Electroporation of planar lipid bilayers L5

Małgorzata Kotulska[1], Przemyslaw Sadowski[1], Peter Kramar[2], Alenka Maček Lebar[2]
[1]Institute of Biomedical Engineering and Instrumentation, Wrocław University of Technology, Poland
[2]University of Ljubljana, Faculty of Electrical Engineering, Slovenia

Duration of the experiments: 45 min
Max. number of participants: 4
Location: Cell culture laboratory 2
Level: Basic

PREREQUISITES

Participants should be familiar with the Safety rules (following the recommendations of the Good laboratory practice). No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

A planar lipid bilayer can be considered as a small fraction of total cell membrane. As such has often been used to investigate basic aspects of electroporation [1,2]; especially because of its geometric advantage allowing chemical and electrical access to both sides of the lipid bilayer. Usually a thin bi-molecular film composed of specific phospholipids and organic solvent is formed on a small aperture in a hydrophobic partition separating two aqueous compartments. Electrodes immersed in these two aqueous compartments allow to measure current and voltage across the lipid bilayer.
Two different measurement principles of planar lipid bilayer's properties can be used: voltage [3] or current clamp method [4]. Planar lipid bilayer from an electrical point of view can be considered as a capacitor, therefore two electrical properties, capacitance and resistance, mostly determine its behavior.

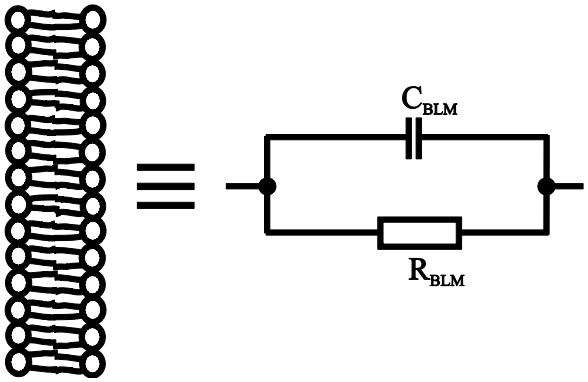

Figure 1. Electrical representation of a planar lipid bilayer.

The aim of the experiment is to build a planar lipid bilayer and to determine capacitance and resistance of the planar lipid bilayer using current or voltage clamp method. In studying basic aspects of electroporation lipid bilayer pore formation and voltage breakdown determination will be observed.

EXPERIMENT

Protocol:

Current clamp method

Form the membrane by the Mueller-Rudin method [5]. Cover the surface of the aperture in a barrier separating two compartments of a measuring vessel with a lecithin solution (20mg/1ml of hexane). After evaporation of hexane, fill compartments with solution consisting of 0.1 mol NaCl, 0.01 mol of HEPES, at pH=7.0. Next, apply a drop of lecithin dissolved in decane (20mg/ml) to the aperture by the micropipette or paint it by a Teflon brush. Observe the membrane capacitance that changes along the membrane formation. When the bilayer is formed, which is indicated by capacitance stabilization,

perform cyclic voltammetry and check the membrane resistance. Next, apply the constant-current on lipid bilayer. This will produce a long-lived electropore, oscillating around its mean diameter. And finally apply a linearly increasing current of the values ranging from 0 to 1 nA to current electrodes and record a transmembrane potential. When you disconnect the current source, the resealing process can be observed. During the experiment you will obtain the time course of the transmembrane voltage and the plot of the programmed current flowing between two current electrodes.

Voltage clamp method

Form lipid bilayer on aperture (d=117 μm) by the Montal - Muller method [6]. Measurement protocol consists of two parts: capacitance measurement and lipid bilayer breakdown voltage measurement. Measure a capacitance of each planar lipid bilayer by discharge method [7]. Then select the slope of linear rising signal and apply it to lipid bilayer. From oscilloscope determine the breakdown voltage (U_{br}) and the lifetime (t_{br}) of planar lipid bilayer.

FURTHER READING:

1. Kalinowski S., Figaszewski Z., A new system for bilayer lipid membrane capacitance measurements: method, apparatus and applications, Biochim. Biophys. Acta 1992; 1112:57-66.
2. Pavlin M, Kotnik T, Miklavcic D, Kramar P, Macek-Lebar A. Electroporation of planar lipid bilayers and membranes. In Leitmanova Liu A (ed.), Advances in Planar Lipid Bilayers and Liposomes, Volume 6, Elsevier, Amsterdam, 2008, pp. 165-226.
3. Koronkiewicz S., Kalinowski S., Bryl K., Programmable chronopotentiometry as a tool for the study of electroporation and resealing of pores in bilayer lipid membranes. Biochim. Biophys. Acta 2002, 1561:222–229.
4. Kotulska M., Natural fluctuationsof an electropore show fractional Lévy stable motion, Biophys. J. 2007, 92:2412-21.
5. Mueller P., Rudin DO., Tien HT., Wescott WC., Methods for the formation of single bimolecular lipid membranes in aqueous solutions. J. Phys. Chem. 1963, 67:534-535.
6. Montal M., Mueller, P., Formation Of Bimolecular Membranes From Lipid Monolayers And A Study Of Their Electrical Properties, Proceedings Of The National Academy Of Sciences Of The United States Of America, 1972, 69:3561-3566.
7. Kramar P, Miklavčič D, Maček-Lebar A. Determination of the lipid bilayer breakdown voltage by means of a linear rising signal. Bioelectrochemistry 70: 23-27, 2007.

NOTES & RESULTS