

The Formation of Stable Transient Pores across Bilayer-coated Capsule Membranes by External Electric Fields¹

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The permeability of NaCl from bilayer-coated capsule membranes is reversibly controlled by an external electric field, because of the formation of stable transient pores in bilayers by transmembrane potentials.

Transmembrane potentials are believed to play a major role in biological processes. When the membrane potential exceeds a threshold value, the lipid bilayer (a poor conducting capacitor) seems to produce transient pores, allowing rapid passage of substances and particles (up to the size of genes).^{2,3} Extensive pulsation experiments of transmembrane potentials have been concluded on such convenient models as suspensions of liposomes³ and planar bilayers.⁴ However, in contrast to biological membranes, in the case of lipid bilayer membranes it has been impossible to induce by an electrical field any long duration (> 1 s), stable transient pores which have not been accompanied by mechanical rupture of the membranes.

Recently, we prepared a bilayer-coated capsule membrane which is formed by physically strong ultrathin nylon membranes and the coating shows characteristics of bilayer vesicles.⁵⁻⁸ We report here that the leakage of entrapped NaCl in such a bilayer-coated capsule membrane is reversibly controlled by an external electric field, and electrically induced, transient pores in bilayers can be stable for several minutes, in contrast to those of liposomes and planar bilayers.

Large nylon-2,12 capsules (diameter 2 mm; membrane thickness 1 μm) were obtained by interfacial polymerization

and dialysed against 0.2 M NaCl aqueous solution. The capsules were coated with synthetic bilayer-forming amphiphiles (sodium didodecylphosphate, $2\text{C}_{12}\text{PO}_4^-$) in dodecane solution at 60 °C by the method described previously.⁵

X-Ray diffraction and electron microscopy confirm that $2\text{C}_{12}\text{PO}_4^-$ amphiphiles on the capsule membrane exist as well-oriented, multiple-bilayers.⁷ The $2\text{C}_{12}\text{PO}_4^-$ bilayer-coat was also shown to have a phase transition temperature ($T_c = 45^\circ\text{C}$) between crystal and liquid crystal, as in the case of suspensions of bilayer vesicles.⁹ Exposure of the capsule to an external electric field was performed as shown in Figure 1; the bilayer-coated capsule was placed between two platinum electrodes (area 1 cm \times 1 cm; separation between the two electrodes 1 cm) connected to a high voltage power supply (d.c., 0–300 V) in deionized water. The permeation of NaCl trapped in the inner aqueous phase was measured by detecting increases in electrical conductance of the outer medium.

Figure 2 shows typical time courses of the NaCl leakage under the intermittent electric field. In the case of the uncoated, semipermeable capsule, the permeation was very fast and not affected by external voltages ranging between 30 and 100 V. In contrast, when the $2\text{C}_{12}\text{PO}_4^-$ bilayer-coated capsule membrane was employed, the permeation of

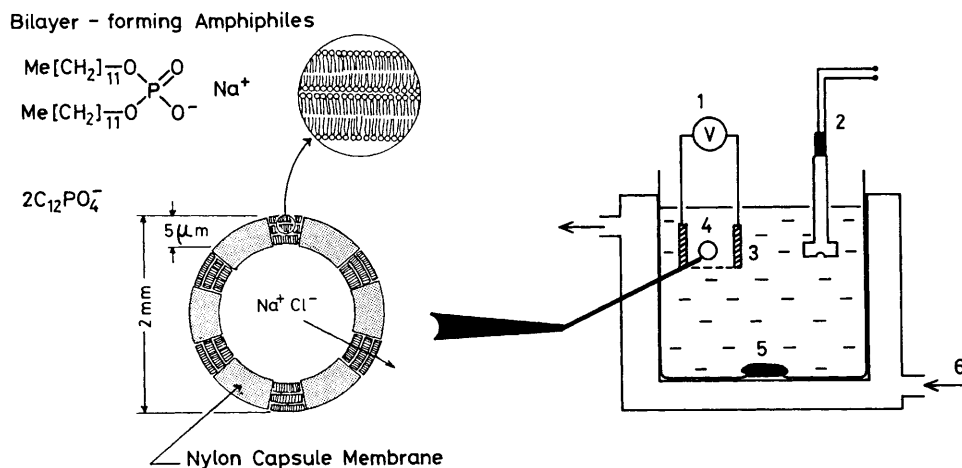


Figure 1. Apparatus for determination of NaCl leakage from the bilayer-coated capsule membrane under external electric fields. 1, High voltage power supply. 2, Conductance cell. 3, Pt electrodes (1×1 cm). 4, Bilayer-coated capsule membrane. 5, Stirring rod. 6, Thermostated circulating water.

