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Stability of free-standing tetraether planar membranes in microchips

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ABSTRACT

Here, we used electrochemical impedance spectroscopy to demonstrate that the polar lipid fraction E (PLFE), a major bipolar tetraether lipid component isolated from thermoacidophilic archaea, can form free-standing planar membranes with remarkable membrane stability in comparison to planar membranes made of diester lipids or triblock copolymers. Stable PLFE planar membranes were generated on micro-pores ranging from 20 to 200 μm in polydimethylsiloxane (PDMS) thin films embedded in a specially made glass/silicon two-chamber microchip. The micro-pore-containing PDMS thin film was fabricated by using a double-side molding approach, which created surface flatness on both sides of the film, removing pore cliffs and thus reducing the variations in the fabrication of free-standing planar membranes. We found that, at 11–39 °C, PLFE free standing planar membranes embedded in the microchips not only did not break, but also exhibited electrical impedance virtually invariant over the entire time period (5–50 h) examined. The stability of PLFE free-standing planar membranes, which is on the order of days and achieved without having lipid polymerization nor using nano-pores, is unusual for naturally occurring lipids. This extraordinary stability is attributed mainly to the strong PLFE-PLFE hydrogen bonding and van der Waals interactions and to the possible interactions between PLFE molecules and the PDMS substrate. This study indicates that the use of PLFE can greatly reduce the instability problem of traditional black lipid membranes and that incorporating unusually stable PLFE planar membranes into microdevices is feasible. In summary, bipolar tetraether lipids such as PLFE are excellent materials to make extremely stable yet biologically relevant free-standing planar membranes suitable for use in chip-based membrane technologies.

1. Introduction

Microchip-based membrane technologies have drawn great attention in both basic research and industry applications in recent years [1–3]. These technologies can be used for biophysical studies of ion channels [2], drug screening [1,4], pathogen and toxin detection [5], concentrating proteins [6], water purification [7], and artificial photosynthesis [8,9].

Free-standing planar lipid membranes (also called black lipid membranes) would be well suited for chip-based membrane technologies because both sides of the planar membrane could be easily accessed, allowing for the measurements of membrane behaviors such as single-channel conductance [10,11]. The major drawback of the traditional black lipid membranes made of diester lipids is their instability. Those membranes are typically suspended over a pinhole of the diameter 0.1–1.0 mm on solid support. Because the surface tension between the membrane and water tends to minimize the membrane area [12], black lipid membranes are prone to mechanical rupture, leading to the loss of membrane electrical resistance typically in 2–4 h [13–16].

For years, great effort has been devoted to increasing the stability of black lipid membranes. One approach is to decrease the pore size in an attempt to reduce the membrane surface area susceptible to surface tension [17]. However, fabrication of nano-pores is limited to hard materials such as alumina or silicon nitride substrate [17] and requires more expensive facilities [18], which may not be readily available in many labs. To our knowledge, fabrication of nanopores on soft materials such as polydimethylsiloxane (PDMS) has remained as a major challenge. Another approach to improve black membrane stability is to use tough membrane materials. Triblock copolymers, for example, have been used to generate free-standing planar membranes with good stability and durability [19]. However, the physicochemical properties of

Abbreviations: BTL, bipolar tetraether lipids; EIS, electrochemical impedance spectroscopy; GDNT, glycerol dialkylcalditol tetraether; GDGT, glycerol dialkylglycerol tetraether; MPL, main phospholipid fraction; PDMS, polydimethylsiloxane; PLFE, polar lipid fraction E; PMOXA-PDMS-PMOXA, poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline); POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine

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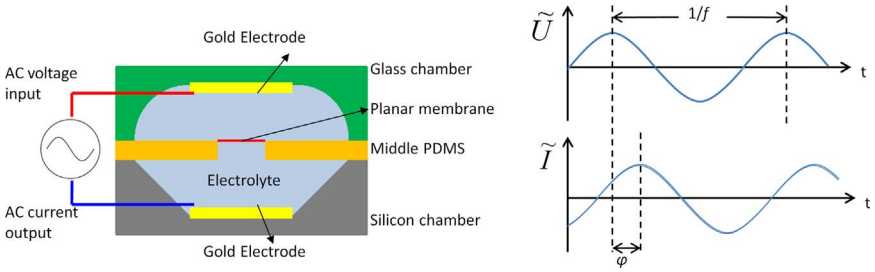

Fig. 1. The micro-device for the EIS measurements of free-standing planar membranes. EIS measurements yield impedance ($Z = \frac{\tilde{U}}{\tilde{I}}$), which is the complex ratio of the voltage ($\tilde{U}$) to the current ($\tilde{I}$) in an alternating current (AC) at a given frequency *f*. The magnitude of the impedance $|z| = Z\, e^{-j\omega\varphi}$, where $\omega = 2\pi f$ and φ is the phase shift. (Note: the scheme is not to scale, and the phase shift in the diagram is just a general illustration of the EIS technique. The actual phase delays detected in our measurements were negative values, ranging from 0° to −80° as previously reported [27]).

triblock copolymers are quite different from those of native lipids, and the thickness of triblock copolymer membranes is typically 10–11 nm [19–21], which is significantly larger than that of mammalian cellular membranes (3.5–4.3 nm) [22]. Since the physicochemical properties of membrane constituents and the membrane thickness can greatly affect the conformation and function of membrane proteins [23], proteins inserted into triblock copolymer membranes may not exhibit the same activities as seen from proteins inserted into natural lipid membranes. As an example, the transition of aquaporin-induced membrane morphological changes in block copolymer membranes is much broader than that in lipid membranes [7]. Therefore, it seems that the key to improve the usage of free-standing planar membranes in microchips for biotechnology applications is not just reducing the pinhole size, but also finding a durable material that would lead to a membrane displaying genuine biological membrane functions yet being able to sustain surface tension between water and the membrane film.

