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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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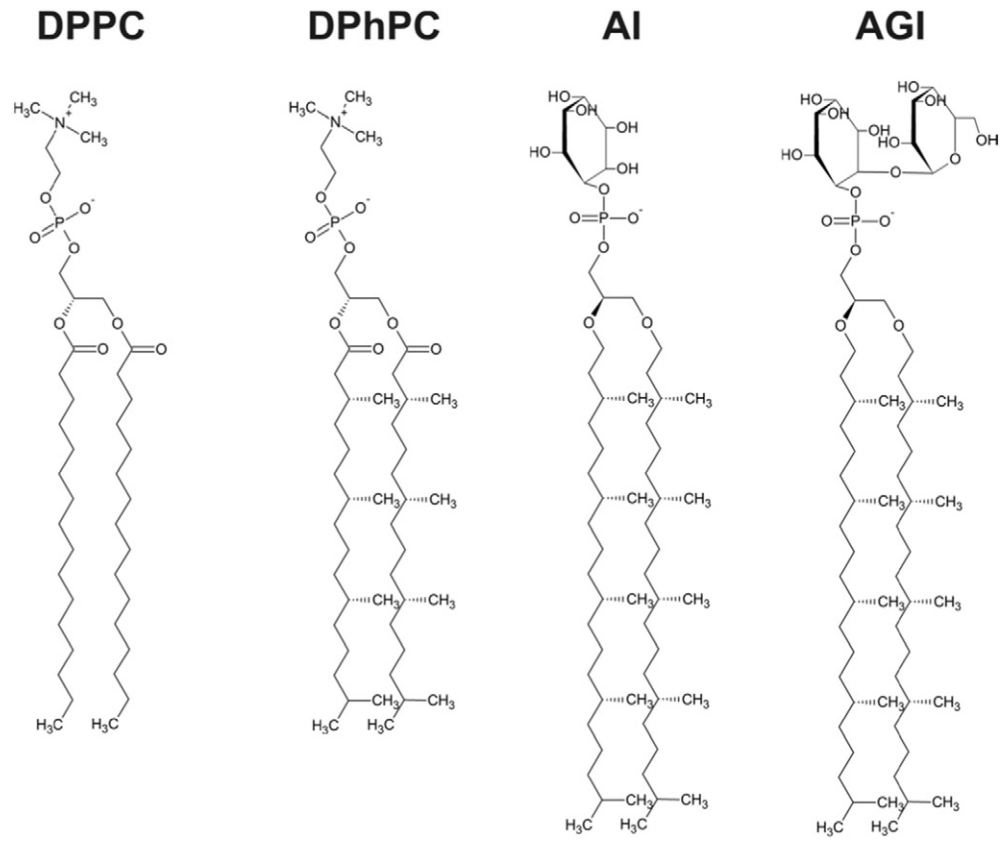

Fig. 1. The chemical structure of the lipid molecules: dipalmitoyl phosphatidylcholine (DPPC), diphytanoyl-phosphocholine (DPhPC) and two components of *A. pernix K1* arheal lipids: 2,3-di-O-sesterterpanyl-sn-glycerol-1-phospho-myo-inositol (AI) and 2,3-di-O-sesterterpanyl-sn-glycerol-1-phospho-1′-(2′-O-α-D-glucosyl)-myo-inositol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

and 10 mM HEPES mixed in the same proportion. Some drops of NaOH were added to obtain the pH of 7.4.

Planar lipid bilayers were formed over a round aperture in a Teflon film of 25 μm thickness, dividing the Teflon chamber in two compartments, each has a volume of 5.3 cm^3. The aperture of 100–200 μm in diameter was made by an electric spark. Aperture was pre-treated with 1 μl of lipid solution and 1.5 μl of solution for creating a torus. Then both compartments were filled with salt solution slightly below the aperture. In each compartment 2 μl of lipid solution was added on salt solution surface. Before folding the level in each compartment above the aperture for the first time, we waited at least 10 min to allow evaporation of solvents from lipid and torus solutions and spreading of the lipids on the salt solution surface. Salt solution was levelled by Syringe pumps (World Precision Instruments, USA). Teflon chamber was immersed in water bath, where temperature was regulated by Thermo CUBE (AMS Technologies AG, Germany) [26]. The temperature in the vicinity of lipid bilayer and in the water bath was measured with two K-type thermocouple probes.

