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Planar lipid bilayers: observing pore creation and extinction

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***Abstract*—From an electrical point of view a planar lipid bilayer can be considered as a non-perfect capacitor; it can be presented as an ideal capacitor in parallel with resistor. In this study the whole measuring system including planar lipid bilayer was modeled by an equivalent electric circuit in Spiceopus software. Such a model gives additional information of experimentally obtained results. In this way we analyze measurements of transmembrane voltage that appears on planar lipid bilayer as consequence of linear rising current. Small voltage drops were obtained before the planar lipid bilayer breakdown. The model showed that effective current on planar lipid bilayer is actually much smaller than the current applied with current generator and should be used in calculations of a conductance related to voltage drops.**

I. Introduction

BIOLOGICAL membranes, the barriers that envelope the cell and its inner organelles, play a crucial role in normal functioning of cells. The simplest model of these biological membranes is a planar lipid bilayer [1]. Because it's geometry it allows chemical and electrical access to both sides of the bilayer, thus physical properties of this model membrane can be easily measured. Usually a thin bimolecular film composed of specified phospholipids and organic solvent is formed on a small aperture in a hydrophobic partition separating two compartments containing aqueous solutions. Electrodes plunged in the aqueous compartments permit the measurement of current and voltage across the planar lipid bilayer [2].

From an electrical point of view a planar lipid bilayer can be considered as a non-perfect capacitor; it can be presented as an ideal capacitor in parallel with resistor. The capacitance and resistance are also the two electrical properties that mostly determine planar lipid bilayer's behavior. A typical resistance is in the range of few gigaohms, but it drops dramatically even if a nanometer-sized hole is present in a lipid bilayer. Formation of pores can be induced by a strong electric field applied to the planar lipid bilayer. Experimental condition governs pores behavior; they may close to intact bilayer or expand over their physical limits and cause breakdown of planar lipid bilayer at break down voltage (U_{br}). The electrical properties (C, R and U_{br}) of the planar lipid bilayer and the behavior of lipid bilayer in electric field depend on the physical properties of the lipids that compose the bilayer. Therefore elasticity modulus and surface tension, for example, can be calculated from the electrical characteristics of the planar lipid bilayer [3].

A number of techniques have been developed to allow investigations of the functions and physical properties of these thin and fragile structures [2]. Electrical measurements are a straightforward way to characterize the barrier function of a bilayer - its ability to prevent the flow of ions. Two electrical measurement methods are commonly used: a voltage clamp and a current clamp method.

When the voltage clamp method is used a voltage signal is applied to the planar lipid bilayer, mostly used to determine breakdown voltage (U_{br}) of the planar lipid bilayer: a step change of voltage [4] or voltage pulse [5-7] as well as linear rising voltage [8], but also some other shape can be applied of the voltage signal [2].

When the current clamp method is used, a current of various shapes is applied to the lipid bilayer [9]. Exposure of the planar lipid bilayer to a constant current (0.1 to 2.0 nA) does not rupture the bilayer rapidly. On both sides of the planar lipid bilayer the charge slowly accumulates building up the transmembrane voltage and when the first pore appears, the transmembrane potential decreases [10]. This prevents further increase of the pore conductivity, which usually starts shrinking. However the pore decrease is also opposed by the negative feedback and the pore (starts) increasing after a while. This chain of events accounts for the pore stabilization and its fluctuations and increases the scatter of U_{br} determined with current clamp method. [11,12].

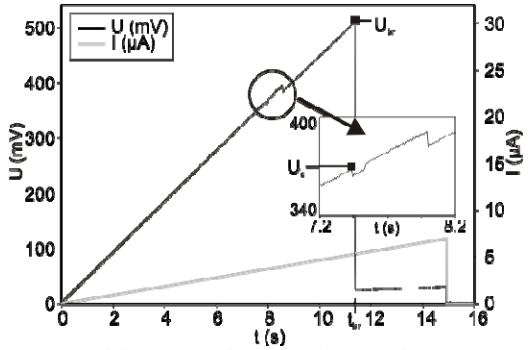

Fig. 1. Trace of the measured transmembrane voltage (U) on planar lipid bilayer exposed to linear rising current (I). At U_{br} the breakdown of membrane were occurred. Arrow points to close-up window of voltage drop U_d.

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Although the pore creation in planar lipid bilayer and their dynamical behavior was already studied [10,13-15] we focus on experimental conditions that allow precise detection of stable pore(s) creation and pore(s) conduction evaluation.

