A STUDY ON PORE FORMATION DYNAMICS IN BIO-MIMICKING MEMBRANE SYSTEM

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Abstract. Transmembrane electrical activities are modulated due to the presence of membrane embedded pores and channels. We have studied the role of lipid membrane which mimics the biological membrane and plays an important role in charge conduction mechanism through membrane. Electrical currents, arising from conduction of ions or charges through field induced pores, fluctuate due to its own dynamics. We have performed statistical analysis on this current fluctuation data under different voltage clamp conditions. The result shows that after a certain cut off value the probability distribution of current fluctuation values follow a power law up to a certain range. Additionally the power law of magnitude of large fluctuations indicates the scale-invariance of size of pores and power law of first time return probability indicates that the dynamical behaviour during transport through pores is correlated and the underlying dynamics during the conduction through pores is analogous to the ion channel dynamics.

Key words: mimetic membrane, lipidic pore, power law, membrane dynamics, electroporation.

INTRODUCTION

Formation of passages between two cellular compartments can appear in two ways: first is the formation of lipidic pores and the second involves a special group of proteins which are responsible for forming pore across the bilayer named as channel proteins [8]. Membrane pores or channels, which connect between two biological compartments, are the fundamentals of various membrane related physiological processes like nerve impulse generation and transmission, passive transport of proton and hydrophilic compounds, etc. Additionally, pore formation shows key relevancy to biotechnological approaches like genetic transformations [13], cell-cell fusion [11], drug releasing phenomena from liposome, etc. [2]. In biological systems specific channels or transporters (as mentioned in the second

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case) mediate the transmembrane ion transport which actually forms electrophysiological property of membrane. However, strong evidences suggest that transmembrane potential is also maintained by passive permeation of ion through the biological membrane, especially in electrically active tissues [15]. Likewise to understand the membrane disruption precisely it is very important to study the properties of lipidic pores in a well defined experimental system of model membrane. Additionally, the precise mechanism of lipidic pore formation including their size, structure, stability, etc. is poorly understood [8, 14, 17] which broadens the scope of research in lipidic pore forming phenomena and the underlying dynamics.

Local disruption of bilayer lipid membrane (BLM) system can be readily achieved by applying electrical or mechanical stresses. Phenomena of BLM-disruption or non-bilayer transient formation under influence of electrical field are termed electroporation [8, 12]. Electroporation broadly covers two types of membrane behaviour under electrical stress: irreversible and reversible electrical breakdown [8]. In the process of irreversible breakdown increasing membrane conductance leads to rupture of membrane and on the contrary in reversible breakdown bilayer structure manages to hold its integrity up to certain experimental conditions like up to a certain voltage [17] and completely discharge the membrane [1]. Experimental and theoretical studies reveal that pore formation through electroporation consists of a sequence of phases like charging phase, creation transient phase, pore evolution phase and finally pore shrinkage phase [12] which definitely indicates a hidden underlying dynamics. A number of methods were proposed to study this dynamics related to pore formation and membrane dynamics [1, 7, 8, 10, 14, 18]. All these studies indicate that the precise knowledge on charge conduction and related pore formation dynamics is still needed to be enriched until a well accepted model arrives.

In our previous study, electrical properties of BLM made of oxidized cholesterol have been reported considering different bathing solutions [3]. These studies indicated that a nonlinear phenomenon exists in the current-voltage characteristic of BLM and this nonlinearity has been characterized by field induced pores including their number and size which are also characterized by nonlinearity. This nonlinearity is also observed in solid film of lipid [9] which confirms nonlinearity as a common feature of an inhomogeneous system [3, 6, 9]. To investigate exact charge conduction mechanism voltage noise spectra analyses have been carried out. Maintaining a constant current through the membrane voltage noises are measured which clearly differentiate between charge conduction mechanism in BLM and solid lipid film [3, 6]. It is already established that size of the pores formed under a current clamp condition is controlled by a feedback mechanism. Additionally, many other related studies have indicated the existence of different pore forming phenomena in voltage and current clamp conditions, but the exact dynamics still remains unknown. To understand the dynamics in a deeper way we have performed fluctuation analysis in the same bio-mimetic system in varying voltage clamp conditions.

MATERIALS AND METHODS

For measuring membrane fluctuation, a model bilayer lipid membrane (BLM) of oxidized cholesterol was formed by painting saturated solution [4, 16] of oxidized cholesterol in n-decane over a very small hole (150 µm) on a Teflon beaker separating two aqueous compartments. Cholesterol was purchased from Sigma Chemicals Co. (St. Louis MO, USA), was oxidized and then recrystallized from n-octane. Platinum electrodes of diameter 0.1 cm were placed across the membrane for the application of electric field.