Planar lipid bilayer electrical properties were measured with electrical system already described by Polak et al. [26]; using a pair of Ag-AgCl electrodes (IVM, USA) immersed in salt solution in each compartment. Current clamp method by means of linearly rising signal was used to measure planar lipid bilayer breakdown voltage. Electrical capacitance was measured using impedance meter Agilent 4284A (HP, USA), that was set to measure parallel resistance and capacitance. All the measurements were done by applying alternating sine voltage with amplitude of 25 mV and frequency of 1 kHz. The electrical capacitance of the system with lipid bilayer C_{SBLM} was measured for each planar lipid bilayer that was formed. After application of linearly rising current signal we measured the electrical capacitance of the system without planar lipid bilayer C_S. The difference in capacitances gives the electrical capacitance of the planar lipid bilayer C_{blm}. This value was then normalized to the area of the aperture to calculate specific electrical capacitance c_{blm} of the planar lipid bilayer. Breakdown voltage (U_{br}) of planar lipid bilayer was measured using linearly rising current signal [26] with steepness k of 300 μA/s (Fig. 2A).

We selected the temperature interval (usually from 19 °C to 56 °C) for each experiment in advance. Heating and cooling rateswere approximately one degree per minute (Fig. 2B). Planar lipid bilayer was formed every 20 to 40 s. In this time interval also specific electrical capacitance and breakdown voltage of a planar lipid bilayer were measured. Due to irregular sampling of measured values during heating and cooling, we grouped the measurements that were obtained in the range of 1°. Each group contains three to fifteen measurements. Data are presented as a mean value ± standard deviation.

3. Results

Specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}) of planar lipid bilayers formed from archaeal lipids in dependence of the temperature on the temperature interval from 19 °C to 56 °C are shown in Fig. 3. Specific electrical capacitance c_{blm} is approximately constant for temperatures up to 40 °C and then gradually decreases above 40 °C. Voltage breakdown U_{br} is more or less constant until 50 °C, while at higher temperatures a considerable drop was observed.

Specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}) of DPPC planar lipid bilayers were successfully measured during the heating regime in temperature range 25 °C to 42 °C only once (Fig. 4). Because capacitance is related to geometrical dimensions of planar lipid bilayer (planar lipid bilayer area and thickness) and it is known that they change significantly with temperature [27,28], we added in the background of Fig. 4 previously published measurements of molecular volume and heat capacity of DPPC [28]. In gel-crystalline phase, in the temperature interval between 25 °C and 33 °C, the specific electrical capacitance is on average 0.29 μF/cm^2. Specific electrical capacitance jumps to an average value 0.44 μF/cm^2 in the ripple phase, at the temperatures between 34 °C and 38 °C. Finally the specific electrical capacitance is on average 0.51 μF/cm^2 in the fluid phase around melting temperature $T_m = 41$ °C. The breakdown voltage U_{br} is approximately 600 mV in the gel-crystalline and ripple phase, but rises to 930 mV in the vicinity of the melting temperature T_m.

Specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}) of planar lipid bilayers formed from DPhPC:DPPC mixture in ratio 80:20 (w:w) in dependence of the temperature between 25 °C and 52 °C are shown in Fig. 5. For comparison the molecular volume and heat capacity of DPPC only [28] are again added in the figure background. The specific electrical capacitance (c_{blm}) gradually increases from 0.30 μF/cm^2 to 0.50 μF/cm^2 in the temperature range 25 °C to 41 °C. Above melting temperature T_m (41 °C) of DPPC, the c_{blm} slightly decreases toward 0.4 μF/cm^2. The breakdown voltage U_{br} is approximately 460 mV in the whole range of measuring temperatures. It can be noticed, that it is slightly higher (500 mV) around melting temperature T_m of DPPC.

4. Discussion

In the present study, we investigate electrical properties of planar lipid bilayers made of archeal lipids isolated from *A. pernix K1*. We measured their specific electrical capacitance and breakdown voltage in dependence of temperature. For comparison, temperature dependence of both electrical parameters was measured also for DPPC planar lipid

