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ELECTROSTIMULATED FUSION AND FISSION OF BILAYER LIPID MEMBRANES

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The interaction and fusion of black lipid membranes have been studied. Two planar bilayers were simultaneously inflated towards each other; when they made contact, spontaneous formation of a 'trilaminar structure' (one bilayer bounded by two bilayers along the perimeter) was observed. Application of a discrete voltage pulse gave rise to the formation of a cylindrical membrane, that is, to the fusion of two bilayers. It is shown that fusion results from electrical breakdown in the contact region of the 'trilaminar structure'.

Fusion of biomembranes has been studied on various model systems such as liposome-cell, liposome-liposome, liposome-planar bilayer. In all these systems, a great number of membrane interactions take place simultaneously; hence, the interpretation of results becomes rather complicated. In this respect, it seems helpful to study the interaction of two black lipid bilayers. These studies have already allowed us to gain some useful information about the nature of the interaction forces and about the structure of the membrane contact region [1–4]. We here present evidence for the applicability of this model system to investigation of single fusion-fission events in lipid bilayers.

Two planar bilayers were formed from lipid solution in *n*-decane (40 mg/ml) on two 1.5 mm holes in Teflon partitions dividing a Teflon cell into three sections (Fig. 1a), containing 10^{-1} M or 10^{-2} M KCl. We studied bilayers of the following compositions: dioleoylphosphatidylcholine (DOPC Serva), DOPC/cholesterol (9 : 1, w/w), azolectin (Associated Concentrates), phosphatidylethanolamine (PE). The working cell was maintained at 30°C. The initial distance between the two planar bilayers, M_1 and M_2, was about 1 mm; it was then gradually reduced by inflation of both membranes. For this purpose, a special calibrated Teflon pivot was raised from section II. The procedure described created a hydrostatic pressure difference, ΔP, forcing M_1 and M_2 to bulge towards each other until they made contact. ΔP depended on membrane tension and usually did not exceed 40 dyn/cm^2. The interaction and fusion of the two bilayers were observed by means of two microscopes, one placed in the plane of membrane contact, the other oriented normally to this plane. In addition, the electrical characteristics of the system were measured (Fig. 1, upper parts). A triangular waveform (amplitude ± 20 mV, sweep rate 100 V/s) was applied to electrode 3 from a generator, G. Currents from electrodes 1 and 2 through the outputs of two operational current amplifiers (CA_1 and CA_2) were recorded on an oscillograph (oscillograms $I_1(t)$ and $I_2(t)$, respectively). The capacitive current $I_2(t)$ appeared as sign-variable rectangular pulses proportional to the capacitance of the M_2 and to the sweep rate. $I_1(t)$, as shown below, underwent qualitative changes, reflecting different stages of M_1-M_2 interaction (see also Ref. 4). We could also find changes in the membrane surface charges during the experiment, using a potentiodynamic method based on the

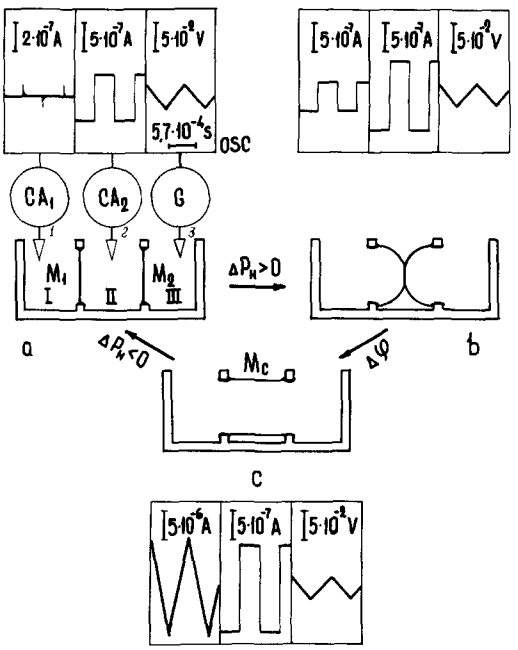

Fig. 1. Experimental pattern of membrane fusion and fission. M_1 and M_2, planar bilayers (DOPC/cholesterol) in 10^{-1} M KCl solution; G, generator; CA_1 and CA_2, operational current amplifiers; 1,2,3, Ag/AgCl electrodes; OSC, oscillograms on the screen of a double-beam oscilloscope; ΔP_H, hydrostatic pressure difference between the inner cell section (II) and two outer sections (I and III); M_c, cylindrical membrane; $\Delta\varphi$, electrostimulation procedure ($\Delta\varphi = 400$ mV).

control of symmetry of capacitive currents [5].

