Available online at www.sciencedirect.com
ScienceDirect
Bioelectrochemistry 70 (2007) 23–27
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Determination of the lipid bilayer breakdown voltage by means of linear rising signal

P. Kramar, D. Miklavcic, A. Macek Lebar *

Faculty of Electrical Engineering, University of Ljubljana, SI-1000 Ljubljana, Slovenia

Received 1 June 2005
Available online 5 April 2006

Abstract

Electroporation is characterized by formation of structural changes within the cell membrane, which are caused by the presence of electrical field. It is believed that "pores" are mostly formed in lipid bilayer structure; if so, planar lipid bilayer represents a suitable model for experimental and theoretical studies of cell membrane electroporation. The breakdown voltage of the lipid bilayer is usually determined by repeatedly applying a rectangular voltage pulse. The amplitude of the voltage pulse is incremented in small steps until the breakdown of the bilayer is obtained. Using such a protocol each bilayer is exposed to a voltage pulse many times and the number of applied voltage pulses is not known in advance. Such a pre-treatment of the lipid bilayer affects its stability and consequently the breakdown voltage of the lipid bilayer. The aim of this study is to examine an alternative approach for determination of the lipid bilayer breakdown voltage by linear rising voltage signal.

Different slopes of linear rising signal have been used in our experiments (POPC lipids; folding method for forming in the salt solution of 100 mM KCl). The breakdown voltage depends on the slope of the linear rising signal. Results show that gently sloping voltage signal electroporates the lipid bilayer at a lower voltage then steep voltage signal. Linear rising signal with gentle slope can be considered as having longer pre-treatment of the lipid bilayer; thus, the corresponding breakdown voltage is lower. With decreasing the slope of linear rising signal, minimal breakdown voltage for specific lipid bilayer can be determined.

Based on our results, we suggest determination of lipid bilayer breakdown voltage by linear rising signal. Better reproducibility and lower scattering are obtained due to the fact that each bilayer is exposed to electroporation treatment only once. Moreover, minimal breakdown voltage for specific lipid bilayer can be determined.

Keywords: Lipid bilayer; Linear rising signal; Capacitance; Breakdown voltage

1. Introduction

Electroporation is characterized by a formation of structural changes within the cell membrane, which are caused by the presence of electric field. These changes, named "pores", increase the plasma membrane permeability, and enable ions and molecules to enter the cell [1]. Reversible electroporation is used to introduce various substances into the cell and has many practical applications like gene therapy, transdermal drug delivery and electrochemotherapy [2–5]. If cell membrane collapses due to too high electric field, the electroporation becomes irreversible. This form of the phenomenon can be used for liquid food and water conservation [6,7]. The applicability of electroporation is broad: from biotechnology and biology to medicine. Each application has its own optimal electrical parameters [8], which has to be determined beforehand [9].

It is stipulated that pores are mostly formed by rearranging of lipid molecules in lipid bilayer structure; if so, planar lipid bilayer is a good model for experimental and theoretical studies [10]. The planar lipid bilayer can be considered as a small part of total cell membrane. Lipid bilayer as an artificial model of the cell membrane can be made of only one type of lipid molecules, their mixtures or even lipids and proteins [11]. Lipid bilayers of different compositions have different electrical properties that are, due to their influence on membrane stability in electric field, important for the use of electroporation.

Planar lipid bilayer stability in an electric field and consequently the voltage that cause bilayer rapture is one of the most

* Corresponding author. Tel.: +386 1 4768 770; fax: +386 1 4264 658.
E-mail address: alenka@lbk.fe.uni-lj.si (A.M. Lebar).

1567-5394/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2006.03.022

important properties of planar lipid bilayers. The breakdown voltage of the lipid bilayer is usually determined by a rectangular voltage pulse. The amplitude of the voltage pulse is incremented in small steps until the breakdown of the bilayer is obtained [12]. Using such a protocol, the number of applied voltage pulses is not known in advance and each bilayer is exposed to voltage stress many times. Such a pre-treatment of the lipid bilayer affects its stability and consequently the determined breakdown voltage of the lipid bilayer [13]. In this study, another approach for the breakdown voltage determination is suggested: using linear rising signal, the breakdown voltage is determined by a single voltage exposure.

2. Materials and methods

2.1. Electrical setup

Our system for following up electroporation of planar lipid bilayers consists of a signal generator, a Teflon chamber and a device, which is used for measurements of membrane current and voltage (Fig. 1).

