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Asymmetric lipid bilayers

Reponse to multivalent ions

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SUMMARY

Asymmetric lipid bilayers are formed by adjoining the hydrocarbon chains of two different lipid monolayers at the air–water interface through an aperture in a teflon partition separating two aqueous phases. It is shown that the addition of Ca^{2+} or poly-lysine to the compartment limited by a monolayer of the neutral lipids glycerol dioleate or phosphatidylcholine results in no modification of the resistance and stability of the membrane, whereas a drastic decrease in both parameters is elicited by the presence of these ions on the opposite compartment containing a monolayer of the negatively charged cardiolipin or phosphatidylserine. The surface-charge dependence of this phenomenon indicates the persistence of the asymmetric lipid distribution in the bilayer after its formation from two different monolayers.

In 1933, Osterhout[1] recorded a membrane potential in *Valonia* and *Nitella* when both outer and inner compartments were composed of the same electrolyte solution (the cell sap) and attributed it to a membrane asymmetry. Since then, considerable efforts have been directed towards the development of asymmetric model membranes; however, in some cases the system chosen has not been relevant to the biological one[2–4] or, in others, the methodology was proposed but no experimental results were published[5–8].

We have recently described the formation of bimolecular lipid membranes by the hydrophobic apposition of the hydrocarbon chains of two lipid monolayers at the air–water interface when an aperture in a teflon septum separating two aqueous phases is lowered through the interface[9]. The formation of asymmetric bilayers was presented and their identical electrical resistance and capacity to the symmetric membranes indicated.

In this communication, the distinct responses of asymmetric bilayers to multivalent ions, when these are added to the compartments limited either by a neutral or a negatively charged lipid monolayer, are reported.

The methods for the formation of the membranes and the study of their electrical properties have been previously reported in detail[9]. For these experiments, the thin teflon partition possessing the aperture was clamped between two halves of a trough and kept stationary. To form a membrane the monolayers were brought into contact by raising the water levels from two syringes located perpendicular to the plane of the monolayers. Best results were obtained by first adjoining one monolayer to the septum and then adjoining the second one. The size of the aperture varied from 0.25 to 0.6 mm in diameter without exhibiting any difference in the reported results, except for the stability which was considerably better when the small apertures were used. To improve the stability of the membranes, the septum was preconditioned by cleaning with *n*-pentane (Hopkin and Williams Ltd, Essex, England) or with pure vaseline, removing its excess with pentane. Glycerol dioleate was from Armak Chemicals Division (Philadelphia, Pa.); egg phosphatidylcholine, bovine cardiolipin and phosphatidylserine were from Applied Science Labs Inc. (State College, Pa.); poly-L-lysine·HBr (mol. wt 127 000) was from Miles-Yeda Ltd (Kiryat-Weizmann, Rehovot, Israel); and other chemicals were of the highest purity commercially available. Glass-redistilled water was used throughout. All experiments were carried out at room temperature (20 ± 2 °C).

Ca^{2+} effects. It has been shown[10,11] that the addition of Ca^{2+} to one of the aqueous compartments separating a hydrocarbon solvent-containing lipid bilayer, composed of negatively charged lipid phosphatidylserine, lowers the membrane resistance and at a certain concentration induces film breakage, whereas the presence of Ca^{2+} in both compartments increases the resistance and stabilizes the membrane. No significant effects are noted on neutral lipid bilayers (*cf.* ref. 12). Fig. 1 illustrates the effects of Ca^{2+} on an asymmetric membrane formed from a neutral lipid monolayer (glycerol dioleate) on one side and a negatively charged lipid monolayer (cardiolipin) on the other. In B, 0.6 mM $CaCl_2$ was added to the compartment limited by the cardiolipin monolayer. 10 min after its addition, the membrane resistance, which was initially $1.2 \cdot 10^7\ \Omega \cdot cm^2$ (Fig. 1A), dropped to $4.3 \cdot 10^5\ \Omega \cdot cm^2$ (Fig. 1B). In contrast, up to 20 mM $CaCl_2$ can be added to the compartment limited by the neutral monolayer without detecting any significant change in resistance ($0.825 \cdot 10^7\ \Omega \cdot cm^2$) (Fig. 1C). Moreover, the membranes became very unstable and prone to breakage in the first situation (Fig. 1B), while no deleterious effect was noted in the latter (Fig. 1C).

