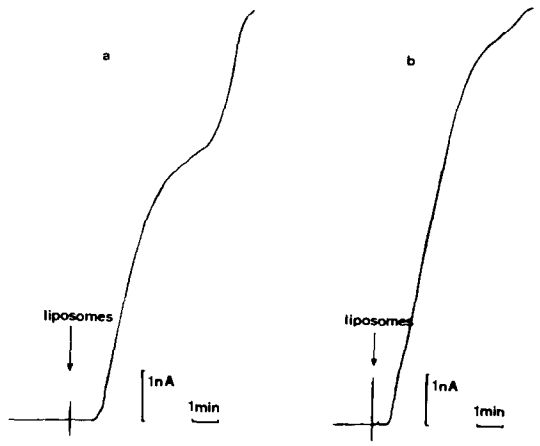

Fig.2. Changes in conductance of glycerol monoleate planar bilayers provoked by the addition of liposomes (2.5 μg lipids/ml) formed of EYPC and PS and contaning valinomycin. The lipid bilayer was formed from either a *n*-decane (a) or squalene (b) solution.

solubilized in *n*-decane (fig.1a) or squalene (fig.1b). In the latter case, the bilayers contain no entrapped solvent [9,10]. No significant change in conductance was observed when cholesterol was absent from the liposomal matrix. Virtually all amphotericin B remains associated with the liposomes [13]. Therefore, our results suggest that the rise in conductance is attributable to the fusion of liposomes with the planar bilayer, with concomitant incorporation of amphotericin B and cholesterol in the black lipid film. The use of solvent-free membranes also indicate that the fusion process is not due to the fusogenic property of *n*-decane [1].

In a second series of experiments, valinomycin was used as the conductance probe, although with this antibiotic an exchange phenomenon may occur between the liposome and the planar membrane. Liposomes formed by EYPC and PS (molar ratio 9/1, respectively) and containing valinomycin (1.5 mol/1000 mol lipid) were added to the same Tris–HCl buffer containing NaCl (100 mM) and KCl (20 mM). When added at a final concentration of 2.5 μg lipid/ml (corresponding to ~5 pmol valinomycin/ml), the liposome increased the membrane conductance (fig.2) at a rate of $5.76 \pm 0.86\ \mu S \cdot cm^{-2} \cdot min^{-1}$ as distinct from $0.51 \pm 0.17\ \mu S \cdot cm^{-2} \cdot min^{-1}$ when the same amount of valinomycin solubilized in ethanol was added immediately to the aqueous medium. The maximal conductance was also much higher when valinomycin was incorporated in the liposomes ($30.14 \pm 7.24\ \mu S \cdot cm^{-2}$) rather than added to the aqueous medium ($0.65 \pm 0.17\ \mu S \cdot cm^{-2}$). Comparable results were obtained when the liposomes containing valinomycin were added to solvent-free membranes (fig.2b). Even when the liposomes were added to yield a final concentration as low as 0.05 μg lipid/ml (corresponding to ~0.1 pmol valinomycin/ml), the conductance was still increased from its basal value of $2.22 \pm 0.24\ nS \cdot cm^{-2}$ to a maximal value of $110 \pm 34\ nS \cdot cm^{-2}$ ($n = 3$). When liposomes without antibiotic were added, the membrane conductance never exceeded $8\ nS \cdot cm^{-2}$ ($n = 3$).

These converging observations indicate that the fusion of lipid vesicles with planar bilayer of glycerol monoleate takes place easily in the absence of Ca^{2+} or other fusogenic agent. These findings are in good agreement with the capacity of glycerol monoleate to induce cell fusion [14]. By comparison with data obtained with other lipid bilayers, they also raise the idea that heterogeneity in the lipid composition of distinct domains in biological membranes could account for a preferential location of such events as exocytosis.

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