Signal generator is a voltage generator of an arbitrary type that provides voltage amplitudes from −5 V to +5 V. It is controlled by costume written software (Genpyrrha), specially designed for drawing the voltage signal that is used for membrane electroporation. The last but not least part of the signal generator is an analogue switch. The switch disconnects the output of the signal generator and switches to the 1 MΩ resistor. The switch is fast, it turns off the signal generator in 2 ns. In this way, a system discharge voltage is measured and consequently the capacitance of the lipid bilayer.

Two Ag-AgCl electrodes, one on each side of the planar lipid bilayer, were plunged into the salt solution. Transmembrane voltage was measured via a LeCroy differential amplifier 1822. The same electrodes were used to measure transmembrane current. Both signals were stored in oscilloscope LeCroy Waverunner-2 354 M in Matlab format. All the signals were processed offline. Chamber is made out of Teflon. It consists of two cubed reservoirs with volume of 5.3 cm^3 each. In the hole between two reservoirs, a thin Teflon sheet with a round hole

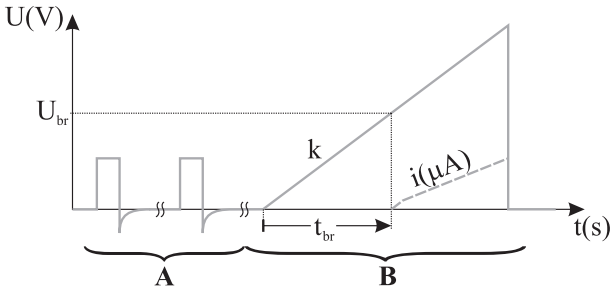

Fig. 2. Measurement protocol: (A) capacitance measurement of lipid bilayer was measured in two steps. In the first step, we measured capacitance of the electronic system without lipid bilayer. Second step was measuring capacitance of electronic system with lipid bilayer and salt solution. (B) Voltage breakdown measurement with linear rising signal.

(105 μm diameter) is inserted. Lipid bilayer is formed by the Montal-Muller method [14].

2.2. Chemical setup

The salt solution was prepared of 0.1 molar KCl and 0.01 molar Hepes in the same proportion. Some droplets of one molar NaOH were added to obtain pH 4.7. The lipids were prepared from POPC (1-pamitol, 2-oleiol phosphatdiholin) in powder form (Avanti Polar-Lipids Inc. ZDA). The POPC was melted in solution of hexane and ethanol in ratio 9:1. The mixture of hexadecane and pentane in ratio 3:7 was used for torus forming.

2.3. Measurement protocol

Measurement protocol consisted of two parts: capacitance measurement (Fig. 2A) and lipid bilayer breakdown voltage measurement (Fig. 2B). Capacitance and the breakdown voltage were determined for each lipid bilayer.

The capacitance of lipid bilayer was measured in two steps (Fig. 2). In the first step, we measured capacitance of the electronic system without lipid bilayer (C_{SYS}). The resistance of oscilloscope probes (1 MΩ) and the special resistor (1 MΩ) were

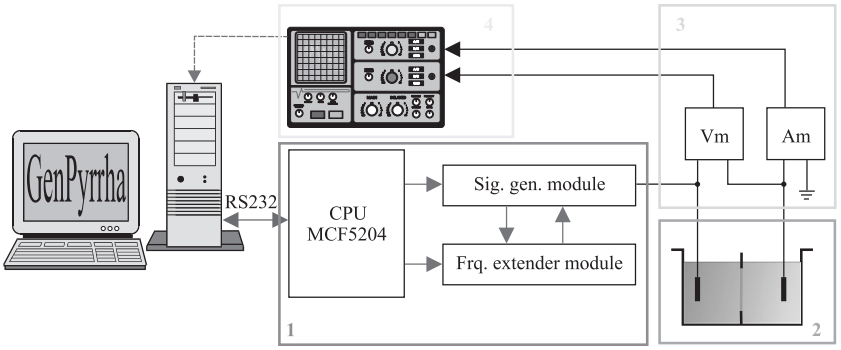

Fig. 1. System for electroporation of planar lipid bilayer. (1) The microprocessor board with MCF5024 processor and two modules. One module generates arbitrary signals and the other that is realized in Xillinx is used for frequency extension. (2) Chamber for forming lipid bilayers and two Ag-AgCl electrodes. (3) Modules for current and voltage amplification. (4) Digital oscilloscope for data storing.

connected in parallel as shown on Fig. 3A. Therefore, the resistance R_{SYS} was approximately 500 kΩ. By finding the time constant τ_{SYS} of the voltage discharge the capacitance C_{SYS} was determined considering the relation

$$C_{SYS} = \frac{\tau_{SYS}}{R_{SYS}}. \quad (1)$$

Second step was measuring capacitance of electronic system with lipid bilayer and salt solution (C_{SBLM}) (Fig. 3B). Equivalent resistor (R_{SYS}) was still 500 kΩ, because the resistance of the lipid bilayer was greater than 10^8 Ω [15].

