Biochimica et Biophysica Acta, 982 (1989) 173–176
Elsevier

BBA 70610

BBA Report

Conductance transition induced by an electric field in lipid bilayers

Mauro Robello and Alessandra Gliozzi

Dipartimento di Fisica dell Università di Genova, Genova (Italy)

(Received 1 August 1988)
(Revised manuscript received 13 February 1989)

Key words Lipid bilayer, Membrane conductance Electric field effect Dipolar interaction

A cooperative phenomenon showing a structural change in the organization of bilayer lipid membranes at a critical value of the applied electric field is presented. The transition is characterized by a sharp increase in conductance. The phenomenon can be observed under current-clamp conditions (rather than the usual voltage-clamp conditions) to avoid rupturing the membrane At a critical potential value the conductance increases and therefore the potential decreases to keep the current constant. Results refer to membranes made of egg ph [illegible] phytanoylphosphatidylcholine and cholesterol/egg PC. It is found that the critical potential at which the transition occurs depends dramatically on pH and ionic concentration, indicating that the electrical properties of the external surface determine the major characteristics of such a transition

In recent years, the important role played by polar heads in lipid organization has been pointed out [1–5] The set of polar heads and bound water of neutral lipids can be regarded as a bidimensional dipole system with a well-defined structure [1,6] Therefore, at a certain critical value, an electric field is likely to induce a sharp variation in the structural organization of the polar heads of a membrane kept at a fixed temperature In a bilayer lipid membrane, the direct consequence of this can be a variation in an electrical parameter like conductance or capacitance However, up to now, no critical phenomenon associated with the presence of a steady electric field has been noted, since a steady potential on the order of 200–300 mV causes the membrane to rupture Higher potentials of the order of 1 V applied for very short time (hundreds of microseconds) cause electric breakdown [7–9] It is generally accepted that this phenomenon is based on the formation of pores in the lipid area of the membrane In this paper we report on complementary experiments in which the formation of new permeation pathways is obtained at a critical value of the electric field

In our experiments the external electric field is applied under 'current-clamp' conditions rather than the usual 'voltage-clamp' conditions At a critical value of the potential, the membrane exhibits a sharp transition to a higher conductance, indicating the formation of aqueous pores

'Solvent-free' (solvent-depleted) membranes were obtained by using a technique that is similar to the one described by Montal and Mueller [7] Monolayers were formed on aqueous solutions, and the double layers were formed on a 200 μm diameter hole in a thin Teflon septum (12 5 μm thick) separating two small Teflon chambers with a capacity of about 2 ml each

The following lipids were utilized: egg PC, diphytanoylphosphatidylcholine, phosphatidylserine from bovine brain (Avanti Polar Lipids), 1-monooleoyl-*rac*-glycerol (Sigma Chemical Co) and cholesterol (Calbiochem) All these compounds were highly TLC purified (> 99%) The electrolytic solutions were prepared using analytical grade salts (Carlo Erba) buffered with Tris-chloride (Trizma, Sigma Chemical Co) All experiments were performed at room temperature

The electric signals were recorded by means of two Ag | AgCl electrodes The system for measuring the current under voltage-clamp conditions and the membrane capacitance has been previously described [11] Under current-clamp conditions, the membrane is in a feedback network of the operational amplifier (Burr Brown 3528 CM), at high impedance (10^{13} Ω), which acts as a current–voltage converter The current–voltage (I–V) characteristics in the current-clamp condition were obtained by sending the membrane a triangular signal,

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Correspondence A Gliozzi Dipartimento di Fisica, Università di Genova, 16146 Genova, Italy

