PHYSICAL REVIEW E VOLUME 52, NUMBER 4 OCTOBER 1995

Nonlinear dc electrical response in a bilayer lipid membrane: Effect of bathing solutions

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(Received 28 November 1994)

The physical origin of a nonlinear electrical response in a bilayer lipid membrane (BLM) separating two aqueous bathing solutions has been attributed by earlier workers to field induced pore formation in the lipid moiety, which provides ionic pathways. Recently, we reported similar nonlinearity in the absence of any ion source, viz., in solid films of lipids. The study ruled out the possibility of pore formation being the only mechanism of transmembrane conductance. Using the BLM of oxidized cholesterol in *n*-decane, we report here our studies on the role of bathing soution on the *I-V* characteristics of these membranes. The nonlinearity is maintained in the presence of an electron donor as well as an electron acceptor in the bathing solution, although there is a distinct difference in the nature of the nonlinearity in the two cases. Our results indicate that three factors, namely, pore formation, the semiconducting properties of the constituent lipid molecules, and the mechanism of transmembrane charge transport, are responsible for the observed nonlinearity.

PACS number(s): 87.22.Bt, 87.22.Fy, 72.15.Cz, 72.80.Le

INTRODUCTION

Charge movement across biological membranes is the key to several natural processes. However, in spite of the increasingly detailed description of ion permeability and membrane structure, the exact mechanism by which ions and other species transport across biological membranes is not yet known. To understand the molecular processes involved in electric conduction in more complex natural membranes, charge transport through an artificially constituted bilayer lipid membrane (BLM) has been studied as a model process by several authors [1–6].

An interesting aspect of the electrical behavior of a BLM in an aqueous bathing solution is the transition from a linear to nonlinear domain above a certain critical field value. It was suggested by earlier workers that the nonlinearity is caused by the transport of charges through pores [1–6]. These pores are formed above a certain field strength as a result of inversion of the spontaneously arising hydrophobic pores in the lipid moiety. The nonlinearity in the *I-V* characteristics arises from a shift in the distribution of these pores toward wider radii with an increase in applied voltage [1]. The critical field at which the structural change occurs is a function of the pH and ionic concentrations of the bathing solution [2], and hence the major characteristics of the transition from linear to nonlinear electrical responses of the BLM are probably monitored by the outer surface of the membrane. The frequency dependence of the bilayer impedance in a BLM predicts several substructural regions within the bilayers where the pores are formed [5].

Glaser and Leikin have shown [4] that the height of the energy barrier to be overcome for pore formation decreases as the square of the applied potential (V). Thus the rate of formation of these hydrophilic pores is proportional to V [2]. The mean size of these pores is a function of V, and in order to cross the membrane through such narrow pores the ions have to overcome another energy barrier, whose height is also a function of V. Thus the applied field influences in a nonlinear way the number, mean size, and conductivity of the pores. Barnett extended the calculation to account for the influence of not only the size but also the shape of the pores in charge conduction [7].

In order to verify the hypothesis of ions being the only transmembrane charge carriers through electrolyte-filled hydrophilic pores, we recently reported our study on the electrical response in thin films of lipids [8]. In spite of the absence of any ion source in this case, the dc *I-V* characteristic curve shows a similar deviation from linearity above a certain field. The Ohmic nature, however, is recovered with the increase in frequency of an applied ac field. This study indicated that besides pore formation, some other characteristic properties of membrane constituent molecules were also responsible for the observed nonlinearity. One such property of lipids is that they are intrinsic semiconductors [9], implying that the nature of charge carriers in the dry state of lipids is primarily electronic [10], and that the nonlinearity is probably governed by this property only.

For lipids (as well as proteins) in the hydrated state, conductivity is predominantly ionic [10,11], owing to the contribution from ionic charge carriers that transport through the hydrophilic pores formed in the lipid matrix. It has been reported that the electrical conductivity of the hydrated BLM can be changed by several orders of magnitude by adding electron acceptors (donors) in the bathing solution [12]. The possible explanation for this change in the conductivity lies in the fact that the pres-

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1063-651X/95/52(4)/4179(4)/$06.00

ence of electron donors (acceptors) in the bathing solution changes the intrinsic semiconducting nature of the lipids to an *n*-type (*p*-type) extrinsic semiconductor [13], thereby increasing the conductivity many times.

To elucidate the role the bathing solution plays in determining the nature of the dc *I*-*V* characteristics in a BLM, here we report our studies using a BLM of oxidized cholesterol in *n*-decane in the presence of an electron acceptor (iodine) as well as an electron donor (acridine orange) in the bathing solution under a dc electric field. Our results show that nonlinearity in the *I*-*V* characteristics at high voltage is obtained in both these cases, although there is a distinct difference in the nature of the transition. The observed nonlinearity has been interpreted in the light of pore formation in the lipid matrix, the semiconducting property of the lipid molecules, and the specific mechanism of transmembrane charge transport in a BLM.