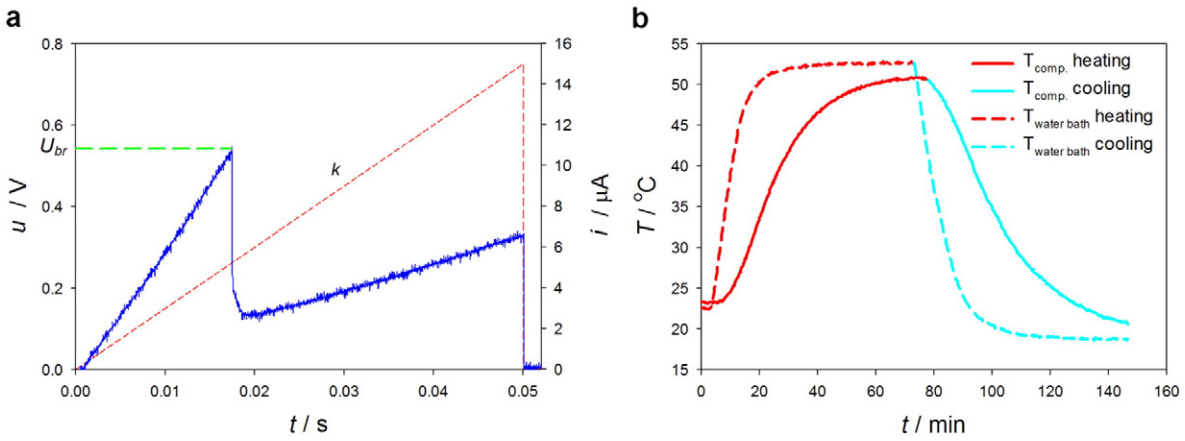

Fig. 2. a) Measurement of breakdown voltage (U_{br}). Current signal of $k = 300$ μA/s is applied to planar lipid bilayer (dashed line). Consequently the voltage on planar lipid bilayer (solid line) is rising until planar lipid bilayer breaks (U_{br}) when voltage drop occurs. b) Measurement cycle of the temperature during heating and cooling regimes while performing experiments in water bath (dashed line) [26] and in the compartment of Teflon chamber where planar lipid bilayer is built (solid line).

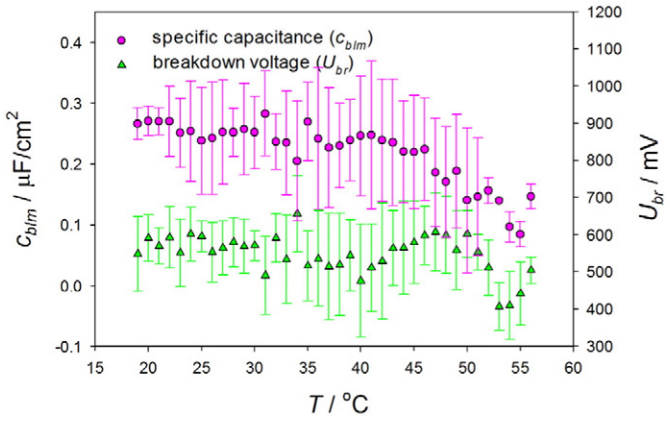

Fig. 3. Specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}) of planar lipid bilayers formed from archaeal lipids *A. pernix K1* in dependence of the temperature between 19 °C and 56 °C.

bilayers and planar lipid bilayers formed from DPhPC:DPPC mixture in ratio 80:20.

We compared breakdown voltages (U_{br}) (Table 1) and values of specific electrical capacitance (c_{blm}) (Table 2) measured at 25 °C and 50 °C with data obtained previously in MD simulation studies [11,12]. Because experimentally measured values and MD simulation results are not comparable directly [29], only the trend of both parameters according to selected temperatures was followed. Mostly all measurements and MD simulations were done at both selected temperatures. The exception is DPPC lipid which was not simulated in gel phase (25 °C) and we also did not find any MD results for DPhPC:DPPC 80:20 mixture. Also, experimental values for DPPC at 50 °C are missing.

Although breakdown voltage of planar lipid bilayers is often measured as well as determined in MD simulations, the difference between both approaches should be pointed out. While in MD simulations we observe the appearance of the first membrane defect [30], in the experiments the complete breakdown of planar lipid bilayer is usually detected. The difference in both observations is significant especially in the cases where exposure of the planar lipid bilayer to the electric field causes appearance of metastable pores that discharge planar lipid bilayer [31]. Moreover, the planar lipid bilayer simulated in MD models is extremely small; usually the factor between simulated planar lipid bilayer area and an area of planar lipid bilayers in experiments is 10^8. Because MD simulations are computationally complex and time consuming, simulated planar lipid bilayers are exposed to extremely high electric field to shorten the time of simulations to hundreds of nanoseconds [32–34]. On the other hand, in our experiments relatively large planar lipid bilayers (100–200 µm in diameter) are exposed to gradually increased electric field for tens of milliseconds. The energy is not used only for pore formation but also for lipid bilayer fluctuations and bending and measurement setup charging. Due to these differences the comparison of the results of both approaches is not straightforward.

