brief communication

Nonlinearity in the I-V characteristics in thin lipid films
Effect of AC and DC fields

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ABSTRACT The current-voltage characteristics of bilayer lipid membranes of oxidized cholesterol separating two bathing solutions have already been extensively studied under a DC electric field. The observed deviation from linearity at high field has been explained by field-induced pore formation, which then act as ion channels in the membrane. Using thin films of oxidized cholesterol and of dipalmitoyl phosphatidylcholine, we have reported for the first time similar deviation from nonlinearity in the DC I-V characteristics when the applied field is above 40 V/cm. Upon application of an AC field, the conductivity increases as square of frequency, while the nonlinear nature of the I-V characteristic curve is still retained at all frequencies up to 5,000 Hz. Our results indicate that besides pore formation, the intrinsic electrical properties of the constituent lipid molecules are also responsible for the observed nonlinearity.

INTRODUCTION

The current-voltage characteristic curves of bilayer membranes of oxidized cholesterol separating two bathing solutions show a deviation from linearity above a certain value of applied electric field, and the transition is characterized by an increase in conductance (1). This value depends on the pH and ionic concentration of the bathing solution, indicating that the major characteristics of such transition are monitored by the outer surface of the membrane (2). The observed nonlinearity in these model systems has been explained by suggesting field-induced transient pore formation in the lipid media (1, 3). The pores that are formed initially by spontaneous fluctuation grow in size as the field intensity increases, and after reorientation of lipid molecules the hydrophobic pores change to hydrophilic ones. These then act as channels for ion transport, thereby increasing the conductivity (4). The frequency dependence of bilayer impedence predicts several substructural regions within the bilayers where the pores are formed (5). As there is no common view on the structure and mechanism of pore formation, extensive studies, both theoretical and experimental, have been done on model systems of planar bilayer membranes (1, 6).

To compare our results with those obtained by others, we have chosen oxidized cholesterol for forming thin lipid films and have observed for the first time, similar nonlinearity in the DC I-V characteristic curves for electric fields above 40 V/cm. Upon application of an AC field, the conductivity increases as square of the frequency while the prominent nonlinear nature of the DC I-V characteristic curve is still retained up to a frequency of 5,000 Hz. Similar results were obtained using thin films of a phospholipid, namely dipalmitoyl phosphatidylcholine. As there is no bathing solution in these cases, we may neglect the number of ions, if any, being transported across the membrane through the field-induced hydrophilic pores acting as ionic channels.

Thus, our experimental results show that nonlinearity appears in the I-V characteristic curves in bilayer membrane separating two bathing solutions as well as in thin solid films of lipids. This indicates that besides pore formation and the nature of the bathing solution, the characteristic semiconducting properties of the lipid molecules are also responsible for the observed nonlinearity.

MATERIALS AND METHODS

Cholesterol and dipalmitoyl phosphatidylcholine were purchased from Sigma Chemical Co. (St. Louis, MO). Cholesterol was oxidized and then recrystallized from *n*-octane (7). AR grade chemicals supplied by E. Merck Ltd. (Worli, Bombay) were used without further purification.

Thin film of lipid (oxidized cholesterol or dipalmitoyl lecithin) was prepared by repeatedly dipping a tin oxide–coated glass plate (courtesy of Professor A. Pal of the Indian Association for Cultivation of Science, Calcutta-700032) in chloroformic solution of the lipid (0.1 M concentration). To remove traces of solvent the film was kept in vacuum for 10–12 h. The experimental sandwich cell consisted of this glass plate containing the thin lipid film (2×2 cm) on which another tin oxide–coated glass plate was placed in such a way so that the lipid film lies between the two conducting faces of the glass plates. Separation as well as insulation between these plates was maintained by placing teflon spacers (~ 50 μm thickness) around the edge of the lipid film. The cell was connected with the external circuit by using a pair of spring clips (Fig. 1). A laboratory made device was used to supply the constant DC voltage to the cell. The AC field was applied using a HIL 2821 function generator (Hindusthan Computers Ltd., Calcutta). Current and voltage (both DC and AC) were measured by using Keithley electrometers (models 614 and 196; Keithley Instruments Inc., Cleveland, OH).

