Confirmation of membrane electroporation from flicker noise

S. De
Department of Physics, Dinabandhu College, Banga, District North 24 parganas, West Bengal, India

R. Basu
Department of Physics, Jagamaya Devi College, 92 SP Mukherjee Road, Calcutta 700 026, India

P. Nandy
Department of Physics, Jadavpur University, Calcutta 700 032, India
(Received 24 March 1999)

Living systems interact with their environment through biological membranes. Charge movement across these membranes plays a key role in several natural processes and has been the subject of intense research. Charge can transport through the hydrophobic barrier of the membrane by means of ion channels, ionophores or electric-field-induced pores. Membrane electroporation results in a nonlinear I-V characteristic of the system. However, nonlinear behavior is exhibited by the majority of the inhomogeneous systems and hence is not a definite proof of pore formation. Noise measurement is a well accepted method of studying the charge transport in inhomogeneous systems and we report here our study of the noise spectral density in a planar lipid membrane, which is a model for more complex biological membranes to study the molecular processes involved in transmembrane charge conduction. Analysis of our results, in the light of similar experiments conducted in various inhomogeneous systems, confirms that nonlinearity here is the signature of membrane electroporation.

INTRODUCTION

The I-V characteristic in planar lipid membranes undergoes a transition from a linear to a nonlinear regime above a certain critical value of the applied bias (V_0). This nonlinearity has been attributed by earlier workers to field-induced pore formation.[1–4] According to this theory, the pores, whose number and size increase with the applied field, provide more polar pathways for ions to pass through the otherwise hydrophobic lipid moiety, which in turn gives rise to the nonlinearity at high field. However, similar nonlinearity has also been observed in thin-solid films of lipids where ionic transport through water-filled pores can be completely ruled out.[5] Hence, nonlinearity of charge conduction in a lipid assembly is not of itself a sufficient criterion to imply the formation of pores.

Nonlinear charge conduction is a common feature of inhomogeneous systems, where at high-field value resistance becomes a function of the applied bias. Noise amplitude in these systems is greater than that in a homogeneous system of comparable resistance, indicating that the microstructure determines the noise spectrum of the system. Thus, a comprehensive view of the microstructure of the inhomogeneous system can be obtained from the noise spectrum, since the noise power depends on the spatial current distribution throughout the system.[6] With this idea, we had earlier carried out noise measurements in thin-solid films of lipids, and the nonlinearity in this system has been attributed to a change in the conduction mechanism at high-field value.[7]

We report here our preliminary results of noise measurements in a planar lipid membrane. In order to investigate how the charge conduction mechanism in this system is modified at high-field value, the noise spectrum obtained here has been compared with the noise spectra obtained in different composite–one-component systems, where the mechanism of charge conduction is well established.[6] The comparison indicates that at high-field value different charge conduction channels are indeed activated, giving rise to the nonlinearity.

MATERIALS AND METHODS

Cholesterol, purchased from Sigma Chemical Company (St. Louis, MO) was oxidized and then recrystallized from *n* octane.[8] Analytical grade chemicals from E. Merck Ltd (Worli, Mumbai, India) were used without further purification. Iodine was purified by resublimation.

The planar membrane was formed by brushing a saturated solution of oxidized cholesterol in *n* decane on a glass pore filter (porosity G-4), separating symmetric bathing solutions containing iodine (concentration 1 mM). Platinum electrodes of 1 cm square were used (interelectrode distance ~6 cm) for the application of a dc electric field across the membrane (for detailed experimental procedure, please see Ref. 9). For the noise measurement, a constant current from a programmable current source (Keithley Model 224) was passed through the sample. (For detailed experimental procedure, please see Ref. 7.)

A modified Hooge's empirical formula for noise spectral density as a function of frequency (f), voltage (V), and chordal resistance ($R=V/I$) is given by[10]

$$S_V = \mathscr{R}(R) V^{\gamma_0+2} 1/f^{\lambda}. \tag{1}$$

The functional dependence of $\mathscr{R}$ is determined by the microstructure of the system.

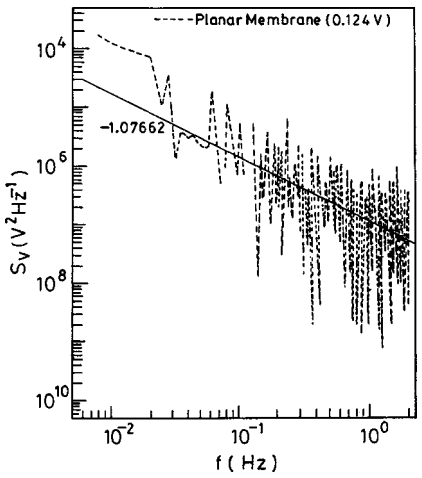

FIG. 1. Log-log plot of noise spectral power $S_V(f)$ vs f for a sample of resistance 4 MΩ. The slope of the line is −1.08, showing the basic $1/f$ nature of the noise spectrum.