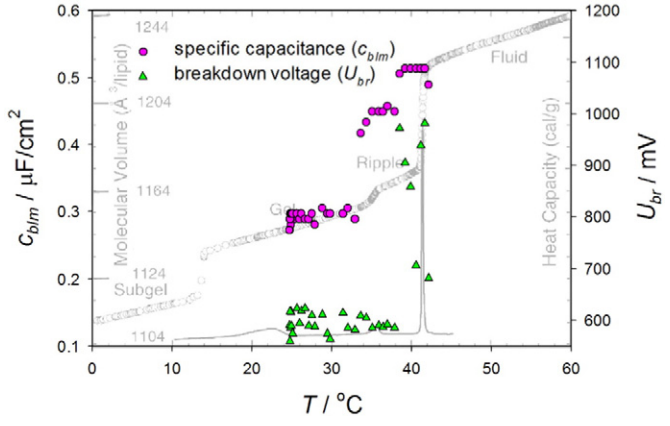

Fig. 4. Specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}) of planar lipid bilayers formed from DPPC in dependence of the temperature between 25 °C and 42 °C. At lower temperatures between 25 °C and 33 °C, the lipid bilayers stay in gel-crystalline phase. At 34 °C till 38 °C, the ripple phase of the planar lipid bilayers is pronounced, while around melting temperature of T = 41 °C, the ripple phase moves to liquid-crystalline phase. For comparison, the results of molecular volume and heat capacity are added.
Adopted from [28].

Experimentally measured breakdown voltage values of archeal planar lipid bilayers are almost the same at 25 °C and 50 °C, while in MD simulations breakdown voltage at 50 °C is lower than at 25 °C (Table 1). From the Fig. 3 it can be clearly seen that also experimentally measured breakdown voltage is lower at the temperatures higher than 50 °C. Therefore we can conclude that results of MD simulations and experimental results have a similar trend at temperatures higher than 50 °C.

Gmajner et al. [9] showed that the archaeosome membranes are heterogeneous, and are composed of components with three types of nanodomains with different fluidity characteristics, which existence is temperature dependent. The presence of these components (nanodomains) gradually and continuously decreases with increasing temperature and above 60 °C the presence of only fluid-like domains has been detected [9]. As was showed by Polak et al. at, higher temperatures, headgroups of AGI and AI molecules are rotated toward the lipid membrane plane [11], which changes structure of the planar lipid bilayer headgroup region. It is possible that because of headgroup rotation more intramolecular hydrogen bonds are created at higher temperatures while at lower temperatures more intermolecular hydrogen bonds are present. Therefore at the presence of electric field water molecules penetrate into lipid molecule headgroup region easily at higher temperatures which can be the reason for easier water wires formation [32,35] and easier formation of the pores observed in MD simulations. Moreover, MD simulations have shown that in the case of archaeal lipids only hydrophobic pores are created [12]. Wodzinska et al. claim that formation of the pore must keep the overall area of the membrane constant [36], which means that hydrophobic pores cannot expand and consequently lead to final planar lipid bilayer breakdown.

At the temperatures below melting temperature, breakdown voltage of DPPC planar lipid bilayer is similar to breakdown voltage of archaeal planar lipid bilayers. According to the results of MD simulation at 50 °C we can expect that first pore in the DPPC planar lipid bilayer at lower voltage than the first pore in archaeal planar lipid bilayer. But due to the fact that in DPPC planar lipid bilayer hydrophilic pores are formed [37], which expand easily, the final breakdown voltage is almost the same as in archaeal lipids. At melting temperature we measured considerably higher breakdown voltage of DPPC planar lipid bilayers than in the gel phase. It is known that at phase transition lipid membranes exhibit tens or even hundreds of milliseconds lasting quantized current fluctuations in pA range at clamp voltages round 100 mV [16,36,38], which lead to higher overall membrane conductivity and permeability. Antonov et al. [39] showed current fluctuations in DPPC planar lipid bilayers at phase transition even in the nA regime. The current fluctuations are proposed to be the consequence of hydrophilic pores or lipid channels. Their open probability and their opening time are voltage depended [40]. It seems that stable hydrophilic pores formed at relatively low voltage locally effectively discharge planar lipid bilayer that cause moderate increase in planar lipid bilayer conductance during current clamp conditions and consequently slower rise of membrane voltage. Therefore overall stability of planar lipid bilayer during phase transition is better than in other phases where current fluctuations were not observed [39]. Due to this reasons final breakdown of planar lipid bilayer at melting temperature is attained at higher voltage, which means that higher breakdown voltage is measured.