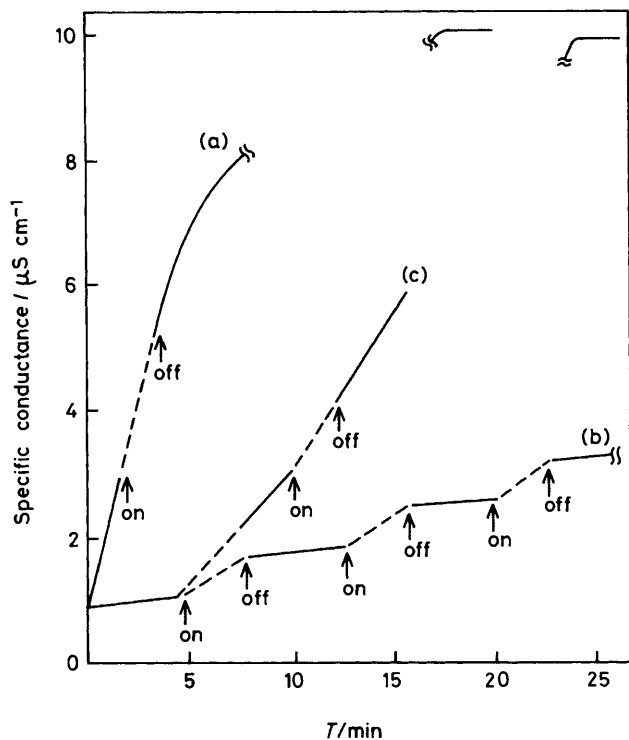


Figure 2. Electrically induced permeability regulation of NaCl across nylon capsule membranes at 25°C . (a) The uncoated capsule under 100 V field. (b) The $2\text{C}_{12}\text{PO}_4^-$ -coated capsule under 60 V field. (c) The $2\text{C}_{12}\text{PO}_4^-$ -coated capsule under 100 V field. The external voltage source was applied at on and ceased at off.

NaCl was reduced markedly because of the high barrier ability of the coating bilayer, and enhanced 15 times (permeability constant, $P = 9.0 \times 10^{-6} \text{ cm s}^{-1}$) under 60 V fields (corresponding to a voltage of 6 V across the membrane with a thickness of $1 \mu\text{m}$; 60 kV/cm) relative to that in the absence of the electric field ($P = 5.5 \times 10^{-7} \text{ cm s}^{-1}$). When the electric field ceased even after a long charging duration (3 min), the permeability reduced to the original slow rate ($P = 5.7 \times 10^{-7} \text{ cm s}^{-1}$). This permeability control could be repeated over and over again without damaging either the coating bilayers or capsule membranes. The electrically-induced permeability