RESULTS AND DISCUSSION

Figure 1 shows a typical noise power spectrum S_V as a function of frequency (f) in a sample with linear resistance $R_0 = 4\ \mathrm{M}\Omega$. The slope of the curve is of the order of unity, which shows the basic $1/f$ feature of the noise spectrum (flicker noise).

The voltage dependence of the noise power at 0.5 Hz (solid symbol) and the resistance ($R = V/I$, open symbol) of this sample are shown in Fig. 2. The slope of the noise curve changes from 1.97 to 1.48 at the onset of nonlinearity ($V > V_0$), where R also deviates rapidly from its linear value, suggesting a strong correlation between the noise power and the nonlinear conductance. The slope from the $S_V - V$ plot may be defined as

$$d(\ln S_V)/d(\ln V) = \gamma + 2.$$

Comparing with Eq. (1), we get

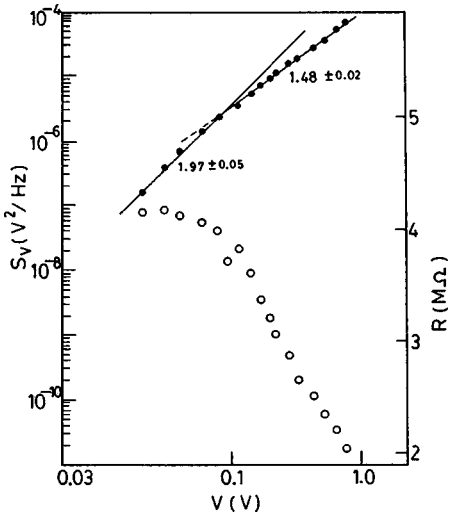

FIG. 2. Typical voltage dependance of noise power S_V at 0.5 Hz (solid symbol) and resistance (open symbol). At the onset of nonlinearity, the resistance decreases, the noise increases and the slope of the noise spectrum drecreases.

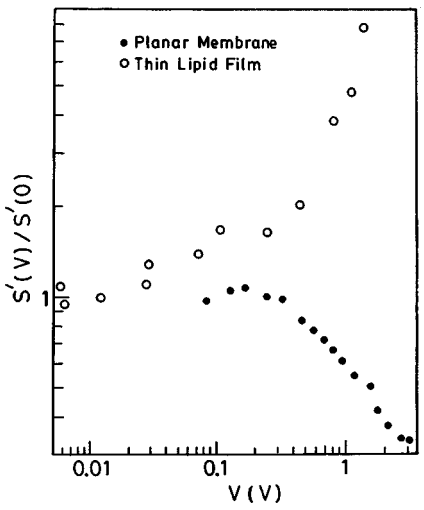

FIG. 3. Plots of normalized $S'(V)$ vs V for thin film of a lipid (open symbol) and a planar lipid membrane (solid symbol). The difference in the noise pattern arises due to the difference in the charge conduction mechanism in these two systems.

$$\gamma = \gamma_0 + (V/\mathscr{R})(d\mathscr{R}/dR)(dR/dV).$$

$$\text{For } V < V_0, \quad dR/dV = 0, \quad \gamma = \gamma_0.$$

$$\text{For } V > V_0, \quad dR/dV < 0.$$

Thus for $V > V_0$, the value of $d\mathscr{R}/dR$ will determine the nature of the noise curve in the nonlinear regime, i.e., whether γ will be less than, greater than, or equal to γ_0. As the functional form of $\mathscr{R}(R)$ depends on the microstructure of the system, it is actually the conduction mechanism that will determine the nature of the $S_V - V$ curve after the onset of nonlinearity. Another interesting observation is that while the resistance decreases in the nonlinear regime by a factor of 2, the noise increases by three orders of magnitude, making noise measurement a more sensitive tool for detecting any change in the conduction mechanism.

Working with thin-solid films of lipid, we obtained similar results, i.e., noise power had a $1/f$ dependence and the slope of the $S_V - V$ plot changed at the onset of nonlinearity.[7] However, there the slope was greater in the nonlinear regime than in the linear regime (1.78 and 1.07, respectively), which is just the opposite of the result obtained with the planar membrane. For the purpose of comparing the noise behavior of the two systems, the generalized relative noise power $S_V/V^{\gamma_0+2} = S'(V)$ at 0.5 Hz normalized by its value in the linear range has been plotted as a function of V in Fig. 3. While $S'(V)/S'(0)$ increases with bias for the thin film, it decreases for the planar membrane.