Such materials could be the bipolar tetraether lipids (BTLs) isolated from crenarcheaota. These materials are chemically inert and able to form stable vesicular membranes with tremendous rigidity and packing tightness [24,25]. In a previous study, we demonstrated that the polar lipid fraction E (PLFE, Fig. S1, Supporting information) [26], a major BTL component isolated from the thermoacidophilic crenarcheaota *Sulfolobus acidocaldarius*, was able to form free-standing planar membranes over 100-μm pores in the PDMS thin films embedded in PDMS chambers located in printed circuit board-based fluidics [27]; however, their membrane stability has not been studied directly.

In the present study, we used electrochemical impedance spectroscopy (EIS) to test if PLFE free-standing planar membranes on micropores in PDMS thin films exhibited long-term stability in comparison to free-standing planar membranes made of diester lipids or triblock copolymers. In order to implement this comparison study, we employed an SU-8 double-side molding approach, in conjunction with the use of the S1813 sacrificial layer, to make flat surfaces on both sides of the PDMS thin film and fabricate a two-chamber microdevice to hold the middle PDMS thin film that accommodates the planar membrane. Our data showed that, at 11–39 °C, PLFE free-standing planar membranes embedded in the microchips exhibited significantly high membrane impedance, which was virtually invariant over the entire time period examined (5–50 h). This result suggests that archaea BTLs such as PLFE are excellent materials to make extremely stable yet biologically relevant free-standing planar membranes suitable for use in microchip-based membrane technologies.

2. Experimental

2.1. Materials

PLFE lipids (Fig. S1) were isolated from *S. acidocaldarius* as described [26]. Diester lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids (Alabaster, AL) (Fig. S2). POPC and PLFE stock solutions (0.2 mg/100 mL) were made in chloroform and chloroform:methanol:water (14:5:1, v/v/v), respectively. Triblock copolymer poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA, MW = 1300–8500–1300 g/mol) was obtained from Polymer Source (Quebec, Canada) (Fig. S2). The stock solution of PMOXA-PDMS-PMOXA (1 wt%) was made in chloroform/toluene (1:1, v/v) [28].

2.2. Fabrication of a micro-device for holding free-standing planar membranes

In order to implement this study, we designed and fabricated a new micro-device, which contains three parts: namely, the top glass chamber (9 mm × 9 mm), the bottom silicon chamber (9 mm × 9 mm), and the middle PDMS thin film (thickness: 480 μm) with a tiny pore (i.e., 20, 100, or 200 μm in diameter) to accommodate free-standing planar membranes (Fig. 1). As shown later, this micro-device was fabricated using MEMS techniques, which are widely known to be robust and reproducible. The resulting micro-device is suitable for EIS measurements of the planar membranes (Fig. 1) and could be useful for studying ion channels and other membrane-bound proteins.

2.2.1. The bottom silicon chamber

The bottom silicon chamber was fabricated from a silicon wafer by photolithography and KOH wet etching, followed by gold electrode deposition (Fig. 2a). We used a 3-in. single-side-polished silicon wafer (thickness: 381 ± 25 μm; Silicon Materials, Glenshaw, PA) with a 1-μm silicon dioxide layer on both sides. One wafer was fabricated to make 49 bottom silicon chambers (9 mm × 9 mm each). Silicon was chosen for making the bottom chamber because silicon offers smooth surface, which is good for coating gold electrode. The general procedure for making one of such bottom silicon chambers is described in Fig. 2a and the fabrication details are given in Supporting information. The gold electrodes in each silicon bottom chamber were soldered to aluminum wires as described in Supporting information.

2.2.2. The top glass chamber

The top glass chamber was fabricated on a standard microscope glass slide (75 mm × 50 mm × 1 mm; Fisherbrand™, Fisher Scientific, Pittsburgh, PA) by photolithography and etched by buffered oxide etchant (BOE), specifically, ammonium fluoride/hydrofluoric acid (Transene Electronic Chemicals, Danvers, MA), followed by gold electrode deposition (Fig. 2b). After metal lift-off, the glass slide was diced into 35 single top glass chambers (9 mm × 9 mm each) using a dicing saw. Glass was chosen to make the top chamber for alignment purposes. The general procedure for making top glass chambers is described in Fig. 2b and the fabrication details are given in Supporting information. The gold electrodes in each glass chamber were soldered to aluminum wires as described in Supporting information.

2.2.3. The middle PDMS thin film

2.2.3.1. Middle PDMS thin films with 100- and 200-μm pores. In our micro-device (Fig. 1), the middle PDMS thin film needs to be flat on both sides in order to bond to the flat surface of the glass (upper) and silicon (lower) chamber and to accommodate the planar membrane over the micropore. The traditional method to make the PDMS thin film is to use epoxy-based negative photoresist SU-8 as the molding layer to make a PDMS replica (e.g., [27]). This method will create PDMS "cliffs" (the orange area in the top-left panel of Fig. 3) at the edge of the SU-8