RESULTS AND DISCUSSION

The I-V characteristic curves for thin films of oxidized cholesterol as well as dipalmitoyl phosphatidylcholine under a DC electric field show deviation from linearity when the electric field is above 40 V/cm. Fig. 2 shows the variation of current with applied voltage for thin film of oxidized cholesterol. The result obtained with bilayer membrane of the same lipid, separating two bathing solutions of 0.01 M KCl, is similar to that for thin film (our unpublished results) and agrees well with the result obtained by others (1). With the application of an AC elec-

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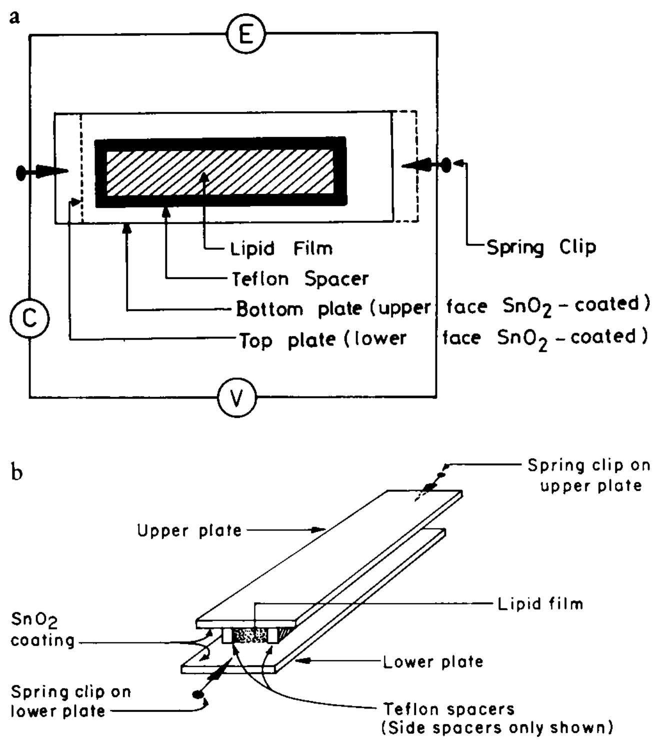

FIGURE 1 A schematic diagram of the experimental cell. The thin lipid film is placed between the two tin-oxide (SnO_2)-coated glass plates; the separation and insulation between the two plates was maintained by teflon spacers of 50 μm thickness. (Figures drawn not to scale.) (*a*) Top view. *E, C, V* are the voltage source and the electrometers for measuring current and voltage, respectively. (*b*) Stereo view.

tric field in the thin film, the I-V characteristic curve retains its nonlinear nature at all values of frequency up to 5,000 Hz (Fig. 2), while the conductivity increases as square of frequency (Fig. 3). (Due to technical limitations, frequencies higher than 5,000 Hz could not be applied.)

From our result and from others (1–6), it is evident that nonlinearity in the current-voltage curve appears both in planar bilayer membrane, separating two bathing solutions, as well as in thin solid films. Fig. 2 shows that there is no break in the I-V characteristic curve up to a frequency of 5,000 Hz. This indicates that an electric field produces a rather progressive effect in thin lipid film which is contrary to the effect that it produces in lipid bilayers. The latter probably arises due to the presence of bathing solution, which serves as the ion source leading to ion transport through the field-induced pores in the membrane. From the dielectric spectroscopy study of electrical conduction in lecithin lipids, both in the hydrated as well as in the dry state, an ionic (proton) conduction mechanism in lipids has been concluded by Szundi (8). However, using systems of different biomolecules, like lipids as well as proteins, Rosenberg and Postow (9) have shown that charge conduction is predominantly ionic in hydrated state whereas it is electronic in the dry state. Our observation partially supports their conclusion.

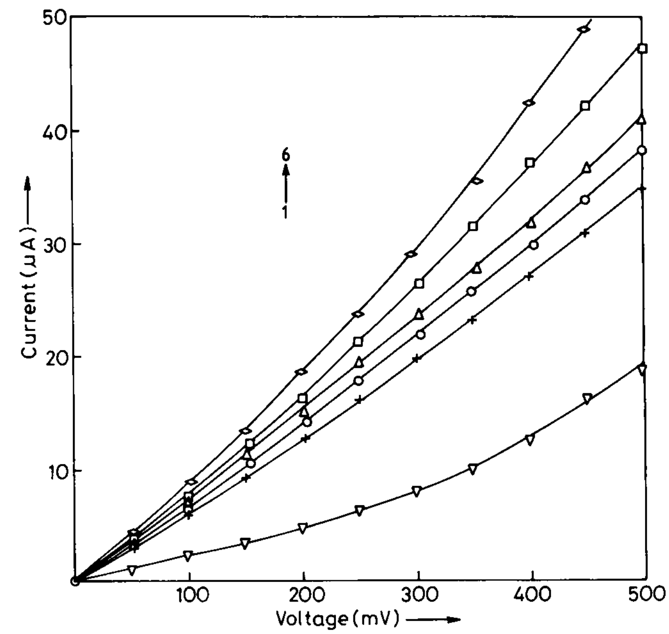

FIGURE 2 Current-voltage characteristic curve in thin solid film of oxidized cholesterol under AC and DC electric fields. Nonlinearity onsets above a critical value of 200 mV, which is equivalent to a field of 40 V/cm. (*1*) DC field; (*2*) 1,000 Hz; (*3*) 2,000 Hz; (*4*) 3,000 Hz; (*5*) 4,000 Hz; and (*6*) 5,000 Hz.