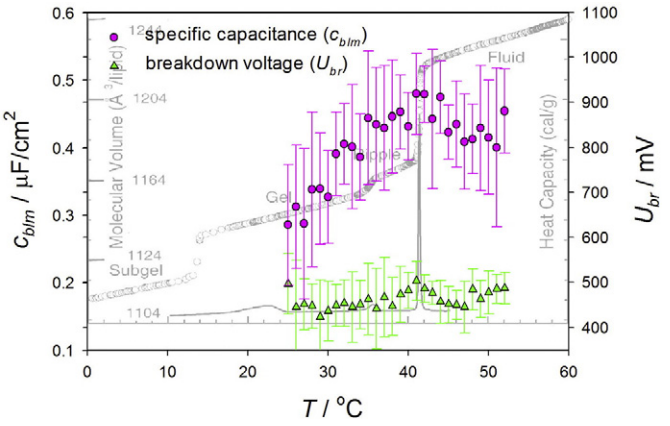

Fig. 5. Specific electrical capacitance (c_{blm}) and breakdown voltage (U_{br}) of planar lipid bilayers formed from mixture 80:20 (w:w) of DPhPC and DPPC respectively in dependence of the temperature. Measurements were done in the temperature interval between 25 °C and 52 °C. For comparison the molecular volume and heat capacity of DPPC are added [28].

Breakdown voltage of DPhPC:DPPC mixture in ratio 80:20 is only slightly higher at DPPC melting temperature, because of low fraction of DPPC lipid in the mixture. These results are in agreement with previous studies, which showed broadening of heat capacity peak and changing of its amplitude for various mixtures of lipids and other additives [36,41–43].

Capacitance is an important electrical parameter of planar lipid bilayer [44]. It is not just a measure of amount of charge that is stored on the lipid bilayer at a certain voltage, but it also reflects the physical state of the lipid system [17]. In all types of experimental studies on planar lipid bilayers specific electrical capacitance serves as an indicator of bilayer quality. In final consideration only bilayers with specific capacitance in the range of hundreds of nF/cm^2 are taken into account. Although specific capacitance of planar lipid bilayer can be measured by various measuring principles [44], all assume planar lipid bilayer as an equivalent circuit made of resistance and capacitance in parallel [17]. In MD simulations the lipid bilayer is considered as an ideal capacitor, which means that parallel resistor is not present. The specific capacitance is calculated as a ratio of applied charge imbalance and created voltage on planar lipid bilayer, normalized to bilayer surface area [45]. Usually experimental values are lower than those obtained in MD simulations [31,46].

Experimental results at 25 °C show that the specific electrical capacitance of planar lipid bilayer made of DPPC and DPhPC:DPPC mixture 80:20 is 0.29 µF/cm^2 while specific electrical capacitance of planar lipid bilayer made of *A. pernix K1* archeal lipids is 0.24 µF/cm^2. Due to the fact that archeal lipid hydrocarbon chains are longer, it is expected that they form ticker bilayers that results in lower electrical capacitance. Moreover archaeal lipid bilayers have a much larger lateral pressure than DPPC lipid bilayers [11], which makes archaeal lipid bilayers more solid and results in low capacitance [17].

Specific capacitance of DPPC planar lipid bilayers was measured in the temperature interval between 25 °C and 42 °C therefore no value is given at 50 °C. The measuring values before and near DPPC phase transition are in line with already published data [17], which states that the capacitance in fluid phase is approximately 1.5 times larger than that of the gel phase. According to our results the factor is 1.75. The specific electrical capacitance even follows intermediate phases before the main phase transition; it increases in steps comparable to molecular volume [28]. MD simulations of DPPC lipid bilayer in temperature range — 23 °C to 77 °C show a transition around 35 °C [47], where considerable increase in area per lipid and decrease in bilayer thickness were observed. This temperature corresponds to the first jump in specific electrical capacitance (from 0.29 µF/cm^2 to 0.44 µF/cm). MD simulations also confirm the existence of distinct structures of DPPC lipid bilayer in the vicinity of melting temperature that corresponds to intermediated phases before the main phase transition. Similar increase in specific electrical capacitance while approaching melting point of DPPC (T_m) was obtained for DPhPC:DPPC 80:20 mixture, but the presence of gel-crystalline and ripple DPPC phases is not visible any more [42]. Specific electrical capacitance at 50 °C is almost two times larger than at 25 °C (Table 2).