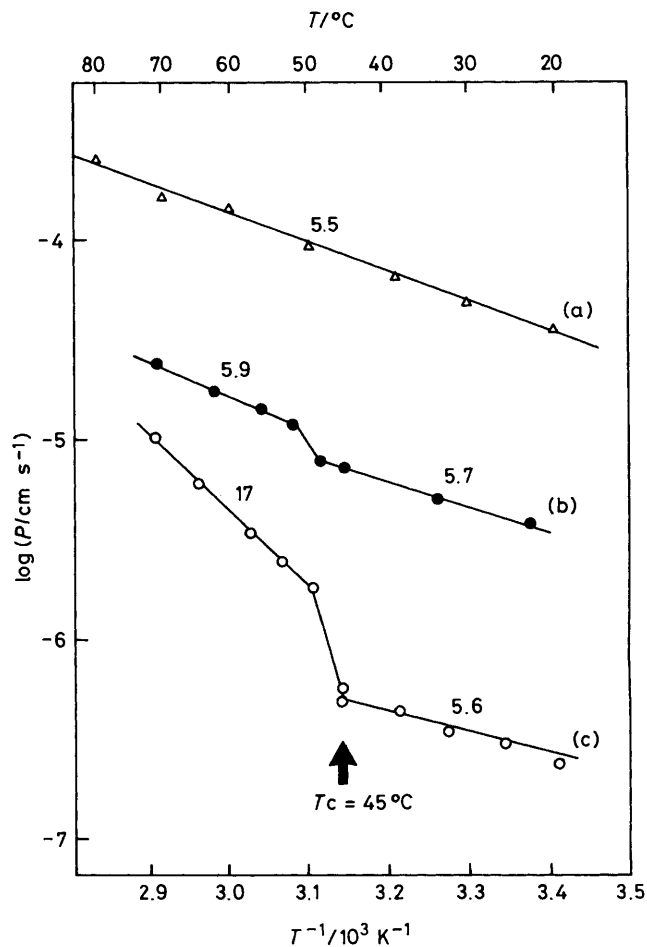


Figure 3. Arrhenius plots of NaCl permeation across nylon capsule membranes. (a) The uncoated capsule. (b) The $2\text{C}_{12}\text{PO}_4^-$ -coated capsule under a 60 V electric field. (c) The $2\text{C}_{12}\text{PO}_4^-$ -coated capsule in the absence of an electric field. Numbers are the activation energies (E_a , kcal/mol) obtained from the slopes. The arrow shows T_c of the coating $2\text{C}_{12}\text{PO}_4^-$ bilayers as obtained from differential scanning calorimetry.

enhancement was proportionally increased with increased electric field in the range 30–80 V. On increasing the voltage

above 80 V or increasing the charging duration beyond 5 min, the NaCl leakage did not revert to the original slow rate, probably because of rupture of the bilayer structures, as shown in curve (c) of Figure 2. The effect of a temperature increase or electrolysis was negligibly small under the conditions employed.

When submitted to such a strong external electric field, the coating bilayer acts as a capacitor because of the poor conductivity of the lipid bilayers, and the transmembrane potential could produce transient pores in the bilayer. Figure 3 shows Arrhenius plots of the permeability of uncoated and $2C_{12}PO_4^-$ -coated capsules with or without the external electric field. The numbers on the graph are the activation energies (E_a in kcal/mol)[†] obtained from the Arrhenius slopes. The uncoated, semipermeable capsule gave a straight line ($E_a = 5.5$ kcal/mol). In the absence of the electric field, the $2C_{12}PO_4^-$ -coated capsule gave a drastic inflection near T_c of the coating bilayers because of the phase transition from a rigid gel to a fluid liquid crystalline state. The permeation mechanism is explained as follows. At temperatures above T_c , the hydrated electrolyte, such as NaCl, permeates through the fluid, hydrophobic bilayer matrix with a relatively high activation energy ($E_a = 17$ kcal/mol). When the bilayer is in the rigid gel state below T_c , permeation through the bilayer matrix becomes difficult, and NaCl permeates slowly through defective pores in the coating bilayers instead. The E_a value below T_c (5.6 kcal/mol), then becomes similar to that of the uncoated capsule membrane ($E_a = 5.5$ kcal/mol), in which the NaCl permeation mainly proceeds by diffusion.

Under the influence of the electric field, the permeation of NaCl was accelerated 3–15 times compared with that in the absence of the electric field over the whole temperature range, and the Arrhenius plot gave only a small inflection near T_c . E_a Values (5.7–5.9 kcal/mol) obtained on application of less than a 60 V field are small, and nearly equal to that in the gel state of $2C_{12}PO_4^-$ bilayers in which NaCl permeates through defective pores of the bilayers. This shows that the transmembrane potential induced by the electric field generates

many transient pores in the coating bilayers, and NaCl permeates rapidly through these pores with a small activation energy.

By using the physically strong, bilayer-coated capsule membrane, stable, electrically induced transient pores are generated for a long period (3–5 min) in bilayers, in contrast to liposomes and planar bilayers, which may provide a new tool for studying the permeation mechanism of large substances such as genes across cell membranes.

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[†] 1 kcal = 4.184 kJ