Formation of lipid–protein bilayers by micropipette guided contact of two monolayers

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Bilayers of a few μm^2 area were formed from lipid-protein vesicles. First, two monolayers are generated from vesicles, which then are brought into local contact by use of a micro-pipette to form a bilayer. Membrane channels of Matrix–Protein and of Colicin A exhibit normal properties in these bilayers. Apart from avoiding solvents completely and of using characterized vesicles as starting material there are two main virtues to this strategy: (1) Current resolution is very high, a few tenths of a pA at 150 μs time resolution; and (2) transport processes can be studied in dependence of surface pressure between 28 and 48 mN/m.

Membrane reconstitution *Planar membrane* *Vesicle spreading* *Surface pressure* *Single channel* *Membrane tension*

1. INTRODUCTION

For the study of ion transport across membranes at a molecular level the appropriate membrane configuration for reconstitution attempts is the 'planar bilayer'. These lipid or lipid-protein bilayers span a circular aperture in a Teflon wall or thin 'septum' which separates two aqueous compartments. To produce such systems mainly three strategies have been devised, namely: (i) the 'black membrane' [1] formed from a droplet of lipid in decane, 'painted' across the hole, which thins down to a lipid bilayer bound by a decane-lipid torus; (ii) the septum is covered with lipid monolayers from both sides which combine to a bilayer within the hole [2]; (iii) vesicle derived bilayers [3], where two monolayers are formed from vesicles and, as in (ii), combined to a bilayer (for a review see [4]).

This report describes a new strategy for forming lipid-protein bilayers. Two monolayers are prepared from vesicles and arranged face to face. A micropipette is used to guide one monolayer on to the other forming a bilayer in a small contact area. This is a technical report on how these bilayers are formed including evidence that the two monolayers indeed combine to a bilayer.

2. MATERIALS AND METHODS

Soya bean lipid was obtained from Sigma, purified and vesicles were made as described [5]. Cholesterol was purchased from Fluka, Buchs Switzerland. Matrix protein was isolated and incorporated into vesicles as described [6]. Colicin A was a gift by Dr F. Pattus (CNRS, Marseille, France); it had been isolated according to [7]. Wilhelmy balances: consisted of a 2 × 2 cm sheet of glass microfibre filter coupled by a double spiral spring to a linear distance-voltage transducer (SS101, Collins Corp., Long Beach, CA). Micropipettes were pulled as described from hard glass capillary tubes (BDH Chemicals Ltd., Poole, UK) [8] and had openings at the tip of 1 to 2 μM. They were used immediately after preparation without further treatment (heat polishing or any type of coating). The pipette holder built according to [8], is plugged into a current amplifier which is mounted on a micromanipulator. Aqueous phases were at room temperature, buffered at pH 7.4 (5 mM *N*-2-hydroxy-

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ethylpiperazine-*N'*-2-ethanesulfonate) and contained either 0.5 M KCl or NaCl.

3. RESULTS AND DISCUSSION

3.1. *Preparation of bilayers*

The preparation of bilayers entails: (i) the formation of monolayers from vesicles (fig.1A); (ii) a particular relative arrangement of two such monolayers (fig.1B); (iii) establishment of local contact by guidance of a micropipette tip (fig.2A) and electrical monitoring of contact formation (fig.2B).

(i) The essentials of monolayer formation from vesicles have been described earlier [3,9,10]. For the present purpose a glass plate is used (fig.1A) at the surface of which 5 to 10 μl of vesicle suspension (1 mg/ml) is distributed. Within about a minute, the exact duration depending on conditions of the vesicle and aqueous phase (Th. Schürholz, H. Schindler, unpublished results) the monolayer surface pressure π reaches values of 15 to 20 mN/m as measured with a Wilhelmy balance. The glass plate is removed and a surface barrier (B) introduced to set the surface pressure π to a particular value.

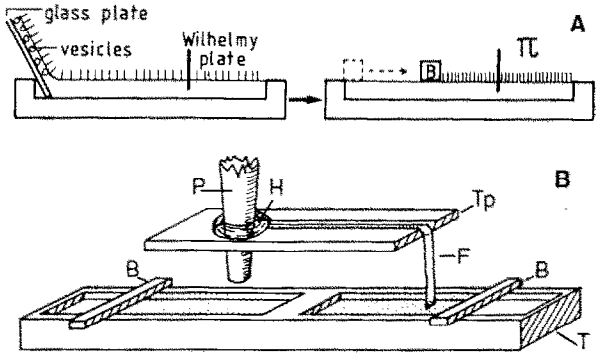

Fig.1. Formation of monolayers from vesicles (A) and arrangement of two such monolayers (B) for bilayer formation. Monolayers (dotted areas in B) are formed (as shown in A) at the two water surfaces (45 × 25 mm) of trough T. The strip of filter paper (F) is fitted into a slot in Teflon plate Tp (12 × 10 × 2 mm) guiding the right monolayer to the surface of pipette P in the water filled hole H (tapered shape with 2.5 mm upper and 1.2 mm lower diameter). The distance between the two monolayers (without pipette) is 1 mm. Surface pressure π of both monolayers was continuously measured (not indicated in B) and could be deliberately changed by movement of barriers B.