Table 1
Breakdown voltage (U_{br}) of planar lipid bilayers.

U_{br} (mV)	Experiments		MD	
	25 °C	50 °C	25 °C	50 °C
A. pernix K1	595	600	5400	4500[a]
DPPC	580	–	–	2200[b]
DPhPC:DPPC 80:20	500	480	–	–

[a] Polak et al. Bioelectrochemistry 100 (2014) 18–26 [12].
[b] Polak et al. J Membrane Biol 246 (2013) 843–850 [37].

Table 2
Specific electrical capacitances (c_{blm}) of planar lipid bilayers.

c_{blm} (µF/cm^2)	Experiments		MD	
	25 °C	50 °C	25 °C	50 °C
A. pernix K1	0.24	0.14	0.67[a]	0.72[a]
DPPC	0.29	–	–	0.94[b]
DPhPC:DPPC 80:20	0.29	0.42	–	–

[a] Polak et al. Bioelectrochemistry 100 (2014) 18–26 [12].
[b] Polak et al. J Membrane Biol 246 (2013) 843–850 [37].

Specific electrical capacitance of planar lipid bilayer is not described only with its area and thickness, but also with its dielectric constant. Mostly, dielectric constant of lipid bilayers is supposed to be 2–3 [48,49], although it is apparent that polarizability of different regions in lipid bilayer is not uniform. Therefore a use of a dielectric profile instead of a single homogeneous dielectric slab was proposed in some studies [50,51]. Nymeyer et al. [52] showed that a dielectric profile of a layer of POPC lipid molecules exhibit at least three regions: lipid tails, with a dielectric constant ~1, the headgroup region, with extremely high dielectric constant ~700, and interfacial region, where water molecules can still be present, with dielectric constant approximately 3. High dielectric constant in headgroup region is related to dipole nature of POPC lipid headgroups. Simple equivalent capacitance of such capacitors connected in series still has low dielectric constant; round 2.8.

According to the results of MD simulations, specific electrical capacitance of archaeal planar lipid bilayers increases with increasing temperature (Table 2) as it is expected from larger area per lipid molecule and thinner lipid bilayer at higher temperature [11]. But experimental results do not follow MD simulation predictions. The specific electrical capacitance of planar lipid bilayers made of archaeal lipids gradually decreases from 45 °C onwards (Fig. 3), it was measured 0.14 µF/cm^2 at 50 °C and it is even lower at higher temperatures.

Similar regions can be proposed also in the case of archaeal planar lipid bilayers, just dielectric constant of the headgroup region is probably lower because the dipoles are not present. Let use approximate dimensions from Polak et al. [11]: 6 nm is a thickness of the lipid bilayer where 1.4 nm is a dimension of lipid tails, with a dielectric constant ~1, 0.4 nm is the thickness of the interfacial region, with a dielectric constant ~3, and 1.2 is an approximate dimension of lipid headgroups, with a dielectric constant ~40. Calculated dielectric constant of an equivalent capacitance in such a case is 2.7. At higher temperatures, the archaeal lipid headgroups are tilt into the lipid bilayer, which shorten dimension of lipid headgroups to a width of sugar molecule (0.7 nm); the hydrophobic tails become more flexible and whole lipid bilayer is therefore thinner (5.5 nm). But because lipid molecules are hydrated to a lesser extent [11], wider region of low dielectric constants can be assumed (lipid tails: 1.8 nm, interfacial region: 0.25 nm). In this case calculated

dielectric constant of an equivalent capacitance is reduced to 2.09. Considering the results of such a simple model, we can conclude, that the reduction of the specific electrical capacitance of archaeal planar lipid bilayers at higher temperatures is possible due to changed local polarizability although planar lipid bilayer becomes thinner.

Specific electrical capacitance and breakdown voltage of planar lipid bilayers that are made of different lipid molecules exhibit complex and nonuniform behaviour at different temperatures. Due to importance of these two electrical parameters for various biotechnological applications, further experimental, theoretical and simulation studies are needed to elucidate structure of planar lipid bilayers at different temperatures as well as their breakdown mechanisms. We would like to emphasize that comparison and combination of the results obtained by different approaches are crucial for understanding of underlying phenomena.

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