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Specific electrical capacitance and voltage breakdown as a function of temperature for different planar lipid bilayers

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ABSTRACT

The breakdown voltage and specific electrical capacitance of planar lipid bilayers formed from lipids isolated from the membrane of archaeon *Aeropyrum pernix K1* as a function of temperature were studied and compared with data obtained previously in MD simulation studies. Temperature dependence of breakdown voltage and specific electrical capacitance was measured also for dipalmitoylphosphatidylcholine (DPPC) bilayers and bilayers formed from mixture of diphytanoylphosphocholine (DPhPC) and DPPC in ratio 80:20.
The breakdown voltage of archaeal lipids planar lipid bilayers is more or less constant until 50 °C, while at higher temperatures a considerable drop is observed, which is in line with the results from MD simulations. The breakdown voltage of DPPC planar lipid bilayer at melting temperature is considerably higher than in the gel phase. Specific electrical capacitance of planar lipid bilayers formed from archaeal lipids is approximately constant for temperatures up to 40 °C and then gradually decreases. The difference with MD simulation predictions is discussed. Specific electrical capacitance of DPPC planar lipid bilayers in fluid phase is 1.75 times larger than that of the gel phase and it follows intermediated phases before phase transition. Increase in specific electrical capacitance while approaching melting point of DPPC is visible also for DPhPC:DPPC mixture.

1. Introduction

Lipid molecules are the main component of cell membranes. Plasma membrane, that separates the interior of the cell from the outside environment, is adapted to living environment of the cell and the functions that the cell has in this environment. Therefore the composition of plasma membrane is not the same in all cells. While phospholipids, glycolipids and sterols are the most common in plasma membranes of eukaryotic cells and bacteria, archaeal membranes contain glycerol ether lipids with saturated chains containing methyl branches. Extreme living conditions, like high temperatures, strong acidity, alkalinity or salinity, determine the unique features of archaeal plasma membrane that are in great extent defined by the structure and properties of archaeal lipid constituents. Therefore archaeal lipids also show broad structure diversity [1,2]. Unique characteristics of archaeal membranes are the reason for diversity of studies suggesting their use in various biotechnological applications [3,4]. Among others, archaeosomes are proposed for using as a drug carrier [5]. In this case drug release could be enhanced by electroporation [6]. Considering such application, the behaviour of the archaeal lipid membrane in electric field is important in addition to membrane's structural and chemical properties.

In this study we focused on lipids that constitute the membrane of the aerobic hyperthermophilic archaeon *Aeropyrum pernix K1*. The detailed structure of constituents, 2,3-di-O-sesterterpanyl-sn-glycerol-1-phospho-1′-(2′-O-α-D-glucosyl)-myo-inositol (AGI) and 2,3-di-O-sesterterpanyl-sn-glycerol-1-phospho-myo-inositol (AI), was elucidated by Morii et al. in 1999 [7]. These two lipids usually compose archaeal membrane in the mol% ratio 91:9. The important feature of both lipids is C_{25}-isopranoid as a hydrophobic part, while the head of the lipid molecule inositol is linked on the phosphate group in AI and glucosylinositol in AGI. As can be seen in Fig. 1, hydroxyl groups are present on all available C-atoms in the sugar rings.

Physicochemical properties of archaeosomes prepared from lipids isolated from *A. pernix K1* were studied by Gmajner et al. [8,9] and Genova et al. [10]. Archaeosomes exhibit large negative surface charge (zeta potential: −50 to −110 mV, increasing with diameter) in broad pH range (2.5 to 12) have low permeability at pH between 5 and 9 while permeability increases moderately with temperature [8]. Differential scanning calorimetry (DSC) has not detected typical gel to liquid phase transition in the temperature range from 0 °C to 100 °C, only broad gradual transition in the temperature range from 0 °C to 40 °C [8]. Electron paramagnetic resonance (EPR) spectra have shown that the archaeosome membranes are heterogeneous, and are composed of components with three types of fluidity characteristics. The presence of each fluidity type depends on pH and temperature. In general, continuous increase in membrane fluidity with temperature has been noticed. Above 60 °C the presence of only fluid-like domains has been detected at pH between 4 and 11 [9]. Genova et al. [10] showed that bending elasticity modulus of the giant vesicles composed of lipids isolated