When M_1 and M_2 existed separately (Fig. 1a), $I_1(t)$ was determined by the voltage drop across the resistance, R_G, of the solution layer between M_1 and M_2 [4]. As the two membranes moved towards each other, their surface areas and R_G increased, causing a gradual increase in the amplitudes of $I_1(t)$ and $I_2(t)$. Their general appearance, however, remained unaltered, even some time after contact between the membranes was observed visually. Then $I_1(t)$ changes qualitatively, acquiring the characteristic rectangular form, the amplitude of pulses being proportional to the visually determined contact area of the membranes (Fig. 1b). The specific capapitance of the contact region for DOPC/cholesterol membranes (0.35 ± 0.02 $\mu F/cm^2$) was close to that of a single bilayer (0.4 ± 0.02 $\mu F/cm^2$). That stage of the membranes' interaction from the moment of visible contact till the current change, we designate here as 'contact', while that after the current change we call 'stuck' (bilayers stuck together). The contact region in 'stuck' state will be designated as $M_{1,2}$. The time interval between the two stages depended on the ionic strength, electrolyte composition and surface charges of the bilayers [6], being usually less than 1 min to 10^{-1} M KCl, irrespective of membrane lipid composition. The 'stuck' state could persist for a long time (more than 1 h). Afterwards, one of the membranes broke, or, occasionally, spontaneous formation of a cylindrical membrane was visually observed (Fig. 1c, see also Refs. 4 and 6), accompanied by a change in the oscillogram from CA_1: now the significant conductance (current) was registered instead of the capacitive current seen before.

There are good reasons to believe that the formation of the cylindrical membrane, that is, the simultaneous joining of M_1 and M_2 and of the volumes they limit, closely resembles cell fusion, and should therefore be called 'full fusion' of the bilayers. Thus, the described transfer between the 1b and 1c states is especially interesting.

We further found that fast fusion of bilayers always occurred after a voltage pulse of definite amplitude $|\varphi|$ had been applied to 'stuck' bilayers (electrode 2 being eliminated from the circuit). For instance, taking $\varphi = 400$ mV, we could observe fusion in less than 50 ms. The mean duration of the voltage pulse application necessary for fusion of 'stuck' bilayers $\bar{\tau}_f$ depended on φ. The plot of log $\bar{\tau}_f$ against φ is shown in Fig. 2 (filled circles). For elucidation of the nature of electrostimulated fusion of bilayers, we measured also mean lifetimes, $\bar{\tau}_1$, as a function of φ for single planar bilayers of the same composition [7] (Fig. 2, open circles).

It can be readily seen from Fig. 2 that over the range of voltages studied, the values of log τ (i.e., electromechanical stability) for the single bilayer and those for $M_{1,2}$ practically coincide. We may conclude, therefore, that in itself, the hydrostatic pressure difference necessary for bringing M_1 and M_2 together has no effect on the stability of $M_{1,2}$, and hence does not promote fusion of adhering bilayers. It should be noted that the application of a potential difference to bilayers in the 'contact' state results in their rupture instead of fusion.

Electrostimulated fusion does not necessarily

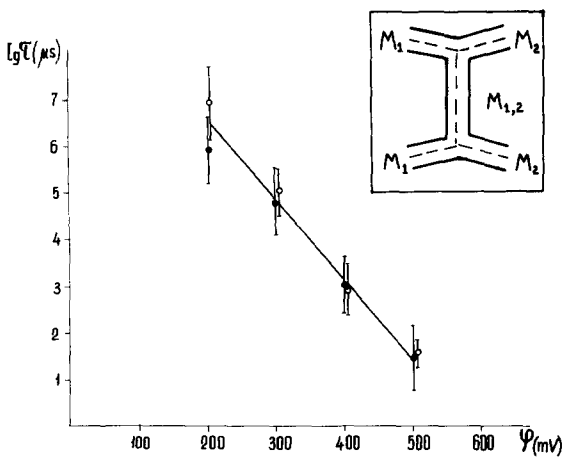

Fig. 2. Comparison of the voltage dependence of the mean lifetime of a DOPC/cholesterol planar bilayer (○) with that of the mean pulse duration necessary for the fusion of two membranes (●). Each point represents the results from at least six measurements. The area of planar bilayer and that of the 'stuck' contact region is approx. $1.7 \cdot 10^{-2}$ cm^2. Experiments were carried out in 10^{-1} M KCl solution. Insert: Trilaminar structure; $M_{1,2}$, single bilayer, formed in the contact region from two monolayers of the interacting membranes, M_1 and M_2.