MATERIALS AND METHODS

Cholesterol, purchased from Sigma Chemical Co. (St. Louis, MO), was oxidized and then recrystallized from *n*-octane [14]. Analytical reagent grade chemicals from E. Merck Ltd. (Worli, Bombay) were used. Iodine was purified by resublimation.

A BLM (100 Å thick) was formed by brushing a saturated solution of oxidized cholesterol in *n*-decane on a 1.5-mm-diameter hole in a teflon cup separating two bathing solutions placed in a plexiglass container (Fig. 1) [15]. Platinum electrodes of 0.1-cm diameter were used for the application of an electric field across the BLM (interelectrode distance 2 cm). Bathing solutions containing iodine and acridine orange (of concentration 1 mM each) were used to study the effects of electron acceptors and electron donors, respectively, on the dc electrical response of a BLM.

A laboratory made device was used to supply the constant dc voltage across the membrane. Current and voltage were measured by using Keithley electrometers (models 614 and 617, Keithley Instruments Inc., Cleveland, OH).

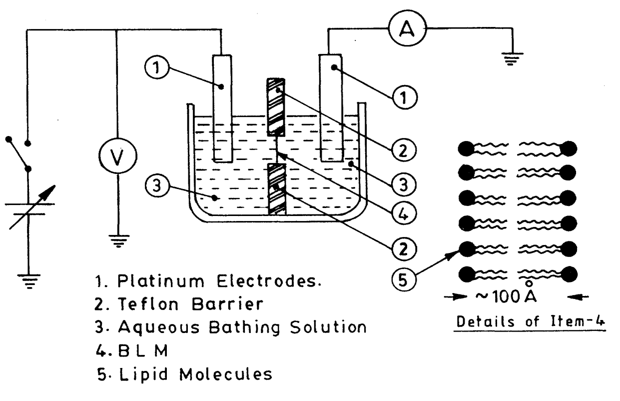

FIG. 1. Scheme of the experimental arrangement.

RESULTS AND DISCUSSION

The voltage drop across the BLM is

$$V_m = V/(1+R_1/R_2) ,$$

where V is the actual voltage applied across the electrodes, R_1 is the resistance of the intervening bathing solution, and R_2 is the membrane resistance which is a function of the applied voltage V. Typical values of these parameters in the presence of an iodine saturated bathing solution and for an interelectrode potential of 50 mV are as follows:

$$R_1 \sim 1.4 \times 10^6 \ \Omega$$

$$R_2 \sim 3.8 \times 10^6 \ \Omega .$$

The calculated value of the electric field across a BLM of 100-Å thickness in this case is 10^4 V/cm.

Even at such high field value there is no significant electroconvection effect as the molecular configuration of the interior of a BLM (an aggregate of hydrophobic fatty acyl chains of lipid molecules with dielectric constant ~2–3) is such that it will not permit charge transport *en masse* across it.

In Fig. 2 we have plotted the dc current I across a BLM of oxidized cholesterol in *n*-decane separating the two symmetric bathing solutions against the applied voltage V. The characteristic curves show a deviation from linearity above a certain field in the presence of either acridine orange (an electron donor) or iodine (an electron acceptor) in the bathing solution (Fig. 2). However, there is a distinct difference between the two in the region where the transition from linear to nonlinear electrical response takes place. In the presence of acridine orange and above the critical field value, the current increases gradually from its Ohmic value [Fig. 2(a)]. This is similar to the behavior noted for a solid film of lipids [8] and in a BLM in an aqueous bathing solution (our unpublished result), as well as in the presence of an electrolyte in the bathing solution [1]. In the presence of iodine, however, the increase of current is stepwise from the linear to nonlinear region [Fig. 2(b)].

Thus our observation is that nonlinearity in the dc *I*-*V* characteristics in a BLM arises as a rule rather than as an exception. In order to elucidate the mechanism of trans-BLM charge transport, it is important to consider what motivates the charge carriers to go through the highly insulating medium of lipid assembly whose dielectric constant is 2–3. The only possible answer is that there must exist polar pathways through which the charge carriers can proceed. In the case of a BLM, for ionic conduction to be possible these paths must be provided by the water-filled randomly distributed pores in the lipid milieu.

Figure 2 reveals that above a certain voltage the Ohmic nature of the *I*-*V* curve changes to a non-Ohmic one, and I becomes a nonlinear function of V. The empirical relation between current (I) and voltage (V) may be written in the form [16,17]