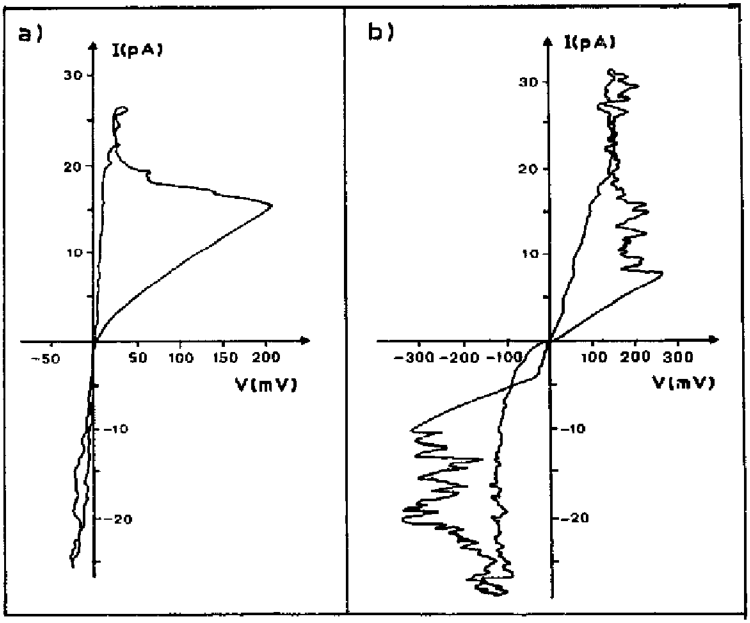

Fig 1 Current–voltage characteristics of membranes made of (a) egg PC/cholesterol (molar ratio 3 1) and (b) egg PC Notice that the presence of cholesterol stabilizes the membrane in the state of higher conconductance In case (b) the phenomenon is almost completely reversible In both cases the ionic concentration was 10^{-1} M KCl at pH 7

usually with an 8–10 min period Moreover, during the I–V cycle, it was also possible to measure the membrane capacitance as a function of the potential This measurement was made after sending a 10 mV peak to peak and 10 kHz input sinusoidal signal through an adder circuit. By using an appropriate decoding circuit, an output signal was obtained that was proportional to the membrane capacitance The measurement sensitivity was of the order of 1%

Fig 1a shows a current–voltage relationship obtained under current-clamp conditions for a 3 1 (mol/mol) egg PC/cholesterol membrane, separating two symmetrical aqueous solutions of 10^{-1} M KCl at pH 7 Fig 1b refers to a pure egg PC membrane under the same experimental conditions As can be seen, the I–V function is initially almost linear but, at a critical value of the potential, the membrane exhibits a sharp transition to a ten-times higher conductance, passing through a state of negative dynamic resistance Since experiments are performed under current-clamp conditions, the increase in conductance occurs together with a decrease in the potential. Moreover, in some cases, the membrane remains in a state of high conductance, even when the current is decreased or inverted (as in Fig 1a), whereas, in other cases (as in Fig 1b), the phenomenon is partially reversible However, the I–V curves do not appear entirely symmetrical about the origin, exhibiting a kind of hysteresis or after-effect Voltage fluctuations which appear after the transition are much higher in egg PC or diphytanoylphosphatidylcholine than in egg PC/cholesterol membranes and their amplitude is reduced by increasing the cholesterol/PC ratio This is probably due to the well-known mechanism of immobilization of phospholipids by cholesterol [12] If the I–V run is repeated several times on the same membrane, the critical potential generally decreases in the second run by 20% and then reaches an almost constant value, while the increase in conductance is always of the same order of magnitude The phenomenon was not affected by reducing the period of the current signal from 10 min to 3 min. Fig 2 presents the results of current–voltage and current–capacitance measurements made on the same membrane at the same time It is widely known that when a potential V is applied to a membrane, the capacitance varies according to the relationship

$$C(V) = C_0(1 + \alpha V^2) \tag{1}$$

where α is a coefficient depending on the amount of

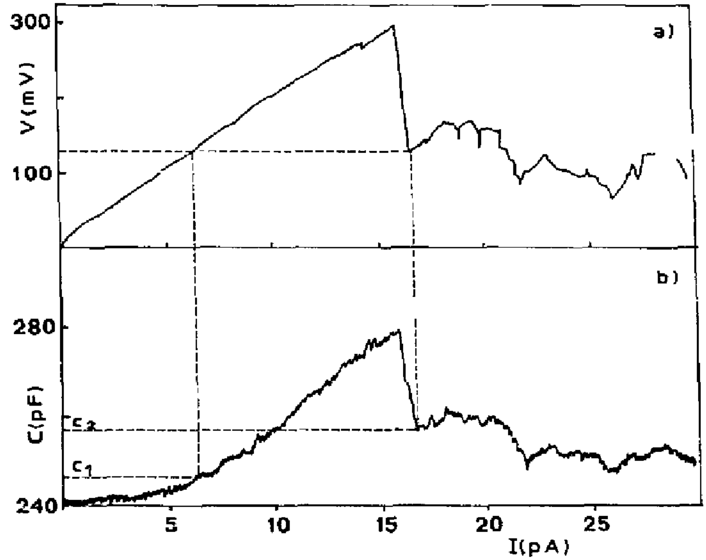

Fig 2 Capacitance–current characteristics for an egg PC membrane (b) The voltage current behaviour for the same membrane has been reported on top (a) Notice that there are two different values of capacitance for the same applied potential before and after the transition Other experimental conditions are as in Fig. 1

