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Electroporation of archaeal lipid membranes using MD simulations

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ABSTRACT

Molecular dynamics (MD) simulations were used to investigate the electroporation of archaeal lipid bilayers when subjected to high transmembrane voltages induced by a charge imbalance, mimicking therefore millisecond electric pulse experiments. The structural characteristics of the bilayer, a 9:91 mol% 2,3-di-O-sesterterpanyl-sn-glicerol-1-phospho-myo-inositol (AI) and 2,3-di-O-sesterterpanyl-sn-glicerol-1-phospho-1′(2′-O-α-D-glucosyl)-myo-inositol (AGI) were compared to small angle X-ray scattering data. A rather good agreement of the electron density profiles at temperatures of 298 and 343 K was found assessing therefore the validity of the protocols and force fields used in simulations. Compared to dipalmitoyl-phosphatidylcholine (DPPC), the electroporation threshold for the bilayer was found to increase from ~2 V to 4.3 V at 323 K, and to 5.2 V at 298 K. Comparing the electroporation thresholds of the archaeal lipids to those of simple diphytanoyl-phosphatidylcholine (DPhPC) bilayers (2.5 V at 323 K) allowed one to trace back the stability of the membranes to the structure of their lipid head groups. Addition of DPPC in amounts of 50 mol% to the archaeal lipid bilayers decreases their stability and lowers the electroporation thresholds to 3.8 V and 4.1 V at respectively 323 and 298 K. The present study therefore shows how membrane compositions can be selected to cover a wide range of responses to electric stimuli. This provides new routes for the design of liposomes that can be efficiently used as drug delivery carriers, as the selection of their composition allows one to tune in their electroporation threshold for subsequent release of their load.

1. Introduction

Archaea are extremophile organisms that optimally grow in extreme environments. They are grouped into halophiles that grow in high salt concentration, methanogens that grow under anaerobic condition, thermophiles that grow at high temperatures and psychrophilic that grow at low temperatures. The cell membranes of these archaea have a unique composition, a high chemical and a high physical stability [1–3]. Compared to simple phosphatidyl-choline (PC) lipids, archaeal lipids have head-groups formed by sugar moieties, ether linkages instead of ester linkages between the head group and the carbonyl region, and methyl-branched lipid tails [2]. *Aeropyrum pernix* is an aerobic hyperthermophilic archaea organism that grows in a coastal solfataric vent at Kodakara, Juma Island, Japan. Its optimal growth environment is at temperatures between 363 K and 368 K, pH 7.0 and salinity of ~3.5%. *A. pernix* cells are spherical with diameters ranging from 0.8 to 1.2 μm [4]. Their membrane is composed of two lipids: 2,3-di-O-sesterterpanyl-sn-glicerol-1-phospho-myo-inositol (AI) and 2,3-di-O-sesterterpanyl-sn-glicerol-1-phospho-1′(2′-O-α-D-glucosyl)-myo-inositol (AGI) at a molar ratio of 9:91 mol% [5]. The core of both lipids is a 2,3-di-o-sesterterpanyl-sn-glycerol ($C_{25,25}$-archaeol) while the polar heads are inositol for AI and glucose for AGI (Fig. 1).

The lipids forming the membranes of such organisms are as such very good candidates as components of liposomes for drug delivery [2,6]. For such applications however, the drug should be ultimately released when the carrier (liposome) reaches the intracellular milieu [7]. One of the methods that can be used to enhance the drug release from the synthetic liposomes is electroporation [8,9]. Electroporation is a phenomenon that affects the stability of lipid membranes since it disturbs transiently or permanently their integrity when these are subject to high voltages (electric fields) [10]. Such a technique is now routinely used in fields as diverse as biology, biotechnology and medicine [11]. For simple membranes, molecular dynamics (MD) simulations have shown that the main effect of high electric fields is to enhance the membrane permeability due to the formation of

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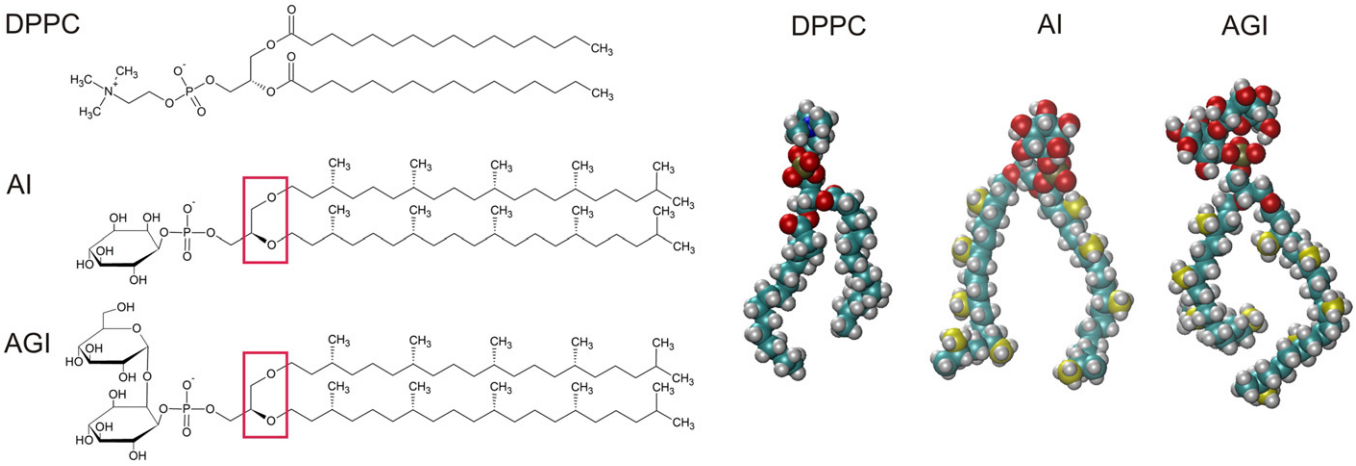

Fig. 1. Representations of archaeal lipids which compose the membrane of Archaea *Aeropyrum pernix* (AI and AGI) and the DPPC lipid.

hydrophilic pores that can be wide enough to transport ions and small molecules [12–17]. When exposed long enough to high fields, lipid bilayers and liposomes can undergo irreversible breakdown [18–20].

