FORMATION OF PLANAR BILAYERS FROM ARTIFICIAL OR NATIVE MEMBRANE VESICLES

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Received 10 October 1980

1. Introduction

The formation of planar membranes from vesicles has been used to study the acetylcholine receptor from *Torpedo* electric organ in planar bilayers formed from native membrane vesicles [1] or from reconstituted vesicles [2] and it allowed reconstitution of porin (or matrix protein) channels as they occur in *Escherichia coli* outer membranes [3]. The objective of this report is to detail the technique, to identify underlying mechanisms, and to explain the prevailing requirement for successful membrane formation that vesicle radii should exceed 50 nm.

2. Materials and methods

Soy bean phospholipids: source, purification, and vesicle formation as in [1]. Vesicle size was stepwise reduced by sonication (Branson sonifier) for different time periods, up to 2 h. Peak sizes were evaluated from electron micrographs after uranyl acetate staining. Samples with mean size variations of >50% peak size were discarded as well as samples with multilamellar vesicles >15% wt.

Dioleoylphosphatidyl choline (DOPC): synthesis, purity, and vesicle formation as in [4]. The resulting vesicle suspensions of differently sized vesicles were analyzed and selected as outlined above. DOPC vesicles of smallest size were prepared according to [5].

Surface tension or monolayer surface pressure was measured in a Wilhelmy ring-balance (Krüss, Hamburg). Bilayer cell, electrical measurements, fabrication of membrane frames was as in [6]. Hepes buffer (10 mM *N*-2-hydroxyethylpipierazine-*N'*-2-ethanesulfonic acid, pH 7.0) and 1 M KCl was used for all vesicle suspensions. Experiments were done at 20°C.

3. Results and discussion

Planar membranes were routinely formed from vesicles in the following way (fig.1): suspensions of vesicles with radii >50 nm and at 1 mg vesicles/ml were filled into the 2 compartments of a membrane cell to just below the aperture in the membrane frame. After ~1 min planar membranes were formed by raising the water levels successively above the aperture. Membranes formed at almost every attempt and no extreme patience or expert knowledge was needed. They were routinely stable for hours, exhibited dielectric breakdown voltages of 300–350 mV, specific resistances of $1-2 \times 10^8\ \Omega/cm^2$ and specific capacitance values of $0.75 \pm 0.05\ \mu F/cm^2$ (for soy bean phospholipid). Reliability of the technique and of bilayer properties was established by >1000 planar bilayers formed from differently composed vesicles, pure lipid vesicles, reconstituted lipid–protein vesicles [3] and native membrane vesicles [1] at various conditions of ionic strength and pH. Besides technical requirements, outlined below, there appeared to be only one principal precondition for successful bilayer formation from vesicles: the vesicle radii had to

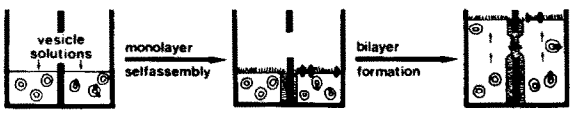

Fig.1. Transformation of vesicles to planar bilayers via spontaneous monolayer formation.

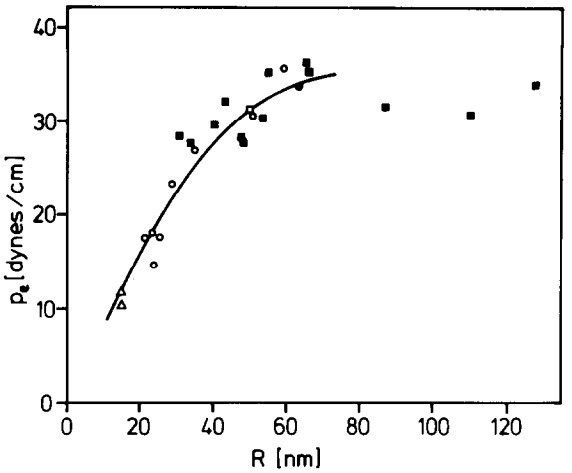

Fig.2. Equilibrium surface pressure p_e at the interface of vesicle suspensions (1 mg/ml) containing vesicles of different radii R. Filled symbols refer to soy bean phospholipid vesicles, open symbols to dioleoyl-phosphatidylcholine vesicles. For details of vesicle formation see section 2.