By finding the time constant τ_{SBLM} of the voltage discharge, the capacitance C_{SBLM} was determined

$$C_{SBLM} = \frac{\tau_{SBLM}}{R_{SBLM}}. \quad (2)$$

The capacitance of lipid bilayer was obtained as a difference between C_{SYS} and C_{SBLM}:

$$C_{BLM} = C_{CYS} - C_{SBLM}. \quad (3)$$

The specific membrane capacitance (c) was obtained by dividing C_{BLM} by the bilayer surface area.

We determined breakdown voltage (U_{br}) of the lipid bilayer by the linear rising signal. The slope of the linear rising signal (k) and the peak voltage of the signal has to be selected in advance. Six different slopes were selected. Breakdown voltage was defined as the voltage at the moment t_{br} when sudden increase of transmembrane current was observed. Time of breakdown t_{br} was defined as a lifetime of the lipid bilayer at a chosen slope of the linear rising signal (Fig. 2).

2.4. Statistics

To compare breakdown voltages and specific membrane capacitances of the lipid bilayers exposed to voltage signals of different slopes (k) Kruskal–Wallis, one-way analysis of variance on ranks test was used. All pairwise multiple comparisons were made by Tukey's test. Descriptive statistics include mean value and standard deviation.

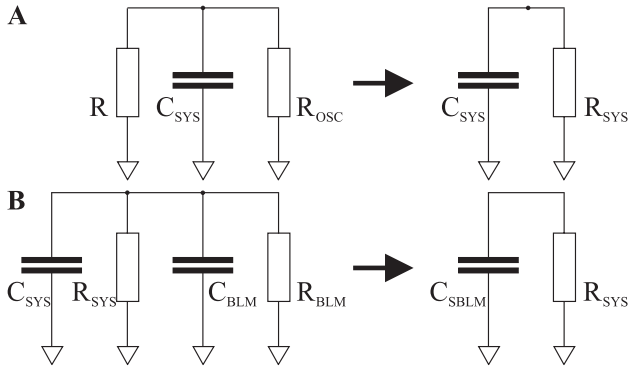

Fig. 3. Electric circuits: (A) system without lipid bilayer and salt solution; (B) system with planar lipid bilayer and salt solution.

Table 1
Specific membrane capacitances (c), breakdown voltages (U_{br}) and lifetimes (t_{br}) for lipid bilayers exposed to linear rising voltage signals of different slopes (k)

N	k (kV/s)	c (μF/cm^2)	U_{br} (V)	t_{br} (μs)
18	4.8	0.5±0.1	0.51±0.02	104±4
6	5.5	0.5±0.1	0.48±0.01	86±3
4	7.8	0.6±0.1	0.49±0.01	61±2
3	16.7	–	0.57±0.01	34±1
5	21.6	0.4±0.1	0.59±0.02	27±1
3	48.1	–	0.74±0.02	15±1

Values given are mean±standard deviation. Number of measurements N in each experimental group is given in the first column.

Using nonlinear regression, a two-parameter curve was fitted to the data

$$U = \frac{a}{1 - e^{-t/b}}, \quad (4)$$

where U was U_{br} measured at different slopes, t was corresponding t_{br}, and a and b are two parameters. Parameter a is an asymptote of the curve, which corresponds to minimal breakdown voltage U_{brMIN} for specific bilayer. Parameter b governs the inclination of the curve.

3. Results

Mean specific membrane capacitances (c) and mean breakdown voltages (U_{br}) of the lipid bilayers with their standard deviations for six different slopes (k) are given in Table 1. Specific membrane capacitances of membranes exposed to linear rising signal of different slopes (k) were in the same range; no statistically significant difference was obtained between different experimental groups. The c for all bilayers included in the study was measured to be 0.5±0.1 μF/cm^2.