Raising the ionic strength of the aqueous solution with monovalent electrolytes prior to the addition of Ca^{2+} to the compartment limited by the charged monolayer considerably reduced its effects on the bilayer; this is the expected finding if the Ca^{2+} effect was due to electrostatic interaction between Ca^{2+} and the phosphate groups of the monolayer, the screening of this charge by increasing the ionic strength of the solution would significantly hinder or even nullify the interaction[13].

Polylysine effects. It has been shown[14] that the addition of polylysine to one of

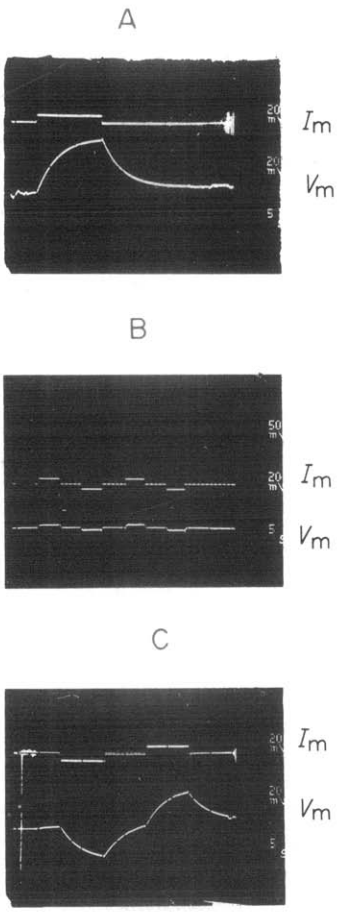

Fig. 1. Effects of Ca^{2+} on asymmetric lipid bilayers. The membranes are formed from one monolayer of glycerol dioleate and one of cardiolipin. The aqueous phase contained 1 mM NaCl and 1 mM Tris–HCl, pH 7.4. The formation of the membrane was followed with the measurement of the membrane capacity by the application of a constant voltage pulse of 20 mV and display of the capacitative current on a storage oscilloscope (for details see ref. 9). After formation, the membrane resistance (R_m) was measured by the application of a calibrated constant current pulse (I_m) and display of the resulting membrane potential (V_m). The clamping circuit used[9] allows switching between constant voltage and constant current modes. (A) Illustrates a membrane potential record as a function of an applied constant current ($8 \cdot 10^{-12}$ A) of the unmodified membrane; in (B), 0.6 mM $CaCl_2$ have been added to the compartment limited by the cardiolipin monolayer; in (C), 20 mM $CaCl_2$ have been added to the compartment limited by the glycerol dioleate monolayer. The aperture area was 0.196 mm^2. Vertical scale: $2 \cdot 10^{-11}$ A/division for current recording, 20 mV/division for potential recording. Horizontal scale: 5 s/division.

the compartments separating a symmetric negatively charged lipid bilayer increases the membrane conductance over 100-fold and confers to the film the property of anionic selectivity. The effects of polylysine on an asymmetric membrane are illustrated in Fig. 2. When $1 \cdot 10^{-8}$ M polylysine is added to the compartment limited by the cardiolipin