solvent contained in the membrane and, to a smaller extent, on the lipid type [13–16] The values of this coefficient range from 2 10^{-2} to 10 V^{-2}. In the membranes considered in the present paper, α is of the order of 1 V^{-2} and C_0 is about 0 6 $\mu F/cm^2$ This fact indicates that although these membranes are considered to be solvent free, small amounts of solvent are still present in the membranes themselves Fig. 2 shows two different effects (i) prior to the transition, the capacitance varies according to the square of the potential, (ii) the transition involves an increase of about 10 pF As an example, Fig 2 compares the values C_1 and C_2 they are related to the same potential before and after the transition, respectively It is worth noting that the difference in these values is almost one order of magnitude greater than the measurement sensitivity. This variation is irreversible and seems to be due to the usual mechanisms by which the capacitance varies with the potential, e g, solvent ejection or formation of microlenses To test this hypothesis, the membrane was subjected to several I–V cycles and the corresponding changes in capacitance were measured Capacitance changes gradually decreased and after 5–6 runs no further capacitance variation (within 1% accuracy) was detected Thus, these membranes, whose specific capacitance is 0.75 $\mu F/cm^2$, can be safely considered to be solvent-free. This experiment highlights two results Firstly, it shows that capacitance changes are indeed due to solvent ejection (or formation of microlenses) and this process seems to occur mainly (but not exclusively) during the transition Secondly, it indicates that small amounts of solvent do not affect the phenomenon Additional experiments performed on membranes with different values of specific capacitance (in the range 0 5–0 7 $\mu F/cm^2$), indicated no correlation between this parameter and the critical potential at which the transition occurs By contrast, a dramatic dependence was observed upon pH and ionic concentration of the external electrolyte solution The critical potential at which the conductance

TABLE I

Critical potential V_c, for the first transition of membranes of different composition in KCl at the indicated conditions

Each number is the mean value of at least 10 membranes

Lipid	Molar ratio (mol/mol)	pH	Concn (M)	V_c (mV)
Diphytanoyl-PC	–	7	10^{-1}	390±70
Egg PC	–	7	10^{-1}	280±70
Egg PC/cholesterol	2 1	7	10^{-1}	270±70
Egg PC/cholesterol	3 1	7	10^{-1}	270±70
Egg PC/cholesterol	3 1	4	10^{-1}	190±70
Egg PC/cholesterol	3 1	3	10^{-1}	140±70
Egg PC/cholesterol	3 1	7	10^{-2}	330±50
Egg PC/cholesterol	3 1	4	10^{-2}	320±60
Egg PC/cholesterol	3 1	3	10^{-2}	310±30

transition takes place in PC and PC/cholesterol membranes in several experimental situations is given in Table I Since pure egg PC has a very low pK, one would expect no pH dependence for such a lipid in the pH range covered by this study, as indeed observed in 10^{-2} M KCl The pH dependency in 10^{-1} M KCl is probably due to adsorption of ions which might vary the electrical properties of the membrane surface

With lipids with different polar heads, e g , monoolein and phosphpatidylserine at pH 7 in 10^{-1} M KCl, a completely different behaviour is observed For monoolein, in 80% of the cases membranes ruptured at about 150–200 mV, while only in 20% of the cases in this potential range was a sudden change in conductance observed, followed almost immediately by the rupture of the membrane Also in these membranes voltage fluctuations up to 100 mV were detected after the transition For phosphatidylserine no transition was observed up to potentials as high as 500 mV

The phenomenon we have described indicates that a sharp variation in the membrane structure has been induced by the external electric field at a critical value of the potential Such a variation gives rise to hydrophilic paths that increase the membrane conductance, which is usually in the range 20–50 pS/cm^2, by one order of magnitude It is worth noting that this phenomenon cannot be observed under voltage-clamp conditions, as the transition to the new state of very high conductance (which can be obtained by extrapolating the higher conductance curve to the critical potential value V_c) would cause the membrane to break

We suggest that the electrical layer, composed of headgroup dipoles on phosphatidylcholine molecules, bound water and ions, rearranges in an external field and this conformational transition affects the permeation properties of the bilayer This interpretation is in agreement with models for the dynamical behaviour of the phosphatidylcholine headgroup [17], which show that there are several local energy minima, in the range 0–5 5 kcal mol^{-1}, with respect to the energy of the global minimum The energy conferred to the headgroup layer by applying to the membrane 200–300 mV is precisely within this range

The authors are indebted to Dr Elisabetta Bianco for her help in portions of the experimental work This work has been supported by a MPI (Ministero della Pubblica Istruzione) grant

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