(ii) Fig.1B shows schematically the trough (T) used for bilayer formation with two vesicle spread monolayers indicated by dotted areas. The monolayer on the right continues along the wet surface of a strip of filter paper (F) to the surface of the water filled hole (H) in the upper teflon plate (Tp). A micropipette (P) filled with buffer, is moved downwards into and through the hole guiding one monolayer against the other.

(iii) One electrode connects the aqueous phase inside the pipette (up: upper phase) to an amplifier as shown in fig.2A. A second electrode provides an input voltage (V_{in}) to the lower aqueous phase (lp). Contact between the monolayers is monitored by applying a voltage sine wave during downward movement of the pipette. Contact formation is evident from a sudden increase of the output signal (see fig.2B). This effective increase in capacitance indicates that contact of the monolayers is not confined to the pipette tip. The pipette is held station-

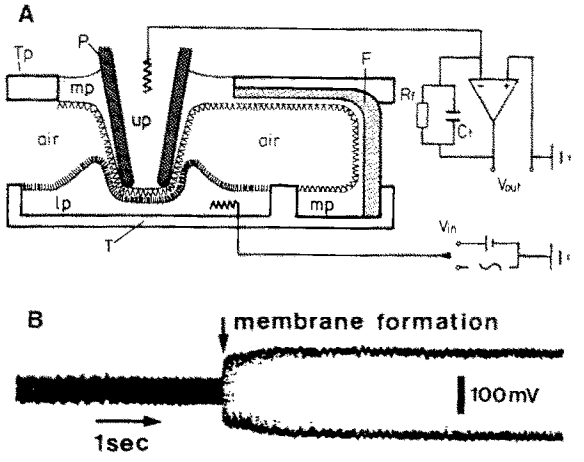

Fig.2. Bilayer formation. (A) Schematic illustration of monolayer arrangement after contact showing three main aspects (evidenced in the text): (i) a bilayer structure at the pipette opening, (ii) a bending of the lower monolayer towards the pipette sidewalls, and (iii) the absence of tight contact between upper monolayer and pipette side walls. Symbols lp, mp and up indicate the three aqueous phases. (B) Time compressed sine wave signal (V_{out}) during downward movement of the pipette until monolayer contact formation. A simplified diagram of the recording system is included in fig.2A, where V_{in} was a 20 mVpp, 100 Hz sine wave and R_f = 10 GΩ. The signal after bilayer formation corresponds to an effective capacitance of 2 pF.

ary as soon as the increase in capacitance is observed. Further downward movement should be avoided because the concomitant increase in capacitance resulted in poorer signal-to-noise ratio in channel traces. Breakage of the bilayer manifests itself by a surge in the output signal of several orders of magnitude.

Stable contact was achieved for a range of surface pressures, from the collapse value of 48 mN/m down to 28 mN/m. At 40 mN/m the bilayer was stable for 10 to 60 min. The voltage of breakdown ranged between 250 and 350 mV (defined by breakage during a continuous ramp of 1 mV/s). The ohmic resistance was as high as 2 to 5 TΩ.

3.2. *Membrane channels*

Direct evidence, that the structure in the area of contact between monolayers is a bilamellar membrane was obtained from studies on membrane channels. Two examples are shown in fig.3. Colicin A is known to form membrane channels by insertion from the aqueous phase into lipid membranes [11]. The channel trace in fig.3A was observed with colicin A in the lower aqueous phase (lp in fig.2A) just after formation of contact at an applied voltage of + 100 mV. The channel conductance of 21 pS, channel closing upon voltage reversal, and the kinetics of channel closing are in quantitative agreement with the characteristics of colicin A channels in SV-bilayers* [12]. The resolution of the channel is quantitated in part b of the figure. At a time resolution of 140 μs (see correlation function G(t)) the current fluctuations of the membrane have a standard deviation of 0.1 pA as can be seen from the amplitude probability function in the figure. This is an improvement by one order of magnitude of resolution compared with bilayers formed by other techniques. This is due simply to the very small size of pipette-supported bilayers. Discrete current events of only a few hundred ions are detectable.