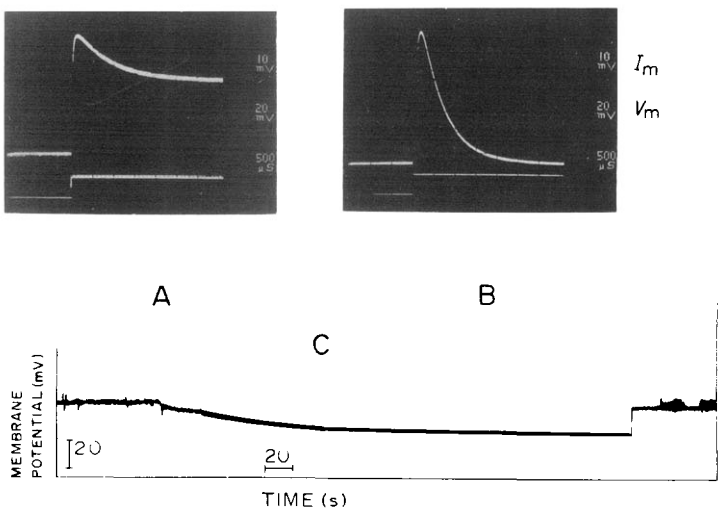

Fig. 2. Effects of polylysine on asymmetric lipid bilayers. Membrane currents at constant voltage (20 mV) in the presence of polylysine. The membrane was formed as in Fig. 1A from one monolayer of glycerol dioleate and one of cardiolipin. The aperture area was 0.048 mm^2. Polylysine was then added to the compartment limited by the cardiolipin (A) or the glycerol dioleate (B) monolayer at a final concentration of $1 \cdot 10^{-8}$ M. The initial phase of the current (spike) corresponds to the capacitative current. The steady component is associated to the ionic current[9]. It should be noted that while the capacitative transients in (A) and (B) are essentially similar, the ionic components are markedly different. From (A), the membrane resistance was calculated as $2.65 \cdot 10^5$ $\Omega \cdot cm^2$. In contrast, no significant modification of the ionic current is detected in (B), where the membrane resistance was calculated as $1.25 \cdot 10^7$ $\Omega \cdot cm^2$. In (C), the membrane was formed as in (A) and (B), in 10 mM NaCl and 1 mM Tris–HCl at pH 7.0; thereafter, $1 \cdot 10^{-8}$ M polylysine was added to the compartment limited by the cardiolipin monolayer. When the effects shown in (A) attained a stable state, a 3-fold salt gradient was established across the membrane by adding concentrated NaCl to the compartment containing polylysine. As a consequence, a potential difference across the membrane developed attaining a steady value of + 20 mV, the sign of the potential indicating that the species carrying the current is anionic. The return of the trace to the baseline indicates the instant when the membrane broke. (A) and (B) Vertical scale: 10^{-9} A/division for current recording, 20 mV/division for potential recording. Horizontal scale: 500 μs/division.

monolayer, the membrane conductance, initially $8 \cdot 10^{-8}$ $\Omega^{-1} \cdot cm^{-2}$ (Fig. 1A) increased to $3.8 \cdot 10^{-6}$ $\Omega^{-1} \cdot cm^{-2}$ (Fig. 2A) and remained stable at that value. Thereafter, a 3-fold salt gradient is installed across the membrane, and as a consequence an anionic membrane potential develops attaining a steady value of + 20 mV (Fig. 2C). As previously shown, this membrane potential varies with the log of the salt activity gradient across the membrane[14]. The surface charge dependence of this phenomenon is indicated by the lack of effect of polylysine when added to the compartment limited by the neutral monolayer (Fig. 2B)[14] and, as in the case of Ca^{2+}, by the masking effect observed when the ionic strength of the bathing solution is raised.

The effects of Ca^{2+} and polylysine can also be demonstrated when cardiolipin is substituted by phosphatidylserine, although these asymmetric membranes are more sensitive to multivalent ions and become very unstable after their adsorption.

These effects of multivalent ions on asymmetric lipid bilayers indicate the persistence of the asymmetric distribution of its lipid components when the membrane is assembled from two different lipid monolayers at the air–water interface. The mixing of the two monolayers during bilayer formation hence appears negligible, if it occurs at all. On the other hand, the rate of transmembrane migration ("flip-flop") of lipid molecules from one monolayer to the other in a bilayer[15] could, in principle, be determined directly from the time dependence of the effects of multivalent ions on asymmetric membranes as those hitherto reported.

The experimental model presented here constitutes a system in which the proposals on the behavior of asymmetric membranes[16,17] and the role of membrane asymmetry in redox and photoenergy transductions[18–20], as well as in other membrane functions (*cf.* refs 12, 21), can be experimentally explored by assembling a bilayer from two lipid monolayers.

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