It seems that the appearance of nonlinearity in the I-V curve does not depend only on the formation of pores and presence of bathing solution, as has been concluded earlier (1–6), but also on some characteristic electrical

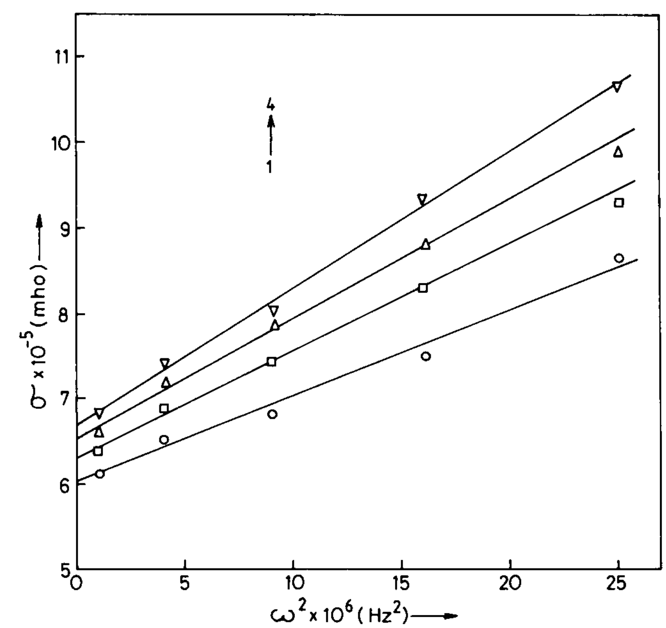

FIGURE 3 Variation of conductivity with square of frequency of the applied AC field in thin solid film of oxidized cholesterol. (*1*) 100 mV; (*2*) 200 mV; (*3*) 300 mV; and (*4*) 400 mV.

properties of the lipid molecules forming the membrane, bilayer, or thin film as the case may be. This, added to the fact that there is a variation of conductance with frequency, indicates that molecular organisation in this system plays an important role (5, 10). Merely representing our experimental cell by a parallel arrangement of resistance and capacitance, the circuit analysis reveals a direct proportionality between the total admittance and frequency, contrary to our result (Fig. 2).

An important characteristic electrical property of lipids, which has been well established, is that they are intrinsic semiconductors (11). It has been reported that presence of several salts, electron acceptors, and donors in the bathing solution can affect the electrical conductivity of bilayer lipid membranes to a large extent (12). This can be explained on the basis of electronic conduction through these membranes. Here the membrane acts as double electrode, oxidizing on one end and reducing on the other (13), while the presence of some of these agents change the intrinsic semiconducting nature of the lipids to an extrinsic one (14). However, the frequency (w) dependence of conductance (σ) in case of intrinsic semiconductors is as follows (15):

$$\sigma = \sigma_0(1 - aw^2\langle\tau\rangle^2);$$
$$a = \langle\tau^3\rangle/\langle\tau\rangle^3,$$

where σ_0 is the DC electrical conductivity, $\langle\tau\rangle$ is the relaxation time averaged over all energy. This expression does not agree with the empirical relation between conductivity and frequency as obtained by us:

$$\sigma = \sigma_0(1 + bw^2),$$

where b is an empirical constant.

Hence the nonlinearity in the current-voltage curve in lipid membranes is not an isolated incident. It is governed primarily by two factors, e.g., formation of pores, which is a function of lipid aggregation properties (3-5) monitored by outer surface of the membrane (2), and the nature of charge carriers, which due to semiconducting nature of the lipid is primarily electronic in the dry state. However for lipids in hydrated state, the conductivity increases to a large extent due to contribution of ionic charge carriers. Again, presence of some specific external agents in the bathing solution can change the intrinsic semiconducting nature of the lipid to an extrinsic one. This may increase the conductivity by several orders of magnitude (12). Lipids are an essential component of biological membranes through which life-sustaining charge transport processes take place. Our observation reported here signifies the importance of their semiconducting property in these processes.

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