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Enhanced Long-Term Stability for Single Ion Channel Recordings Using Suspended Poly(lipid) Bilayers

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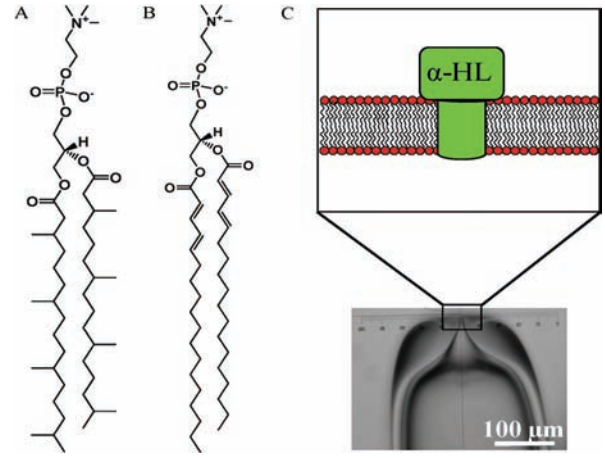
Suspended phospholipid bilayers, also known as black lipid membranes (BLMs), are an important tool for investigating the activity of reconstituted ion channels.[1] Ligands can be added to the buffer on either side of a BLM, allowing binding to both sides of a transmembrane ion channel to be probed. However, long-term monitoring of ion channel activity using BLMs composed of naturally occurring lipids is limited by the inherent instability of the bilayer. The lipids are self-associated via a network of relatively weak intermolecular forces; as a consequence, membrane rupture usually occurs in <24 h.[2] Herein, we demonstrate that lipid polymerization is an effective strategy to significantly extend BLM lifetimes.

A number of approaches have been utilized to improve the physiochemical stability of BLMs, including miniaturization of the aperture across which the BLM is suspended,[1b,3] sandwiching the BLM between agarose layers,[4] and doping the membrane with stabilizing agents (e.g., cholesterol).[5] The enhanced stability obtained using these approaches is attributed to introduction of additional noncovalent intermolecular interactions or minimization of stresses that disrupt intermolecular interactions. For example, suspending a BLM across a glass nanopore minimizes the membrane surface area susceptible to rupture.[2] While these types of BLMs are more stable than a conventional construct (e.g., a bilayer suspended across an ~100 μm aperture in a Teflon sheet), their long-term robustness is inherently limited by the noncovalent nature of their assembly. Stabilization by lipid polymerization is a strategy that addresses this limitation.[6]

Reactive lipid monomers have been used to prepare both linear and cross-linked poly(lipid) networks.[7] A wide variety of polymerizable lipids have been synthesized, differing in the type of the reactive moiety, its location in the lipid, and the number per lipid.[7] Much of the initial work in this field was performed using bis-diacetylene lipids which tend to produce relatively rigid polymers with numerous defects.[8] Polymerization of BLMs prepared from mixtures of nonpolymerizable and monodiacetylene lipids resulted in a moderate increase in membrane lifetime (from 13 to 31 min), and single channel recordings of gramicidin A in these BLMs were measured.[9] An earlier study showed that the activity of α-hemolysin (α-HL) was maintained after partial linear polymerization of a BLM composed of a monodiacetylene lipid; however stability data were not reported.[10]

Poly(dienes) are rubber-like materials;[7] thus dienoyl lipids appear to be a better choice for creating a stable poly(BLM) that maintains the activity of incorporated ion channels. Among these are bis-dienoyl phosphatidylcholine (bis-DenPC; Scheme 1) and bis-sorbyl phosphatidylcholine (bis-SorbPC); both readily form poly(lipid) vesicles and planar supported bilayers with markedly increased stability to surfactants, solvents, drying, and long-term storage.[8] Furthermore, poly(bis-SorbPC) planar membranes have been utilized for reconstitution of functional bovine rhodopsin.[11]

Scheme 1. Structures of (A) Diphytanoyl Phosphatidylcholine (DPhPC) and (B) Bis-dienoyl Phosphatidylcholine (bis-DenPC) and (C) Schematic of an Ion Channel Functionalized BLM Suspended across the Opening of a Micropipet[a]



[a] The aperture of the pipet ranges from 5 to 20 μm.

In an effort to significantly improve the long-term stability of single ion channel recordings, we fabricated and characterized BLMs composed of poly(bis-DenPC). BLMs were formed across apertures prepared using borosilicate glass pipettes (Scheme 1 and Supporting Information (SI)). Aperture diameters ranging from 5 to 20 μm (I.D.) were formed by first breaking the pipet and then using a microforge to anneal the tip. The inner and outer surfaces of pipettes were covalently modified with 3-cyanopropyldimethylchlorosilane to provide a moderately hydrophobic surface that supports formation of a lipid monolayer.[2] BLMs were formed using a method similar to that of Montal and Mueller.[12] Briefly, solutions of lipid (DPhPC or bis-DenPC) were dried and resuspended in *n*-decane (~10 mg/mL). This solution (3 μL) was applied to the pipet tip and allowed to dry, prior to backfilling the pipet with recording buffer (1 M KCl, 5 mM HEPES, pH 7.5). The pipet was mounted onto a holder and the lipid solution was reapplied, followed by immersion into a bath containing recording buffer. Successful BLM formation was verified by an increase in the resistance monitored using a patch clamp amplifier.

