Biochimica et Biophysica Acta 1798 (2010) 21–31

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

The influence of different membrane components on the electrical stability of bilayer lipid membranes

Iris van Uitert, Séverine Le Gac, Albert van den Berg *

BIOS the Lab-on-a-Chip group, MESA+ Institute for Nanotechnnology, University of Twente, Postbus 217, 7500 AE Enschede, The Netherlands

ARTICLE INFO

Article history:
Received 9 April 2009
Received in revised form 1 October 2009
Accepted 6 October 2009
Available online 14 October 2009

Keywords:
Electroporation
BLM
Cell membrane
Pore formation
(Phospho)lipid
Channel protein

ABSTRACT

A good understanding of cell membrane properties is crucial for better controlled and reproducible experiments, particularly for cell electroporation where the mechanism of pore formation is not fully elucidated. In this article we study the influence on that process of several constituents found in natural membranes using bilayer lipid membranes. This is achieved by measuring the electroporation threshold (V_{th}) defined as the potential at which pores appear in the membrane. We start from highly stable 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) membranes (V_{th} ~200 mV), and subsequently add therein other phospholipids, cholesterol and a channel protein. While the phospholipid composition has a slight effect (100 mV$\leq V_{th} \leq$290 mV), cholesterol gives a concentration-dependent effect: a slight stabilization until 5% weight (V_{th} ~250 mV) followed by a noticeable destabilization (V_{th} ~100 mV at 20%). Interestingly, the presence of a model protein, α-hemolysin, dramatically disfavours membrane poration and V_{th} shows a 4-fold increase (~800 mV) from a protein density in the membrane of 24×10^{-3} proteins/μm^2. In general, we find that pore formation is affected by the molecular organization (packing and ordering) in the membrane and by its thickness. We correlate the resulting changes in molecular interactions to theories on pore formation.

1. Introduction

A cell membrane consists of a (phospho)lipid matrix defining its structure and shape and serving as a substrate for membrane proteins. Phospholipids are composed of two main parts: (i) a hydrophilic head consisting of a backbone molecule (either glycerol or sphingosine), a phosphate and a polar group and (ii) two "parallel" hydrophobic chains (saturated or unsaturated and of various lengths). Due to their amphiphilic structure, phospholipid molecules self-assemble into micelle or bilayer structures when placed in aqueous solutions. The properties of phospholipids are determined by the length of the hydrocarbon chains, the amount of unsaturations present in the chains, the molecular shape and the nature of the head group. These distinct properties of phospholipids affect their packing density and consequently the fluidity and stability of the membranes. Another important lipidic constituent of the cell membrane is cholesterol that typically represents 30% mole of the lipid matrix. Cholesterol greatly affects the fluidity of the membrane by establishing specific interactions with the hydrocarbon chains and head groups of the phospholipids [1]. The last main component found in cell membranes is proteins which account for 50% of the membrane weight [2]. The so-called transmembrane proteins span the membrane and present hydrophilic parts eventually protruding on both sides and a hydrophobic part located in the hydrophobic core of the membrane.

A cell membrane forms an impermeable barrier to foreign entities, among which genes, drugs, particles, and certain dyes. For many applications, such as DNA transfection or drug research, it is necessary to transport these substances into a cell, and this requires the transient permeabilization of the cell membrane. A commonly used technique for this purpose is electroporation [3-5]. Thereby, pores are temporarily created in the membrane upon application of a high external electric field (kV/cm), usually short DC pulses (μs–ms range) or exponentially decaying pulses [6]. When the imposed transmembrane potential reaches a threshold value of about 0.2–1 V, a rearrangement in the molecular structure of the membrane occurs, leading to the formation of pores and a substantial increase in the cell's permeability to ions and molecules. These last few decades, the popularity of the technique of electroporation has been increasing, notably for cell transfection [7,8]. However, the overall success rate of the process remains low: using batch electroporation typically only 40% to 70% of the cells are viably electroporated [9], most of them remain viable while being unaffected by the electrical stimulus and a small amount dies. Indeed, the whole process is difficult to control at

Abbreviations: DPhPC, 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine; PS, Porcine brain l-α-phosphatidylserine; PI, Bovine liver l-α-phosphatidylinositol; PE, Bovine heart l-α-phosphatidylethanolamine; PC, Phosphatidylcholine; Ch, Cholesterol; BLM, Bilayer lipid membrane; V_{th}, Electroporation threshold voltage

* Corresponding author. Tel.: +31 53 489 2724; fax: +31 53 489 3595.
E-mail address: a.vandenberg@utwente.nl (A. van den Berg).

0005-2736/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2009.10.003

the level of a cell population as the outcome of the electroporation process depends on a number of uncontrollable parameters, such as the cell size, shape and "vulnerability" [6].

One approach to increase the success rate of electroporation experiments, consists of getting a better understanding of the mechanism(s) of pore formation and identifying key-parameters for this process through experimental work and/or theoretical modelling. Studies on the mechanism of pore formation provide insight into the pore location and into the sequence of molecular events leading to pore formation. For instance, the comparison between the electrical breakdown of cell membranes and lipid membranes has demonstrated that the pores originate in the lipid matrix of the membrane [10]. Furthermore, molecular dynamics simulations have demonstrated that the formation of a pore proceeds in three steps upon application of a potential across the membrane. In the first stage, the electric field is locally enhanced, causing water defects in the bilayer structure. In the second stage, water molecules form a water file that spans the bilayer by establishing hydrogen bonds with each other. In the last stage, molecular rearrangement of the phospholipids in the vicinity of this water defect occurs and phospholipid molecules move towards this water channel to give a hydrophilic pore lined with phospholipid head groups [11,12]. Pores start to form nano- to microseconds after application of the electric field [12-15], expand in a few milliseconds and close again in seconds to minutes in cells and milliseconds to seconds in artificial membranes [10,14,16,17]. The diameter of an electropore varies from 0.5 to 400 nm [12,13,15,18].