In order to test channel activity of integral membrane proteins, one monolayer was formed from

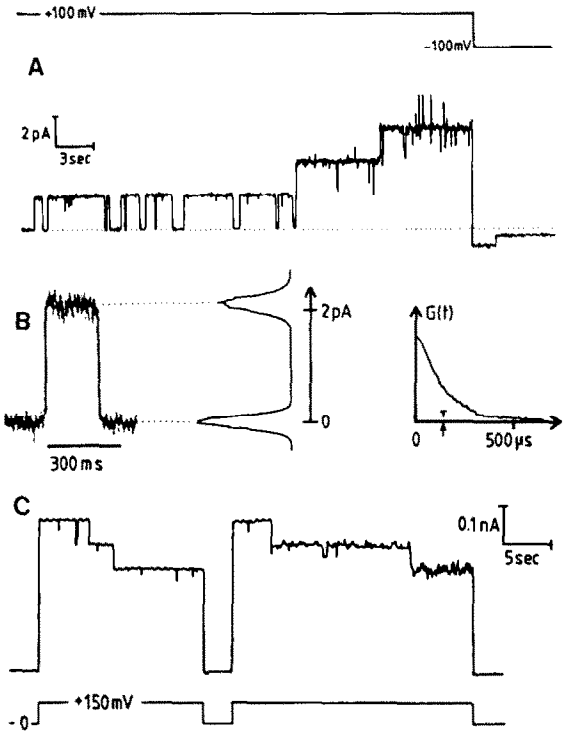

Fig.3. (A) Current trace of colicin A channels. Colicin A was injected (10 μl of 100 μg/ml) into the lower aqueous phase after bilayer formation. The surface pressure of both monolayers was set to 42 mN/m. The solution contained 0.5 M KCl. Positive voltage refers to positive potential at the side containing colicin A. R_f = 10 GΩ. (B) Current resolution. The middle part shows a probability density function of current amplitudes during single channel events of colicin A (left). The standard deviation of background fluctuations is 0.1 pA which compares to 2.1 pA channel current. The corresponding time resolution is 140 μs estimated from the autocorrelation function G(t). For probability and correlation analysis the model SAI-43A from Honeywell (Denver, CO) was used. (C) Current record of matrix protein channels. The lower monolayer was formed from vesicles containing 1 matrix protein trimer per 10^5 lipids (soya bean lipids and cholesterol, 6:1 weight ratio). Aqueous phases contained 0.5 M NaCl. Surface pressure was 42 mN/m in both monolayers.

lipid vesicles containing Matrix Protein (Porin) isolated from *Escherichia coli* outer membranes. Upon contact formation between this monolayer and a lipid monolayer channel activity was observed as shown in fig.3C. The channel conductance, the closing of channels with voltage and channel

* For an easy distinction between the two strategies to form bilayers from vesicle derived monolayers we suggest the names: PV-bilayer (Pipette supported, Vesicle derived bilayer) and SV-bilayer (Septum supported, Vesicle derived bilayer, see strategy (iii) in section 1)

re-opening at zero voltage agree with the data obtained for Porin in SV-bilayers [6] at the same conditions. Porin channels were also observed when *Escherichia coli* outer membranes were directly used to form one monolayer (data not shown) in agreement with corresponding data on SV-bilayers [6].

The potential of this technique to study surface pressure dependences of membrane properties and of ion transport processes is under current investigation.

3.3. *Technical notes*

(i) Any attempt to replace the upper continuous monolayer by lipid films associated with the pipette surface failed. For example, a pipette was partly covered with a monolayer by moving its tip into and out of a lipid monolayer at the surface of an aqueous phase. While lowering the pipette bilayers formed which, however, never lasted longer than 1 min (typically a few seconds), irrespective of whether or not the film was allowed to dry onto the glass surface. Even a reservoir of lipid at the glass surface, dried from lipid in a solvent (hexane) did not improve this result. Only if solvent was not allowed to evaporate, stable bilayers formed, which were probably bound by a lipid solvent torus. This is in accord with the recent report on bilayer formation at the tip of a micropipette, where the pipette was punched through a black membrane in presence of a lipid-decane solution [13]. In earlier attempts, two monolayers, separated by a decane phase, were successfully combined into a bilayer [14]. (ii) Using two continuous monolayers as in fig.1B and 2A apparently there remains a thin film of water between the pipette wall and the upper monolayer provided that the exposed surface of the pipette is small (narrow air gap). This can be inferred from the measured resistance (typically 10 GΩ) between aqueous phases up and up (up to a 100-fold transient decrease of this resistance could be induced by pipette movements). This waterfilm appears to be essential for stable bilayer formation in that it prevents tight association of the upper monolayer to the glass surface. (iii) Suction or positive pressure applied to the pipette interior brought no gains. There was no significant influence of glass type (soft glass, hard glass) or glass surface of the pipette (heat polishing [8] or etching with 1% HF) on bilayer formation or stability.

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