require an external electric field. In fact, when the charged bilayer, M_1 (azolectin, surface charge $\epsilon_1 = 3 \pm 0.5$ μC/cm^2, surface potential $\varphi_{s_1} = 87$ mV), and the neutral bilayer, M_2 (DOPC/cholesterol, $\epsilon_2 = 0$, $\varphi_{s_2} = 0$), 'stuck' in 10^{-2} M KCl, the surface potential difference was 90 ± 8 mV, being quite close to $\varphi_{s_1} - \varphi_{s_2} = 87 \pm 10$ mV. These asymmetrical membranes fused spontaneously considerably more often than those produced when both M_1 and M_2 were formed either from azolectin or from the DOPC/cholesterol mixture (see also Ref. 4). This may be accounted for by the fact that $M_{1,2}$ is now exposed to a reasonably strong internal electric field. Measurement of surface charges showed that the cylindrical membrane formed by spontaneous or electrostimulated fusion of charged and uncharged bilayers has an intermediate surface charge which correlates with the area ratio of the non-contacting parts of M_1 and M_2 in the 'stuck' state before fusion.

Bringing together the walls of a cylindrical membrane by lowering the Teflon pivot in section II, we may induce membrane fission; the system reverts to the original state (i.e., Fig. 1c reverts to Fig. 1a). In contrast to fusion, fission does not require electrostimulation: it takes place spontaneously, provided the opposite walls of a cylindrical membrane are sufficiently close to each other. The fusion-fission cycle may be repeatedly fulfilled for all bilayer compositions studied.

Our data lead to a definite conclusion concerning the structure of the $M_{1,2}$ region (see Fig. 1b) required for electrostimulated fusion. The closeness of the specific capacitance and electromechanical stability of $M_{1,2}$ to those of the separated bilayers (M_1 and M_2) shows that $M_{1,2}$ is none other than a single bilayer; hence, we observe here the spontaneous formation of a so-called 'trilaminar structure' (see also Refs. 1, 4 and 8). The results obtained with neutral (M_1) and charged (M_2) bilayers prove that a single bilayer, $M_{1,2}$, is formed from monolayers of different membranes. The formation of a 'trilaminar structure' has been also reported for biomembranes [9].

The identity of lifetimes of $M_{1,2}$ to those of a single bilayer M_1 (or M_2) together with the strong dependence of τ_1 and τ_f on φ may account for the almost certain formation of cylindrical membranes in our experiments. Indeed, the voltage drop across $M_{1,2}$ is twice as large as that across M_1 or M_2 beyond the 'stuck' region. Therefore, the ratio of the lifetimes, τ_1/τ_f, is about $10^{\alpha\varphi/2}$, where α is the log $\bar{\tau}_f(\varphi)$ slope from Fig. 2. The higher φ is, the greater is the probability of the rupture of $M_{1,2}$ prior to the non-contacting parts of M_1 and M_2 that is, of M_1-M_2 fusion.

The coincidence of $\bar{\tau}_f(\varphi)$ and $\bar{\tau}_1(\varphi)$ also suggests that electrostimulated fusion of membranes results from irreversible electrical breakdown of the 'stuck' region, that is, from the formation of a hydrophilic inverted pore, the radius of which exceeds the critical value [7]. Worth noting is that the cylindrical membrane may be formed equally well by piercing $M_{1,2}$ with a glass or metal needle.

Some authors have suggested [8,10] that the intramembrane electric field plays a significant role in cell fusion in vivo. The spontaneous fusion of neutral and charged bilayers observed in our model system agrees with this assumption. It has been shown recently [10,11] that an external electric field applied to the contacting cells causes their fusion. This phenomenon is likely to have wide application in biotechnology [12]. According

to Zimmermann et al. [12], electrostimulated cell fusion results directly from the electrical breakdown of lipid bilayers of contacting cell membranes.

The pulse duration necessary for fusion strongly depends on voltage, in agreement with Ref. 12. It is shown in Ref. 13 that the mean lifetime of the lipid bilayer is reciprocal to bilayers area. Taking this into account, and extrapolating the $\log \bar{\tau}(\varphi)$ curve (Fig. 2) to $\varphi = 0.8$ V, we obtain for a cell contact region of approx. 10^{-6} cm^2 area a lifetime equal to approx. 10 μs. This value is close to the duration of the pulse for $\varphi \approx 0.8$ V necessary for electrostimulated cell fusion [12]. This agreement indicates the applicability of a simple two-bilayer model system for exploration of the mechanism of electrostimulated cell fusion, as well as of different stages of membrane interaction, preceding fusion.

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