Breakdown voltage U_{br} increased with increasing slope of the linear rising voltage signal. U_{br} measured at slope 4.8 kV/s is not statistically different from U_{br} measured at slope 5.5 kV/s

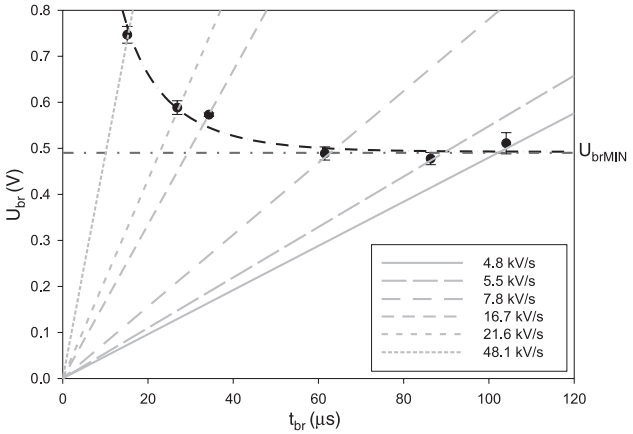

Fig. 4. The breakdown voltage (U_{br}) (black dots) of lipid bilayers as a function of lifetime t_{br}. The gray lines on the figure show six different slopes (k) of applied liner rising voltage signal. Black dashed curve represents two parameters equation fitted to data (Eq. (4)). An asymptote of the curve (a) with the value of 0.49 V corresponds to minimal breakdown voltage U_{brMIN} for lipid bilayers made of POPC.

($p=0.009$) and it is not statistically different from U_{br} measured at slope 7.8 kV/s ($p=0.305$). U_{br} measured at slope 5.5 kV/s is not statistically different from U_{br} measured at slope 7.8 kV/s ($p=0.941$). Also U_{br} measured at slopes 16.7 kV/s and 21.6 kV/s are not statistically different ($p=0.872$). All other pairwise multiple comparisons exhibited $p<0.001$; these means that U_{br} of all other experimental groups differ significantly.

The data are also presented in graphical form (Fig. 4). The parameters a and b of the curve (4) are 0.49 V and 14.77 μs, respectively. An asymptote of the curve (a) with the value of 0.49 V is considered as minimal breakdown voltage U_{brMIN} for lipid bilayers made of POPC.

4. Discussion

Breakdown voltage is one of the most important properties of a lipid bilayer when biomedical and biotechnological applications of electroporation are under consideration. Although planar lipid bilayer differs in a number of characteristics from the biological membrane, it is believed that general picture of the electroporation is the same [16]. The aim of this study was to present a different measuring protocol for determination of the lipid bilayer breakdown voltage, which would avoid multiple exposure of the lipid bilayer to a voltage stress.

According to our measuring protocol, each lipid bilayer is exposed to voltage signal only twice. First bilayer capacitance is determined by a rectangular voltage signal of 0.30 V. Capacitance measurement reveals an intact lipid bilayer before inducing its breakdown. In the next step, lipid bilayer breakdown is induced by linear rising voltage signal.

Evans et al. used similar approach in their experiments on lipid vesicles [17]. They applied tension at different loading rates and they found out that tension needed for membrane rupture increases with increasing loading rate. These results are in line with the results of our study, with different stimulating signal. On the other hand, Satkauskas et al. compared effectiveness of different electrochemotherapy protocols in vivo on mice [18]. They used different durations and amplitudes of rectangular voltage pulses and observed nonlinear dependence between amplitude (strength) and duration of the signal for the same electrochemotherapy effectiveness.

In our study, we selected six different slopes of linear rising voltage signal due to already known experimental evidence that lipid bilayer lifetime is dependent on the applied voltage [12,19] and that the lipid bilayer breakdown voltage is dependent on the lipid bilayer pre-treatment [13]. Our results show that lifetime of lipid bilayer depends on the slope of linear rising voltage signal and that also the breakdown voltage is a function of the slope of the linear rising voltage signal; it increases with increasing slope. This is comparable to results obtained by Evans et al. [17]. According to theoretical model proposed by Joshi and Schoenbach [20], the pore generation rate depends exponentially on the membrane voltage; therefore, in the case of steep voltage signal, breakdown voltage was achieved after shorter pre-treatment. On the other hand, also because of shorter pre-treatment of the lipid bilayer in the case of steep voltage signal the corresponding breakdown voltage was higher. Strength-duration two-parameter curve (Eq. (4)) was fitted to experimental data and an asymptote corresponded to minimal breakdown voltage U_{brMIN} of specific lipid bilayer (POPC).

Troiano et al. [12] have used the same lipids (POPC) in their experimental studies. With considerably long rectangular voltage pulses (10 s), they had detected breakdown voltage of 0.17 V. This value however is much lower then the U_{brMIN} obtained in our study (0.49 V). Because the entire pre-treatment (number of applied voltage pulses) of the lipid bilayers in their experiments is not known, it is possible that much low breakdown voltage was detected.