II. Materials and Methods

A. Experimental setup

The chamber, where planar lipid bilayers are formed, consists of two reservoirs with a round aperture (117 μm in diameter) in a separating thin Teflon sheet (25 μm). Planar lipid bilayers are formed by the Montal-Muller method [16].

All measurements are performed by measuring system described by Kalinowski *et al*. [15]. The system consists of two modules. The first module is capacity to period converter, used for measuring planar lipid bilayer capacitance. The second module is a potentiostat-galvanostat for planar lipid bilayer studies under current clamp. Both modules are controlled with a personal computer. Four Ag-AgCl electrodes, two current electrodes and two reference electrodes are used.

The salt solution is prepared of 0.1 M KCl and 0.01 M Hepes in the same proportion. Some droplets of 1 M NaOH are added to obtain pH 7.4. The lipids are prepared from POPC (1-pamitoyl 2-oleoyl phosphatidylcholine) in powder form (Avanti Polar-Lipids Inc. ZDA). Lipids are melted in solution of hexane and ethanol in ratio 9:1. The mixture of hexadecane and pentane in ratio 3:7 is used for torus forming.

B. Measurement protocol

Measurement protocol consisted of capacitance measurement, observation of pore(s) development on planar lipid bilayer and finally its breakdown voltage determination. The capacitance was normalized to the surface area of the orifice to calculate the specific capacitance of the planar lipid bilayer (c_{BLM}).

To observe pore's development and behavior five different slopes k_I were selected: 300 nA/s, 500 nA/s, 1 μA/s, 4 μA/s, 8 μA/s, 10 μA/s and 20 μA/s. Breakdown current and voltage were defined as the current/voltage at the moment t_{br} when sudden drop of the voltage was detected (Fig. 1).

C. Modeling planar lipid bilayer experimental setup

The whole measuring system was modeled by an equivalent electric circuit (Fig. 2). We used Spiceopus software (www.spiceopus.si) to simulate the equivalent electric circuit behavior under our specific experimental conditions [17]. In SPICE model current was applied by current generator I_0. Experimental setup is modeled in three parts: chamber, planar lipid bilayer and pore(s). C_{sys} is the capacitance of the chamber and R_{sys} is the resistance of the chamber. Planar lipid bilayer was modeled by capacitance C_{BLM} and resistance R_{BLM}. The appearance of pore(s) (R_P) in the planar lipid bilayer was simulated by introducing a voltage controlled switch in the R_P branch. An intact planar lipid bilayer was represented by an open switch while the planar lipid bilayer with pore(s) was represented by a closed switch.

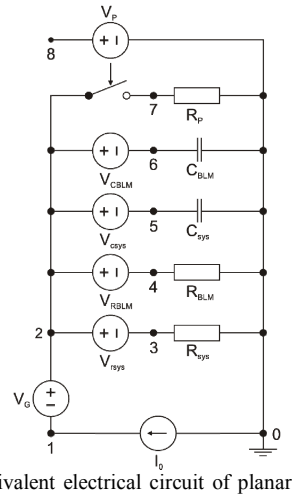

Fig. 2. Equivalent electrical circuit of planar lipid bilayer and measurement system with current source. Pore that appears in planar lipid bilayer is modeled with resistor R_P. Pore creation is controlled by voltage controlled switch.

Spiceopus software uses the node-voltage analysis. To measure the currents flowing through various parallel branches of the circuit, an independent voltage source (V=0) was added in series with the element of the branch (Fig. 2).

The amplitude and the shape of the current in the model correspond to measured experimental traces. Due to the fact that experimentally obtained voltage traces should correspond to voltage trace in the model the value of R_{BLM} was set to 10^8 Ω, which is a typical value found in the literature [20] and the value of membrane capacitance was set to 0.51 μF/cm^2; according to previously obtained experimental measurements.

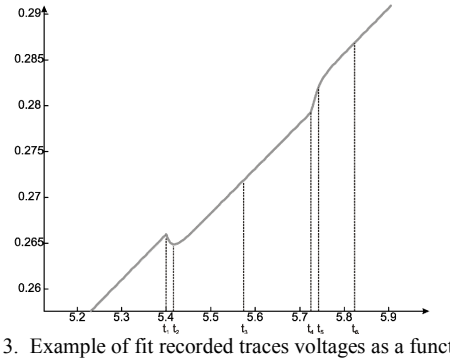

Fig. 3. Example of fit recorded traces voltages as a function of time.