$$I = \sigma_1 V + \sigma_2 V^2 ,$$

where σ_1 is the Ohmic and σ_2 is non-Ohmic conductivi-

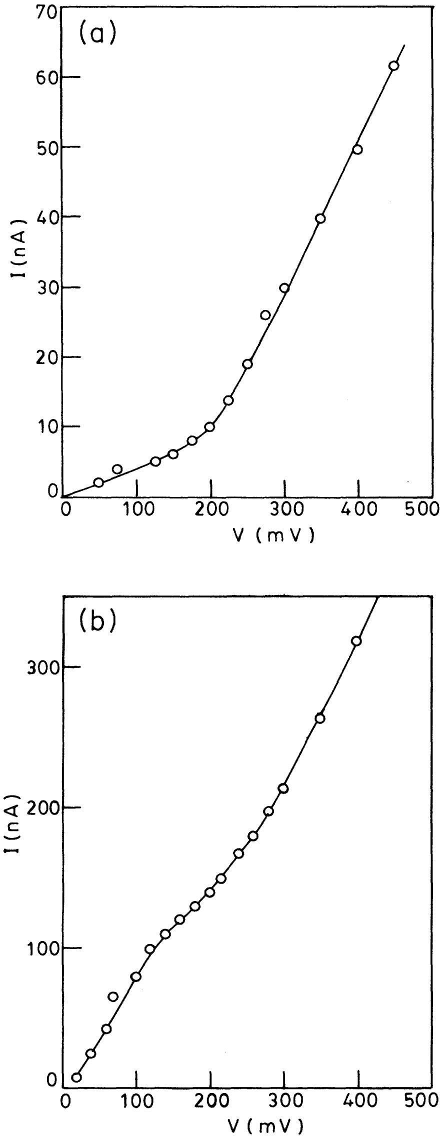

FIG. 2. Current-voltage characteristic curves in the BLM of oxidized cholesterol in *n*-decane in an aqueous bathing solution in the presence of (a) acridine orange (concentration: 1 m*M*), and (b) iodine (concentration: 1 m*M*).

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To explain the charge transport phenomenon through a binary mixture, Gefen *et al.* put forward two models [16], one of which is the nonlinear random resistor network (NLRRN) model. This model was used by Gefen *et al.* [16] in the case of a discontinuous thin gold film to describe in-plane propagation in a two-dimensional matrix consisting of a set of nonlinear random resistors (hence the name) that are linked in a gridlike structure. The model has also been successfully applied by Chakrabarty, Bardhan, and Basu [17] to explain charge transport across a three-dimensional system of a carbon-wax mixture of low conductance.

Our preliminary investigation shows that this model can perhaps be applied to describe trans-BLM charge transport, the rationale for which is as follows. Thermal fluctuations in the bilayer structure produce transient defects that become filled with water from the bathing solution [1]. These pathways in the otherwise nonconducting lipid moiety are in all probability randomly oriented and may be considered to serve as microscopic components of the NLRRN model. At higher field values these microscopic components aggregate to form pores, the size, frequency, and conductivity of which are nonlinear functions of voltage [4].

In order to explain the difference in the pattern of nonlinear *I*-*V* characteristics in the presence of electron acceptors or donors in the bathing solution, we note that the extrinsic semiconducting nature of the lipid moiety is different in the two cases. While the electron donors decrease the band gap energy E_g and induce an *n*-type semiconductivity in the lipid aggregate structure, the effect of electron acceptors is to induce a *p*-type semiconductivity along with a decrease in E_g. In both cases there is a substantial rise in the conductivitities [12].

Now let us consider the case when iodine is present in the bathing solution. The band gap energy E_g in this case decreases from 2.02 to 0.98 eV [12], and the lipid ensemble acquires a *p*-type semiconductivity, implying that the majority charge carriers are holes. Initially the current increases with voltage owing to hole conduction. At higher values of the applied field, ions from the bathing solution move toward the BLM, which by virtue of its double electrode behavior [18] injects electrons at the lipid-water interface. These electrons tend to recombine with the holes. At a critical field the effect of recombination becomes significant and the current reaches a steady value. When the process of recombination is over, both electrons and holes contribute to conduction, and the current increases with increasing voltage.

The situation is altogether different when acridine orange, an electron donor, is present in the bathing solution. The majority charge carriers in this case are electrons. At higher voltages, when there is an injection of electrons at the interface, the current continues to increase with an increase in the applied field.

Thus from our experimental observations with different BLM systems and with thin solid films of lipids, we may conclude that the possible factors which contribute to the nonlinear electrical response in these systems are as follows.

(a) Pore formation in the lipid aggregates in the hydrated state, the number, size, and conductivity of these pores being nonlinear functions of the applied voltage [1,4,5]. The repulsive action among the ions moving through the pores attenuates the ion current through the membrane. The attenuation factor, which is a measure of the interionic interaction, is a function of ion concentration, dielectric constant, and pore geometry [19].

(b) The characteristic semiconducting property of the lipid molecules changes from an intrinsic to an extrinsic one in the presence of electron donors and acceptors in the bathing solution [12]. This property monitors the finer details of the *I*-*V* characteristic curves of the BLM in the presence of a bathing solution.

(c) pH and ion concentration of the bathing solution, as the critical field value is a function of these parameters [2].

The BLM is a widely accepted model for biological membranes through which most of the life sustaining charge transport processes take place. In this context our observations reported here and the conclusions drawn therefrom are of significance for a better understanding of the related biological processes.

ACKNOWLEDGMENTS

We are thankful to Professor E. Sackmann for the gift of the BLM chamber, and to Dr. K. Bardhan, Dr. S. Basak, and Dr. A. Nandy for stimulating discussions. We are grateful to the following Central Government agencies for providing us with financial assistance: University Grants Commission, Council of Scientific and Industrial Research, and Department of Science and Technology.

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