Our measurement instruments are Keithley Sourcemeter 2400 and Keithley Electrometer 6514. We acquired data by a programmable IEEE488 communication port using Lab VIEW software & CEC488 GPIB card. BLM formation and data acquisition operation were performed on a Vibration Isolation table within Faraday Cage (Harvard Apparatus) to avoid mechanical and electrical fluctuation. The fluctuation of current with time was recorded under voltage clamp conditions without using any filter and the voltages range from –125 mV to +125 mV. The experiment was repeated several times and the fluctuation patterns obtained were similar for all the cases. Distilled water is used for bathing medium and all data are taken at room temperature.

RESULTS

For analyzing the fluctuation data in oxidized cholesterol membrane we have clamped the membrane [4] at various voltage settings. For each clamped voltage-set more than 100,000 data are taken for ease of statistical calculations.

POWER LAW ANALYSIS

Time trace of the data had shown a continuous fluctuation (Fig. 1). For measuring the magnitude of fluctuating current, a base line was drawn for the entire time series by taking running average of eleven contiguous data points and the magnitude of fluctuations was measured over that baseline. Then frequency count was performed to calculate the probability distribution $p(I)$ of magnitude of current throughout the whole data taking a constant bin size as mentioned in the figure caption.

Figure 2 describes that in all cases the probability distribution of large fluctuation above a certain value of current magnitude shows a power law $P(I) = A\ I^{-\alpha}$ (A = proportionality constant) [5] and below this value there is a flat region which does not show any power law. We define this critical value as the cut off value and it can be estimated from the figure itself. Large currents (above the cut off value) are due to the passage of a large number of ions or charges and power law indicates that a large number of ion passes through the pores in a correlated way. Whereas small current (below the cut off) is due to the movement of a few number of charges or ions in a random way and hence it does not show any power law.

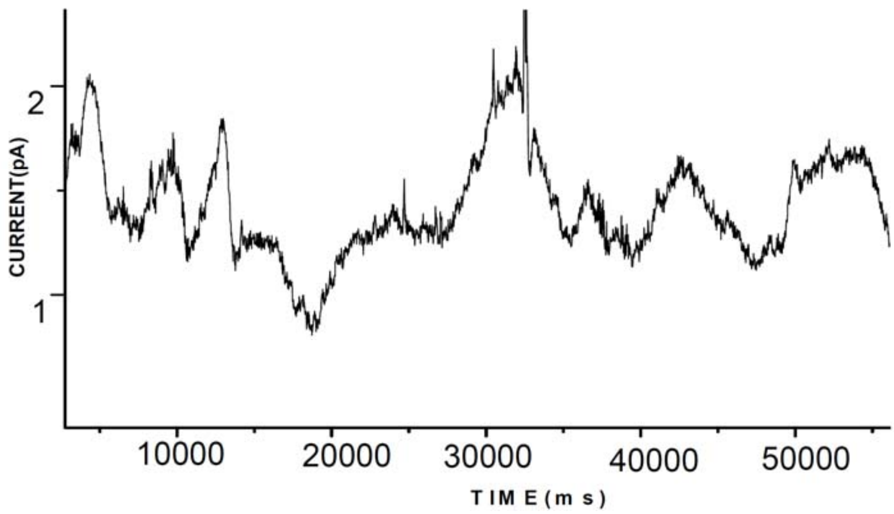

Fig. 1. Continuous fluctuation of membrane current at +50 mV voltage clamp condition.

We have estimated the cut-off values for each voltage set which dictates that the cut-off value increases up to a certain clamped voltage range followed by a sudden decrease (Fig. 3).

STUDY ON TIME TRACE

Assuming that the large current fluctuations and the pore formation are strongly related, an extension of the above mentioned investigation is followed by studying the probability distribution of recurrence time (τ defined as the time difference between two successive large fluctuations) of the time trace of large currents above the cutoff values. Figure 4 shows that probability distribution of recurrence time also follows a power law distribution $P(\tau) = \beta\tau^{-\beta}$ ($\beta = 2.12 \pm 0.12$).