exceed 50 nm+. This requirement could be related to the surface pressure at the vesicle suspension interface. With increasing vesicle size the surface pressure was found to increase towards a limiting value (p_{equiv}) (fig.2) for soy bean phospholipids (filled symbols) and dioleoyl-phosphatidylcholine (open symbols). The formation of stable bilayers from vesicles required that the surface pressure values at both interfaces approached p_{equiv} for which vesicle radii had to exceed 50 nm. The solid line in fig.2 is a predicted relation between surface pressure and vesicle size obtained from an analysis of the spontaneous formation of a monolayer at the surface of a vesicle suspension in [4]. The monolayer was shown to reach a lipid exchange equilibrium with the vesicles in the subphase. The vesicle size dependence of the surface pressure at equilibrium p_e (see fig.2) was evidenced to reside in the limited size of the vesicle which effects the rate of lipid exchange. For sufficiently large vesicles (radius >50 nm), the limited size effect becomes negligible so that the energy state of the lipids in the monolayer can be regarded to be 'equivalent' to that of lipids in the bilayer because the monolayer is in equilibrium with basically a planar bilayer. From this it is expected that two monolayers at p_{equiv} (equivalence pressure) form a stable bilayer when they are apposed during bilayer formation. These conclusions from the analysis in [4] match well these empirical relations between vesicle size, surface pressure and success of bilayer formation, so that the above 'equivalence concept' provides a basis of understanding to the successful transformation of vesicles to planar bilayers. Moreover, this concept is based on the equilibrium between the monolayer and each vesicle as such (as a phase) so that the surface pressure is expected to be independent of the concentration of vesicles in the subphase, which is indeed found (fig.3) within the range studied.

Besides the principal requirement concerning vesicle size there were technical provisions to take for successful bilayer formation from vesicles. The two major technical aspects are:

(i) The rate of equilibration between monolayer and vesicles, depending on vesicle concentration (fig.3) should be fast enough to hold the surface pressure close to p_{equiv} when the membrane is formed during which the surface pressure tends to drop due to the increase in monolayer area (fig.1). This is ensured by using ≥0.5 mg vesicle/ml (fig.3)*. Multilamellar or aggregated vesicles should be avoided as they reduce considerably the equilibration rate. The rate was found to

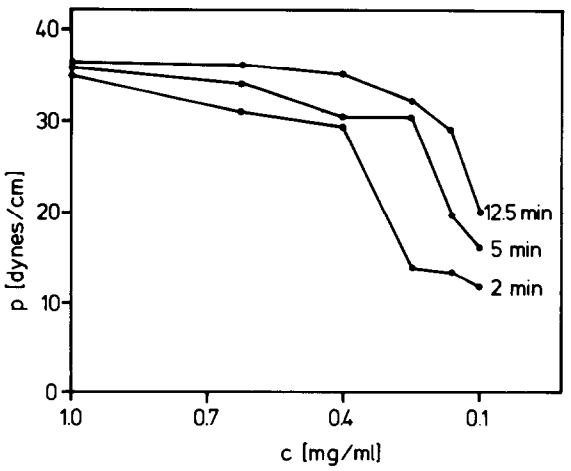

Fig.3. Surface pressure p at the interface of vesicle suspensions for different vesicle concentrations at 3 times after clearing the interface by aspiration. Soy bean phospholipid vesicles of 60 nm radius were used.

+ This criterion is not related to a certain fixed surface pressure but to p_{equiv}, which was found to vary with vesicle composition (to be published elsewhere)

* The monolayer surface pressure could temporarily be raised above p_{equiv} by integration of monolayers from the surfaces of vesicle suspension droplets deposited onto the interfaces of the membrane cell. This facilitates bilayer formation

increase slightly with increasing [KCl].

(ii) Irregularities around the perimeter of the aperture in the teflon septum should be <1 μm for 10 μm teflon thickness and deviations from planicity should be <10 μm as routinely achieved by the technique in [6]. Pre-treatment of the septum is necessary and may be either by hexadecane or triglyceride, deposited to a thin continuous film around the aperture.

Acknowledgements

The author thanks A. Frank for expert technical assistance. This investigation was supported by grant 3.390–0.78 from the Swiss National Science Foundation.

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