The values for C_{sys} and R_{sys} are unknown, so they were determined by fitting voltage in the model to experimentally measured voltage. Similarly the appearance of pore(s) (R_P) in planar lipid bilayer was determinate. In all experimental conditions voltage to voltage drop ratio was much higher than signal-to-noise ratio of measured voltage.

III. Results and Discussion

In our study 63 experiments on planar lipid bilayers were analyzed. Mean specific capacitance value of planar lipid bilayers c_{BLM} with standard deviation was determined to be

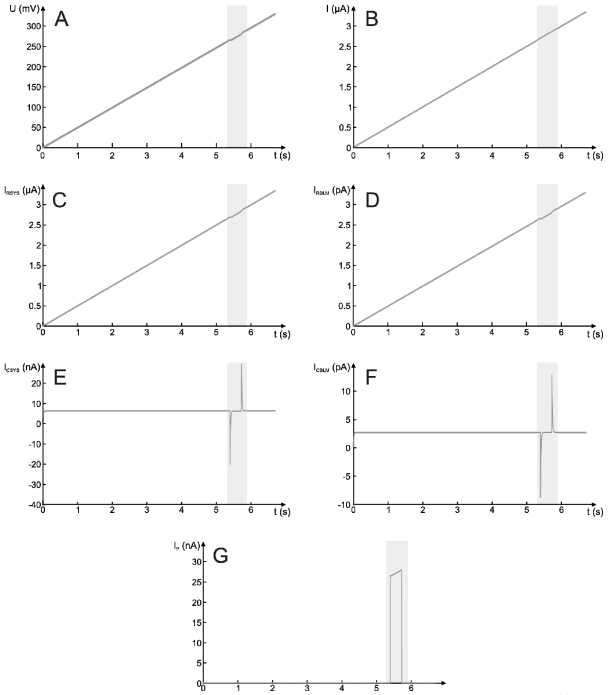

Fig. 4. Traces obtained by SPICE simulation of planar lipid bilayer with the experimental system. A) Voltage on the membrane, which is the same in all branches. B) Applied current by means of current source generator. C) The trace of the current that flows through the system resistance R_{sys}, which predominantly lasts to the chamber. D) Current trace that flows through the planar lipid bilayer. E) The trace of the current trough capacitance of the experimental setup C_{sys}. F) Trace of the current trough capacitive part of planar lipid bilayer. G) Current trough the pores modeled in SPICE software by the branch which contains voltage controlled switch.

0.51±0.16 μF/cm^2. Breakdown voltage needed for planar lipid bilayers rupture did not exhibit dependence on the slope of linear rising current signal. The mean value of all breakdown voltages (U_{br}) with standard deviation was measured to be 0.32 ± 0.04 V.

Exposing planar lipid bilayer to a linearly increasing current the voltage builds across the membrane and induces events indicative of membrane poration and subsequent resealing of the membrane (Fig. 1). Out of these 63 experiments, small voltage drops were observed in 31 experiments. Mostly one single voltage drop, but even up to three separate drops were observed on each voltage curve. All together, we recorded 44 small voltage drops. The exceptions were the steepest two current signals (10 and 20 μA/s) where no voltage drop was present. According to their shape, they were divided into two categories: (1) drop only and (2) drop & reseal (Table I). If the voltage dropped to a finite value U_d and did not rise back, the drop was categorized as "drop only". If the voltage dropped for a finite value U_d and rises back continuing on the same slope as before the drop after a certain time interval, the drop was classified as "drop & reseal".

Usually such voltage drop events are quantified as:

$$G = \frac{dI}{dU}. \qquad (1)$$

Using this equation (1) the conductance of typical voltage drop was couple of mS. Such huge conductance should present very large pore that is not realistic. Hence we modelled the complete electronic system, chamber and planar lipid bilayer using an equivalent electrical circuit to determine individual contribution of each part. With SPICE model all 63 measured voltage traces were modelled. The values that gave the best fit were C_{sys} = 105 ± 54 nF and R_{sys} =100 ± 5 kΩ. Finally, R_P i.e. the resistance of pores was adjusted to each of experimentally obtained voltage drops.

The values of the currents in all branches (Fig. 4) are given in Table II. On planar lipid bilayer only small part of all applied current is presented before voltage drop (share is 2 10^{-18}). When voltage drop appeared, the share of the applied current that flows directly across the pores is only 10^{-14}.

The model showed that effective current on planar lipid bilayer is actually much smaller than the current applied with current generator. Therefore true pore conductance should be calculated using the current from the model I_p, transmembrane voltage U and equation 1. Distribution of pores conductance is presented in Fig. 5.