The change in conductivity may be written as

$$\delta\sigma = e\mu\,\delta n + en\,\delta\mu.$$

Thus nonlinear conduction at high field may arise either from a change in the existing charge conduction mechanism or from some new charge conduction channels being created at high field, allowing more charge carriers to pass through the system.

Our next question is under what conditions is the nonlinearity in the conduction accompanied by a decrease in resis-

tance as well as in relative noise. Using a simple resistive circuit, Nandi *et al.*[6] have shown that, in general, this occurs when parallel resistances are added to the circuit. According to the dynamic random resistor network (DRRN) model of Gefen,[11] in a network of conducting and nonconducting bonds, nonlinearity arises when, above a certain critical value of the bias, some of the insulating bonds start to become conducting. This situation has similarity with the case of charge conduction in lipid membranes. Here the thermally created water-filled pores are the randomly placed conducting elements. The onset of nonlinearity is triggered when at high field the lipid molecules start orienting to provide more ionic pathways parallel to the existing ones. This reduces the resistance and decreases the relative noise.

At this point we further compare our noise spectral measurements with those obtained by Nandi *et al.*[6] using composite systems like carbon-wax and disordered but one-component systems like conducting polymer and V_2O_4. We notice that the nature of the noise spectrum for a planar membrane, namely, the $1/f$ dependence and the decrease in slope of the $S_V - V$ plot at the onset of nonlinearity, is similar to that obtained for the carbon-wax mixture. A satisfactory explanation of this behavior has been obtained by Nandi *et al.*[6] by applying Gefen's DRRN model.

The planar lipid membrane furnishes the basic structure of the biological membrane, and transmembrane charge conduction is vital for the survival of living systems.[12] However, the semipermeable nature of its interior to the passage of most water-soluble molecules, and the fact that some of the lipid heads bear a charge, and that the membrane is surrounded by two conducting electrolyte solutions, allow us to schematize the lipid layer with its conducting inner and outer media as a capacitor.[13] The ionic strength of the bathing solution, the charge of the interface, and the magnitude of the field are determinant factors for the formation of holes.[14] In this context our observations and the conclusions drawn are of primary significance, and can be extrapolated with proper caution and a good understanding of the biological system to explain the transmembrane charge conduction process, responsible for the survival of the living world.

ACKNOWLEDGMENTS

We are grateful to Dr. K. K. Bardhan and Dr. C. D. Mukherjee not only for allowing us to use their laboratory facilities but also for stimulating discussions held on several occasions. We are also thankful to Dr. U. N. Nandi for his help with the experiment, and to Dr. A. Nandy and Dr. R. Krimholtz for further helpful discussions. The work was supported by a grant from the Department of Science and Technology of the Govt. of India.

[1] E. Brynda, L. Nespurkova, and S. Nespurek, Czech. J. Phys., Sect. B **35**, 873 (1985).
[2] M. Robello and A. Gliozzi, Biochim. Biophys. Acta **982**, 173 (1989).
[3] R. Glaser and S. Leikin, Biochim. Biophys. Acta **940**, 275 (1988).
[4] R. Ashcroft, H. Coster, D. Laver, and J. Smith, Biochim. Biophys. Acta **730**, 231 (1983).
[5] D. Bhattacharya, R. Basu, A. K. Ghosh, A. Manna, A. Nandy, and P. Nandy, Biophys. J. **64**, 550 (1993).
[6] U. N. Nandi, C. D. Mukherjee, and K. K. Bardhan, Phys. Rev. B **54**, 12 903 (1996).
[7] S. De, R. Basu, U. N. Nandi, C. D. Mukherjee, K. K. Bardhan, and P. Nandy, Phys. Rev. B **56**, 13 627 (1997).
[8] R. Basu, S. De, S. Nayar, S. Das, A. K. Ghosh, and P. Nandy, Phys. Rev. E **52**, 4179 (1995).
[9] S. Das, R. Basu, A. K. Ghosh, M. Minch, and P. Nandy, J. Photochem. Photobid, A **85**, 161 (1995).
[10] F. N. Hooge, T. G. M. Kleinpenning, and L. K. J. Vandamme, Rep. Prog. Phys. **44**, 479 (1981).
[11] Y. Gefen, W. H. Shih, R. B. Laibowitz, and J. M. Vigianno, Phys. Rev. Lett. **57**, 3097 (1986).
[12] E. Sackmann, *Structure and Dynamics of Membranes*, edited by R. Lipowsky and E. Sackmann (Elsevier, Amsterdam, 1995).
[13] M. Grattarolla and G. Massobrio, *Bioelectronics Handbook* (McGraw Hill, New York, 1998).
[14] H. D. Betterton and M. P. Brenner, Phys. Rev. Lett. (to be published).