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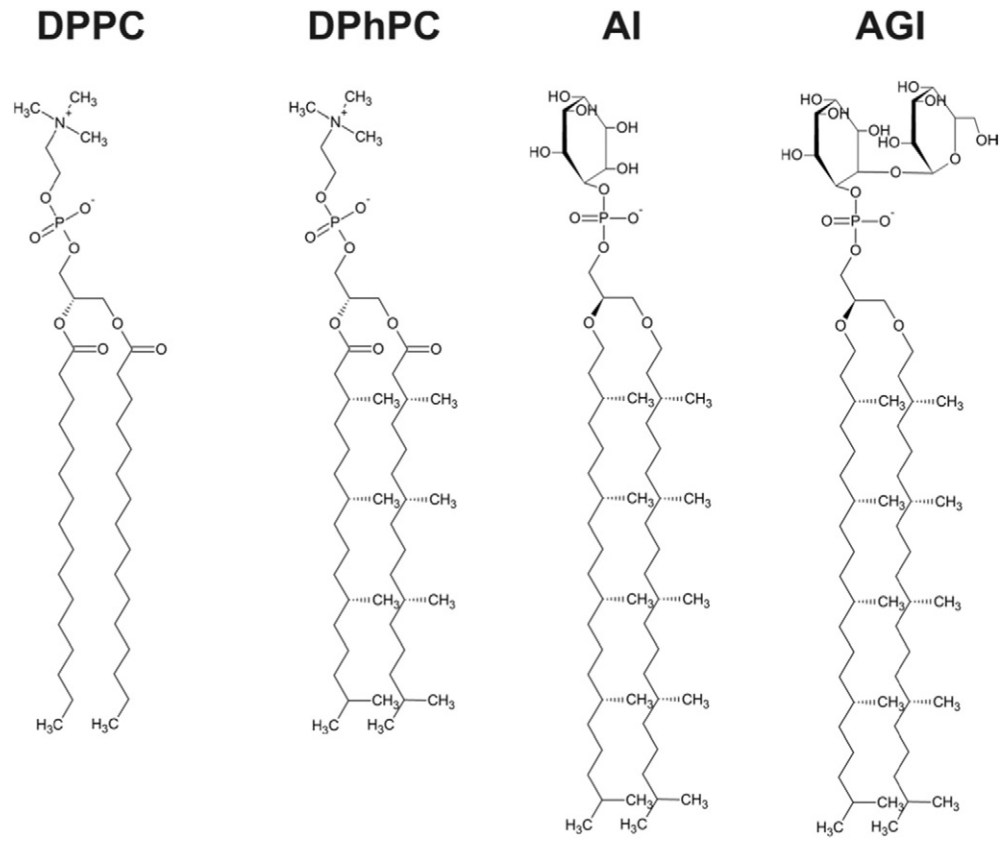

Fig. 1. The chemical structure of the lipid molecules: dipalmitoyl phosphatidylcholine (DPPC), diphytanoyl-phosphocholine (DPhPC) and two components of *A. pernix K1* arheal lipids: 2,3-di-O-sesterterpanyl-sn-glycerol-1-phospho-myo-inositol (AI) and 2,3-di-O-sesterterpanyl-sn-glycerol-1-phospho-1′-(2′-O-α-D-glucosyl)-myo-inositol (AGI).

from *A. pernix K1* is $1.89 \cdot 10^{-19}$ J at 27 °C, meaning that at this temperature archaeal membranes have similar elastic properties as membranes composed of eukaryotic lipids.

The AGI/AI bilayers, that mimic lipid structure of archaeal *A. pernix K1* membrane, have been modelled in MD simulations by Polak et al. [11,12], where structural characteristics have been studied and the behaviour of the bilayer in electric field. Good agreement of the electron density profiles resulted from MD simulations and small angle X-ray scattering (SAXS) has been obtained at 25 °C and 50 °C. Like other lipid bilayers also AGI/AI bilayers react to external electric field by pore formation. The MD simulations showed, that relatively large voltage (5.2 V at 25 °C) is needed for pore formation and that archaeal lipids do not migrate toward the interior of the hydrophobic core to stabilize the pore edge, which means that only hydrophobic pore is formed.

In our present study we investigate electrical properties of planar lipid bilayers formed from lipids isolated from *A. pernix K1*. We measured their specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}), i.e. the voltage that causes the planar lipid bilayer irreversible rupture [13], as a function of temperature. For comparison, temperature dependence of specific electrical capacitance and breakdown voltage was measured also for dipalmitoyl phosphatidylcholine (DPPC) bilayers and bilayers formed from mixture of diphytanoyl-phosphocholine (DPhPC) and DPPC in ratio 80:20. All lipids were carefully selected according to their chemical structure (Fig. 1). In the headgroup of all lipids phosphate group is present; additionally, DPhPC and DPPC incorporate choline, while inositol/glucoinositol is present in archaeal lipids (AI and AGI). Headgroups are linked to hydrocarbon chains by ester links in DPhPC and DPPC lipids, on the other hand ether links are present in archaeal lipids. Hydrocarbon chains in DPhPC and DPPC lipids are of the same length (C16), but they are straight in DPPC and highly methylbranched in DPhPC. Similar but longer (C25) highly methylbranched isopranoid chains are present also in both archaeal lipids. DPPC is an extensively studied lipid that exhibits a clear gel-fluid phase transition at 41 °C [14]. The increase in lipid bilayer capacitance and in intensity of current fluctuations was shown at phase transition temperature [15–17]; while according to our knowledge, breakdown voltage at phase transition has not been measured. The electrical properties of DPhPC have been studied at room temperature [18–23], but not in broader range of temperatures. It also has to be noted that DPhPC does not show phase transition from gel to fluid phase over a temperature range from −120 °C to 120 °C [24].

In this article we present the behaviour of specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}) as a function of temperature in the range 19 °C to 56 °C for planar lipid bilayers made of lipids isolated from *A. pernix K1*, DPPC and DPhPC:DPPC mixture in ratio 80:20. Additionally, we compare the experimentally obtained values of both parameters with previously published data from MD simulation studies. The important differences in the two approaches are pointed out and discussed.

2. Materials and methods

Planar lipid bilayers were formed following the method described by Montall and Mueller [25] from lipids extracted from archaea *A. pernix K1*, DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) and mixture of DPhPC (1,2-diphytanoyl-sn-glycero-3-phosphocholine) and DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) in ratio 80:20. Extraction of archaeal lipids was done at University of Ljubljana, Biotechnical Faculty, Slovenia. Lipidis DPPC and DPhPC were purchased from Avanti Polar Lipids, USA. Lipids were dissolved at concentration of 10 mg/ml in a mixture of hexane (Sigma-Aldrich, USA) and ethanol absolute (Sigma-Aldrich, USA) in ratio 9:1. Solution for forming a torus was prepared from mixture of hexadecane (Fluka, Germany) and pentane (Fluka, Germany) in ratio 3:7. Salt solution was prepared from 100 mM KCl