Another crucial aspect towards understanding the process of electroporation is the identification of key parameters affecting the stability of membranes. Experimental and theoretical studies have shown that this strongly depends on the composition of the membrane: the structural properties of the phospholipids found therein and the presence and amount of other membrane constituents. For instance, the electroporation threshold depends on the bilayer thickness, and consequently on the length of the phospholipid hydrocarbon chains [19,20]. Another key-factor is the presence of non-zero intrinsic monolayer curvature phospholipids in the membrane. Such lipids have a conical shape with either a large head group (positive intrinsic monolayer curvature) or a small head group (negative intrinsic monolayer curvature) compared to the hydrocarbon chains. These molecules cause packing defects in the membrane that facilitate the process of pore formation [21,22]. Interestingly, it has been demonstrated that the head group charge does not influence the membrane stability although one could expect that electrostatic interactions (repulsion or attraction) between different head groups would result in a change in the packing density of the membrane [19,20]. A second membrane compound of importance is cholesterol; it affects membrane properties not only through specific interactions with the hydrocarbon chains [23-27] but also because of its negative intrinsic monolayer curvature [21,28-30]. Depending on the nature of the phospholipid molecules and the amount of cholesterol used this effect can be two-fold [24,31-33]. The last main component found in natural membranes, peptides and proteins, also influences the stability although this phenomenon is still poorly understood. For instance, Troiano et al. [34] showed that the presence of small peptides such as gramicidin A and D in the membrane decreases the probability of pore formation. Recent molecular dynamics studies have corroborated these results [35,36].

Alternatively, external (natural or synthetic) molecules can also be employed to modulate the membrane properties: they strengthen molecular interactions between phospholipids or loosen the intermolecular structure of the membrane. For instance, both the detergent octaethyleneglycol mono n-dodecyl ether ($C_{12}E_8$) [37] and the solvent dimethylsulfoxide (DMSO) [38] decrease the stability of the membranes by acting as a positive curvature molecule. Additionally, the surfactant sodium dodecyl sulfate (SDS) reduces the membrane stability by lowering the interactions between phospholipid molecules due to its incorporation in the cell membranes [39]. On the other hand, the addition of the surfactant poloxamer 188 leads to an increase in the membrane stability; this is caused by either specific interactions with the phospholipids or by the formation of an insulating layer at the membrane surface, behaving as a shunting layer for the applied potential [40].

These different results suggest that the molecular aspects of the composition of the membrane are important for the creation of pores and the electrical stability of the membranes, and that the interactions between the different molecules in the membrane are key-parameters in the process of pore formation.

In this article, we aim to get a better insight into the mechanism of electropore formation. For that purpose, we investigate the influence of the membrane composition and of its individual constituents on its (electrical) stability. Our approach consists of using artificial membrane models, bilayer lipid membranes (BLMs) and assessing the membrane stability by measuring the electroporation threshold. This threshold is defined as the potential at which pores are observed in the membrane. The basic "building block" we use to prepare the membranes is a synthetic phospholipid, 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC). This phospholipid is composed of two identical saturated hydrocarbon chains functionalized with methyl groups and a relatively large head group. It yields densely packed and very stable membranes. In a first stage, we investigate to which extent the electrical stability may be affected by the properties of the individual phospholipids present in the membrane. For that purpose, BLMs are prepared using a mixture of two phospholipids; DPhPC mixed with other phospholipids found in natural cell membranes that are unsaturated and have a non-zero intrinsic monolayer curvature. Following this, we examine how cholesterol affects pore formation in DPhPC membranes, and finally the contribution of one type of channel proteins, α-hemolysin, on the same process.

2. Materials and methods

2.1. Chemicals

Lipids (1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC), bovine heart l-α-phosphatidylethanolamine (PE), bovine liver l-α-phosphatidylinositol (PI), porcine brain l-α-phosphatidylserine (PS) and cholesterol (Ch) (Fig. 1)) are purchased at Avanti Polar Lipids (Alabaster, AL). The protein α-hemolysin is purchased at Sigma-Aldrich (St. Louis, MO). KCl, Hepes, Tris and n-decane are purchased at Sigma-Aldrich (St. Louis, MO). Chloroform is purchased at Merck Chemicals (Darmstadt, Germany). Deionized water (18.2 mΩ×cm) which is used for all solution preparation and cleaning procedure is obtained using a MilliQ system (Millipore, Billerica, MA).

2.2. Measurement set-up

A conventional bilayer system (Warner Instruments, Hamden, CT) is used for BLM experimentation. This system comprises of a delrin cup and chamber containing two round compartments. The cup is inserted in the *trans*-compartment of the chamber, and the compartments are connected via a 150 μm aperture in the cup across which BLMs are prepared. Both compartments contain 1 mL buffer solution and Ag/AgCl electrodes (used for the electrical characterization of the BLMs). Electrical measurements are carried out with an Axopatch 200b patch-clamp amplifier (Molecular devices, Sunnyvale, CA), applying voltages and measuring currents across the bilayer. Data-acquisition is performed with LabVIEW and a PCI-6259 data acquisition card (National Instruments, Austin, TX).

2.3. Preparation and characterization of BLMs

All lipids are used as chloroform-based solutions. DPhPC and PI are purchased at 10 mg/mL solutions in chloroform. Other lipids (PS, PI,