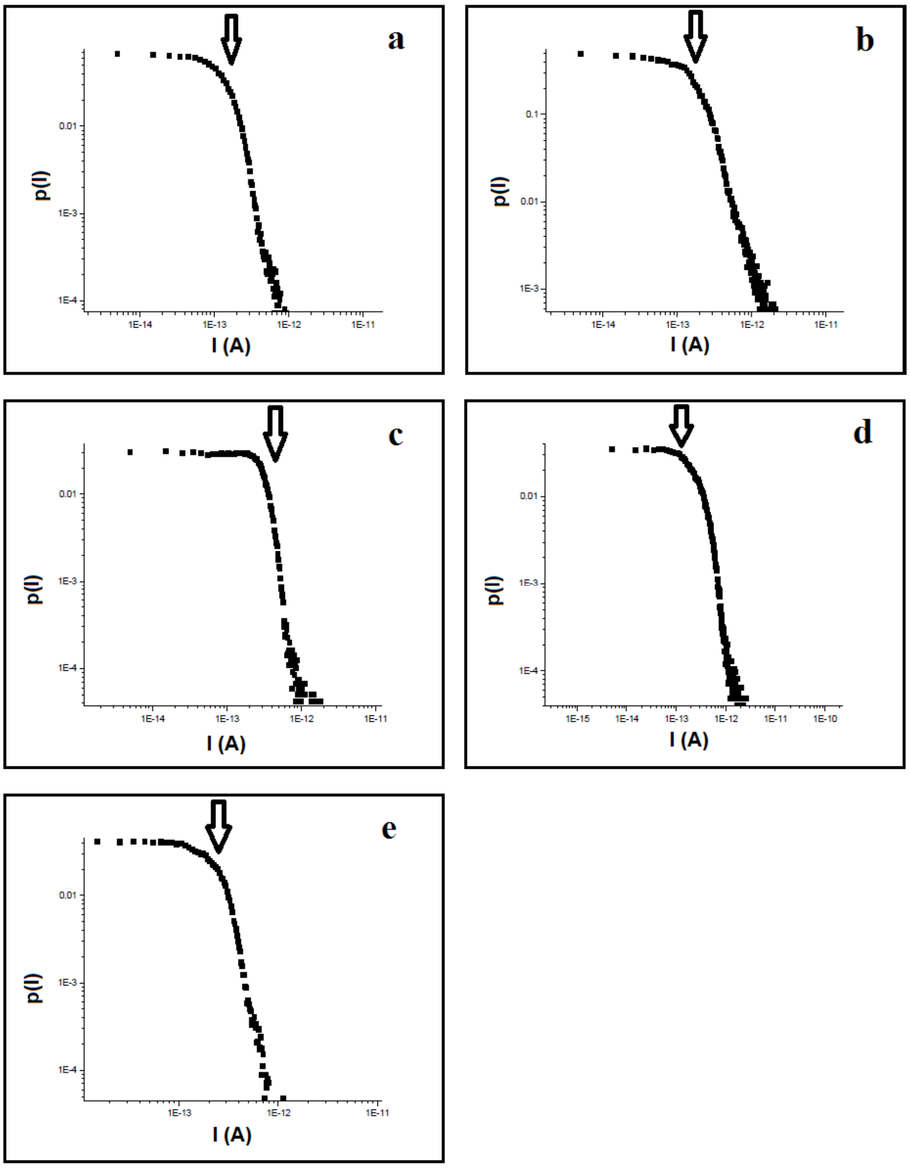

Fig. 2. Existence of power-law in probability distribution of fluctuation magnitude clamped at–25mV (a), –50mV (b), –75mV (c), –100mV (d), –125mV (e). Bin size used for all the calculations is 1E–14.

DISCUSSION AND CONCLUSION

From the statistical analysis of voltage clamping data we have found that in all voltage range magnitude of fluctuation follows a power law above a cut-off value. And that cut-off value increases with the increase of clamped voltage and suddenly drops at higher voltage like 100 mV which clearly indicates a higher range of cut-off value which is very nearly equal to that of the physiological range of voltage. This perhaps justifies the use of bilayer lipid membrane as a biomimetic equivalent of a biological membrane.