IV. Conclusion

Lipid membranes are sensitive to high electric field, which causes either reversible or irreversible electroporation. The phenomenon is under remarkable consideration of scientific community due to broad specter of electroporation based applications in medicine and biotechnology. In this paper we focus on experimental conditions that allow precise detection of stable pore creation and its (their) conduction evaluation.

At current-controlled experiments the restricted charge supply gradually charges the membrane, eventually reaching the critical point at which the pores are created and electroporation occurs. The limited charge supply does not allow uncontrollable charge flow through pores. Therefore pores creation and their disappearance can be studied.

In the past the pores on planar lipid bilayer was

TABLE I
VOLTAGE DROPS AS A FUNCTION OF THE LINEARLY RISING CURRENT SLOPES. THE NUMBER OF MEASUREMENTS FOR EACH CURRENT SLOPE, THE NUMBERS OF EXPERIMENTS THAT EXHIBIT VOLTAGE DROPS, AMONG WHICH THE NUMBER OF DROP & RESEAL EVENTS AND THE NUMBER OF DROP ONLY EVENTS ARE PRESENTED.

Current slopes (k) μA/s	All	Total drops	Drop & reseal	Drop only
0.03	4	4	0	6
0.05	9	6	5	3
0.1	5	1	1	0
0.2	8	3	3	2
0.5	13	10	8	6
4	5	4	1	5
8	5	3	2	2
10	6	0	0	0
20	8	0	0	0

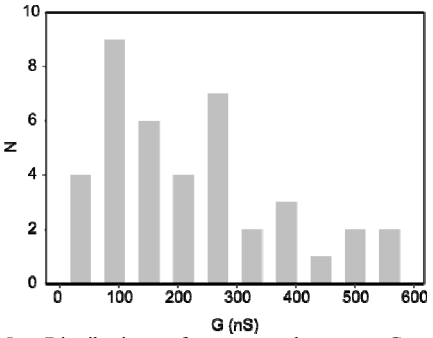

Fig. 5. Distributions of pores conductances $G = 1/R_P$ extracted from SPICE model.

experimentally observed by several authors [10,13-15]. In their studies mostly fluctuations (irregular small changes) of transmembrane voltage or current were observed and explained as widening and shrinking of a single pore. By exposure of planar lipid bilayer to linear rising current we wanted to avoid long lasting fluctuations of transmembrane voltage. Therefore the probability of pore dynamics was limited.

The trace of drop which is result of pore(s) appearance depends on an experimental system specially a chamber where the planar lipid bilayer is formed. Therefore the electric circuit SPICE model was used to determine the properties of the chamber and evaluate the change of planar lipid bilayer conductance observed in voltage drop. Applied current is predominantly used for charging the Teflon chamber and only small part affects the planar lipid bilayer. This brings to lower time resolution of the system. Therefore we could observe only pores conductance that is great enough.

The median value of pore conductance was measured to be 100 nS. The results of molecular dynamic simulation on POPC showed that the conductance of one stable pore was 160 pS [18]. Taking into account this value the estimated number of pores obtained in our experiments was 625, which means that the density of the pores was 578 10^8 1/m^2.

Glaser *et al.* estimated the number of the pores on planar lipid bilayes in the range from 0.2 10^5 to 4.0 10^5 according to the parameters of the pulse that where pulse length and amplitude [19]. Due to their experimental conditions this values corresponds approximately 2000 10^8 1/m^2. Estimation of the density of the pores has been done also on dense cell suspensions [20]. Described results are in range with our results.

TABLE II
PARAMETERS OF VOLTAGE TRACE SHOWN IN FIG. 3 OBTAINED BY SPICE MODEL (FIG. 4).

	t_1	t_2	t_3	t_4	t_5	t_6
t (s)	5.40	5.42	5.57	5.72	5.74	5.84
U (V)	0.27	0.26	0.27	0.28	0.28	0.29
I (μA)	2.70	2.71	2.78	2.86	2.87	2.92
I_{RSYS} (μA)	2.69	2.68	2.75	2.83	2.86	2.92
I_{RBLM} (pA)	2.66	2.65	2.72	2.79	2.82	2.88
I_{CSYS} (nA)	6.20	2.49	6.14	6.14	13.00	6.20
I_{CBLM} (pA)	2.71	1.09	2.68	2.68	5.68	2.71
I_P (nA)	0.00	26.49	27.17	27.92	0.00	0.00

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