Based on our results, we suggest determination of lipid bilayer breakdown voltage by linear rising signal. Better reproducibility and lower scattering are obtained due to fact that each bilayer is exposed to electroporation treatment only once. Moreover, minimal breakdown voltage for specific lipid bilayer can be determined.

References

[1] E. Neumann, S. Kakorin, K. Toensing, Funadamentals of electroporative delivery of drugs and genes, Bioelectrochemistry and Bioenergetics 48 (1999) 3–16.
[2] A.R. Dent, V. Preat, Transdermal delivery of timol by electroporation through human skin, Journal of Controlled Release 88 (2003) 253–262.
[3] D. Ferber, Gene therapy: safer and virus-free? Science 294 (2001) 1638–1642.
[4] L.M. Mir, S. Orlowski, Mechanisms of electrochemotherapy, Advanced Drug Delivery Reviews 35 (1999) 107–118.
[5] M. Cemazar, I. Wilson, G.U. Dachs, G.M. Tozer, G. Sersa, Direct visualisation of electroporation-assisted in vivo gene delivery to tumors using interval microscopy—spatial and time dependent distribution, BMC Cancer 4 (2001) 81–87.
[6] G.W. Gould, Biodeterioration of foods and an overview of preservation in the food and dairy industries, International Biodeterioration & Biodegradation 36 (1995) 267–277.
[7] M.C. Vernhes, A. Benichou, P. Pernin, P.A. Cabanes, J. Teissie, Elimination of free-living amoebae in fresh water with pulsed electric fields, Water Research 36 (2002) 3429–3438.
[8] M. Puc, S. Corovic, K. Flisar, M. Petkovsek, J. Nastran, D. Miklavcic, Techniques of signal generation required for electropermeabilisation. Survey of electropermeabilisation devices, Bioelectrochemistry 64 (2004) 113–124.
[9] A. Macek Lebar, G. Sersa, S. Kranjc, A. Groselj, D. Miklavcic, Optimisation of pulse parameters in vitro for in vivo electrochemotherapy, Anticancer Research 22 (2002) 1731–1736.
[10] M. Fosnaric, V. Kralj-Iglic, K. Bohinc, A. Iglic, S. May, Stabilization of pores in lipid bilayers by anisotropic inclusions, Journal of Physical Chemistry. B 107 (2003) 12519–12526.
[11] H.T. Tien, A. Ottova, Planar lipid bilayers (BLMs) and their applications, Elsevier, New York, 2003, Ch. The Lipid Bilayer Concept: Experimental Realization and Current Applications, pp. 1–74.
[12] G.C. Troiano, L. Tung, V. Sharma, K.J. Stebe, The reduction in electroporation voltages by the addition of surfactant to planar lipid bilayer, Biophysical Journal 75 (1998) 880–888.
[13] I.G. Abidor, V.B. Arakelyan, L.V. Chernomordik, Y.A. Chizmadzhev, V.F. Pastushenko, M.R. Tarasevich, 246-electric breakdown of bilayer lipid membranes: i. The main experimental facts and their qualitative discussion, Bioelectrochemistry and Bioenergetics 6 (1979) 37–52.
[14] M. Montal, P. Muller, Formation of bimolecular membranes from lipid monolayers and study their electrical properties, Proceedings of the National Academy of Sciences of the United States of America 69 (1972) 3561–3566.
[15] H.T. Tien, Bilayer Lipid Membranes (BLM), Marcel Dekker, inc., New York, 1974.

[16] M. Tarek, Membrane electroporation: a molecular dynamics simulation, Biophysical Journal 88 (2005) 4045–4053.
[17] E. Evans, V. Heinrich, F. Ludwig, W. Rawicz, Dynamics tension spectroscopy and strength of biomembranes, Biophysical Journal 85 (2003) 2342–2350.
[18] S. Satkauskas, D. Batiuskaite, S. Salomskaite-Davalgiene, M.S. Venslauskas, Effectivness of tumor electrochemotherapy as a function of electric pulse strength duration, Bioelectrochemistry 65 (2005) 105–111.
[19] A. Macek Lebar, G.C. Troiano, L. Tung, D. Miklavcic, Inter-pulse interval between rectangular voltage pulses affects electroporation threshold of artificial lipid bilayers, IEEE Transactions on Nanobioscience 1 (3) (2002) 116–120.
[20] R.P. Joshi, K.H. Schoenbach, Electroporation dynamics in biological cells subjected to ultrafast electrical pulses: a numerical simulation study, Physical Review E 62 (2000) 1025–1033.