Obviously, electroporation of membranes depends on their lipid composition [21]. A molecular level insight about the phenomena has been gathered from MD simulations of lipid bilayers subject to large transmembrane voltages. Most studies concerned the electroporation of phosphatidylcholine (PC) based lipid bilayers [22–26] and the large body of data showed that the electroporation thresholds depend on the type of lipid considered. The presence of increasing cholesterol amounts in lipid membranes was also shown to increase the electroporation threshold [27,28]. Recently, we considered dipalmitoyl-PC (DPPC), and diphytanoyl-PC (DPhPC)-ester and -ether based bilayers [24], comparing therefore lipids with acyl chains and methyl branched chains, and lipids with ether or ester linkages, which changes drastically the membrane dipole potential. We have shown that the electroporation thresholds of these bilayers depend not only on the properties of their component hydrophobic tails but also on the "electrical" properties of the membrane, i.e. its dipole potential.

Archaeal lipids from *A. pernix* present an additional feature: their head group possesses either inositol or glucose moieties. It is unknown how such lipids when forming bilayers would behave under high voltages. It is also interesting to determine how their stability can be modulated by changing the lipid composition, e.g. by adding a third component. This is precisely what we are investigating in the present paper. We first determine the structural characteristics of archaeal lipid bilayers by confronting small angle X-ray scattering data performed on unilamellar vesicles to MD simulations of bilayers of the same composition. We proceed then to study these bilayers electroporation.

2. Material and methods

2.1. Growth of A. pernix K1

The optimum conditions for maximizing *A. pernix* biomass were obtained when $Na_2S_2O_3 \times 5H_2O$ (1 g of per liter) (Alkaloid, Skopje, Macedonia) added Marine Broth 2216 (Difco™, Becton, Dickinson and Co., Sparks, USA) at pH 7.0 (20 mM HEPES buffer) was used as a growing medium in 1 L flask at 365 K (for details see [29]). After growth, the cells were harvested by centrifugation, washed and lyophilized.

2.2. Isolation and purification of lipids, and vesicle preparation

The polar lipid methanol fraction composed of approximately 91% AGI and 9% AI [5] (average molecular mass, 1181.42 g mol^{-1}) was purified from lyophilized *A. pernix* cells, as described previously [30]. After isolation, the lipids were fractioned using adsorption chromatography [31], and the polar lipid methanol fraction was used for further analysis. Organic solvents were removed under a stream of dry nitrogen, followed by the removal of the last traces under vacuum. For mixed lipid liposomes, the appropriate mass of archaeal $C_{25,25}$ lipids and DPPC were dissolved in chloroform and mixed together in glass round-bottomed flasks. The lipid film was prepared by drying the sample on a rotary evaporator. For preparation of a pure DPPC lipid film, chloroform/methanol (7/3, v/v) was used as solvent. The dried lipid films were then hydrated with warm (~318 K) 20 $mmol.l^{-1}$ HEPES buffer, pH 7.0 or deionized water (milliQ). The mol% of the archaeal $C_{25,25}$ lipids in the mixed archaeal-DPPC liposomes was: 100, 95, 90, 75 and 50. Multilamellar vesicles (MLV) were prepared by vortexing the lipid suspensions vigorously for 10 min. MLV were further transformed into large unilamellar vesicles (LUV). After six freeze (liquid nitrogen) and thaw (warm water) cycles, the liposomes were pressure-extruded 21 times through 400-nm polycarbonate membranes on an Avanti polar mini-extruder (Avanti Polar Lipids, Alabastr, Alabama, USA), at between 323 and 333 K. The total lipid concentration in all SAXS experiments was 10 mg/ml.

2.3. Small-angle X-ray scattering measurements

Small-Angle X-Ray Scattering (SAXS) measurements were performed on the Kratky compact camera (Anton Paar KG, Graz, Austria) [32], which was modified to enclose the focusing multilayer optics for X-rays (Göbel mirror; Osmic). The camera was attached to a conventional X-ray generator Kristalloflex 760 (Bruker AXS GmbH, Karlsruhe, Germany) equipped with a sealed X-ray tube (Cu K_α X-rays with a wavelength $\lambda = 0.154$ nm) and operating at 40 kV and 35 mA. The samples were measured in a standard quartz capillary with an outer diameter of 1 mm and wall thickness of 10 μm. The scattering intensities were detected with the position sensitive detector PSD-50 M (M. Braun GmbH, Garching, Germany) in the small-angle regime of scattering vectors $0.1 < q < 7.5$ nm^{-1}, where $q = 4\pi/\lambda \cdot \sin(\vartheta/2)$, ϑ representing the scattering angle. In order to get reliable measuring statistics, each sample was measured for a period of 20 h. Prior to the data analysis by the inverse Fourier transformation method, the scattering data were corrected for the empty capillary and solvent scattering, and put on the absolute scale using water as a secondary standard [33].

2.4. Evaluation of SAXS data

SAXS data were evaluated using the Indirect Fourier Transformation (IFT) technique [34,35]. Even through the overall dimension of the lipid