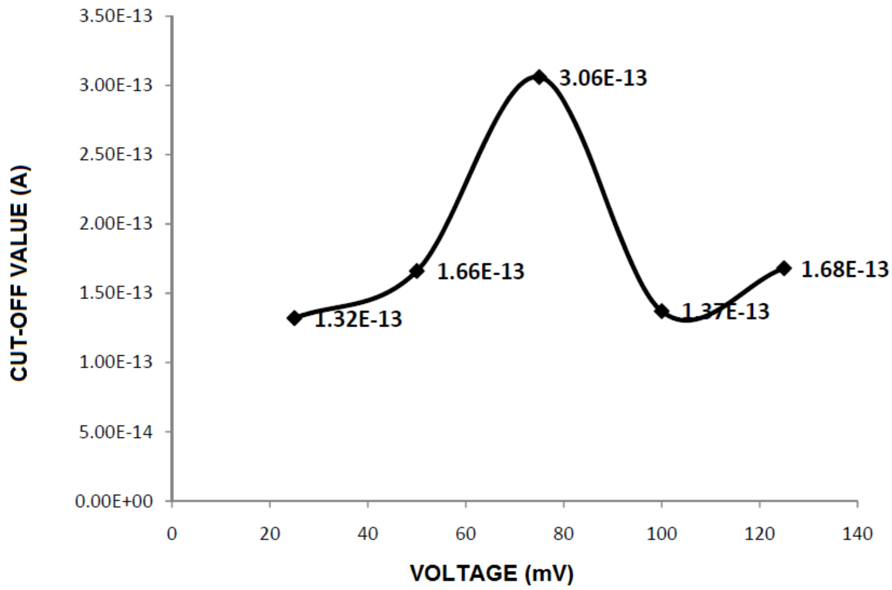

Fig. 3. Graphical representation of cut-off against increasing clamped voltage.
The graph shows that the cut-off value increases up to 75 mV followed by sudden drop.

From the study of distribution of recurrence time of return probability of large fluctuation we have confirmed that probability distribution of recurrence time for large fluctuation above the cutoff value holds power law. The occurrence of power law of both magnitude and recurrence time is direct reflection of the dynamics of complex hierarchical properties of the system and due to the lack of fine tuning in scaling (both magnitude and time). This reveals that the system evolves to critical states irrespective of initial condition and without fine tuning parameter. The underlying dynamics during the charge transport through lipid membrane pores is recognized as self organized criticality (SOC). There are several literature references [1, 8, 14, 17] on the dynamics of formation of pores and conduction through it. We have used statistical analysis of current through the pores and explored a general prescription of the charge conduction dynamics through the pores which is SOC and it is analogous to the dynamics of ion channel which is another pathway of charge conduction through the membrane.

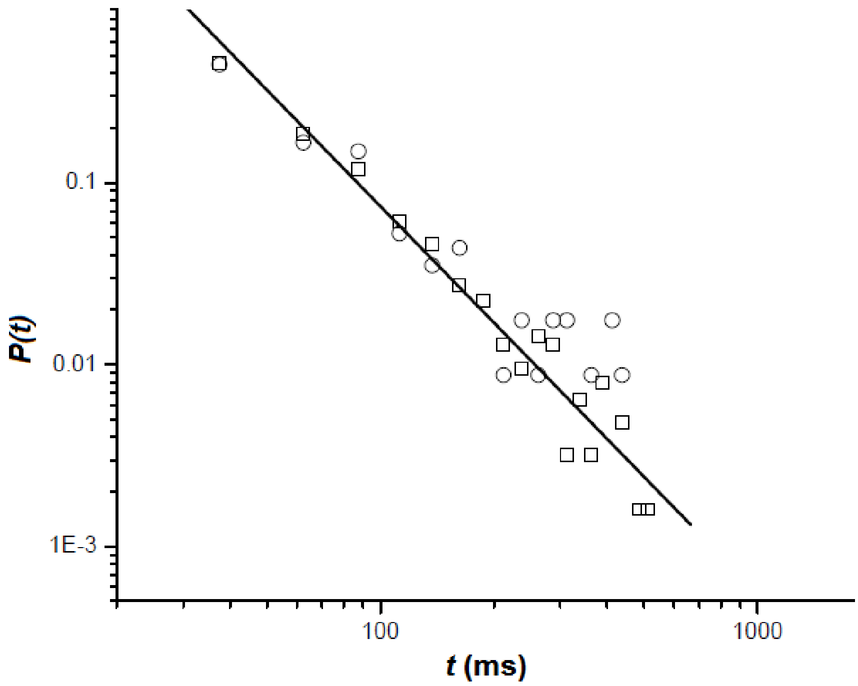

Fig. 4. First time return probability is obeyed by a power law above cut-off magnitude and above twice of cut-off magnitude. The hollow circles and the squares correspondingly indicate the data points for the fluctuation above cut-off magnitude and above twice of cut-off magnitude.

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