In the absence of a BLM, pipet resistances ranged from 10 to 100 kΩ and were dependent upon the aperture diameter. Upon BLM formation with DPhPC or bis-DenPC, the resistance values were 3.6 (±0.4) (n = 5) and 4.2 (±0.6) (n = 5) GΩ/cm^2, respectively (also see SI). The statistical equivalence of these values indicates that the dienoyl moieties in bis-DenPC do not measurably increase bilayer permeability. Subsequent UV irradiation (see SI) of bis-DenPC to yield cross-linked BLMs resulted in a 5−10% increase in conductance, whereas no increase was observed upon irradiation of DPhPC BLMs (see SI). This increase likely arises from defects

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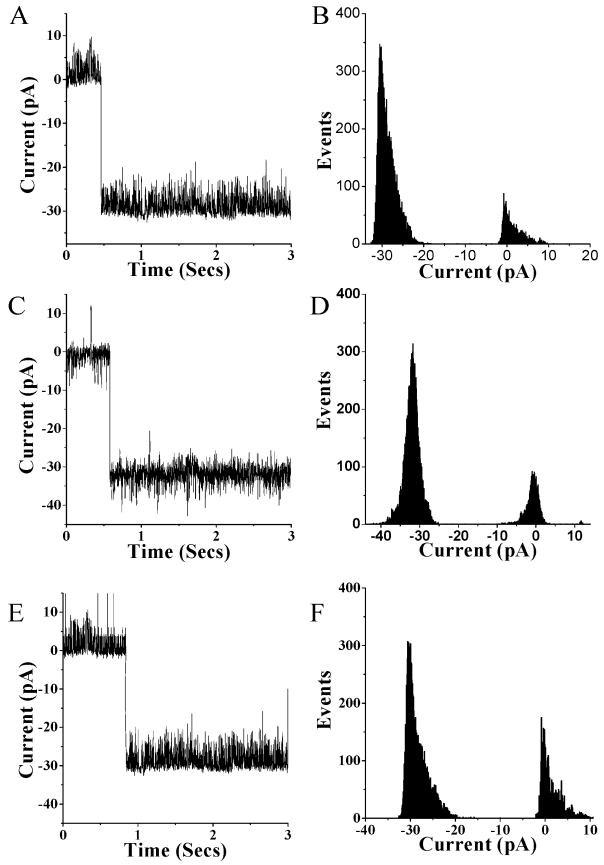

Figure 1. Single ion channel recordings of α-HL in DPhPC (A), bis-DenPC (C), and poly(bis-DenPC) (E) BLMs. All-points histograms of α-HL channel recordings in DPhPC (B), bis-DenPC (D), and poly(bis-DenPC) (F) BLMs.

in lipid packing generated upon cross-linking and/or changes in the material properties of the bilayer, e.g., the curvature (bending) elastic modulus.

The stability of BLMs was investigated by monitoring the conductance of BLMs prepared using DPhPC, bis-DenPC, and poly(bis-DenPC). Pipettes with BLMs were stored in recording buffer and measured at defined intervals. The mean BLM lifetime was 240 ± 60 min ($n = 4$) for bis-DenPC compared to 260 ± 30 min ($n = 4$) for DPhPC. In contrast, conductances indicative of an intact BLM were maintained for ca. 3 weeks (22 ± 4 days, $n = 4$) for poly(bis-DenPC). We also explored the effect of passing BLMs repeatedly across the air-buffer interface. Unpolymerized bis-DenPC and DPhPC BLMs degraded after 3 ± 1 and 2 ± 1 passes ($n = 12$ for each type of BLM), whereas no BLM degradation was observed for poly(bis-DenPC) BLMs after 30 passes ($n = 5$). Conductance data are listed in the SI.

BLMs were functionalized with α-HL (Scheme 1), a protein that forms a heptameric pore complex upon insertion into lipid membranes.[13] The resulting ca. 1 nS pore[5] has a molecular weight cutoff of ca. 3400 Da[14] and is well characterized in BLMs. Following BLM formation, the pipet potential was adjusted to −40 mV and α-HL (3 μL of 0.5 mg/mL) was added to the bath (500 μL of recording buffer) in contact with the exterior surface of the BLM. Upon insertion of the first α-HL pore, the buffer was exchanged to minimize the probability of multiple channel insertions. Insertion of an α-HL pore is observed as a stepwise, negative decrease in the measured current (Figure 1). For multiple channel experiments (see SI), longer incubation times were allowed. Single channel recordings were performed with α-HL inserted into DPhPC, bis-

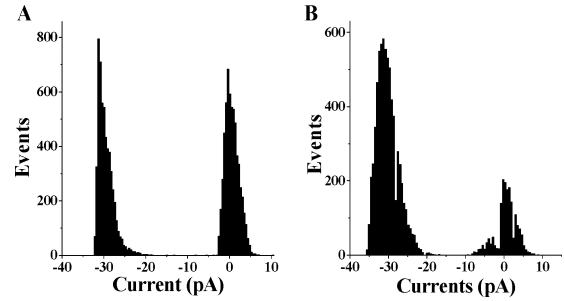

Figure 2. All-points histograms of single α-HL recordings in a poly(bis-DenPC) BLM on day 1 (A) and day 7 (B). Between recordings, the pipet was stored in recording buffer at 4 °C.

DenPC, and poly(bis-DenPC) BLMs (Figure 1); in the latter case, lipid polymerization was performed after α-HL insertion. In all three types of BLMs, openings of ca. −30 pA were obtained, which correlates well with published data.[5] Using poly(bis-DenPC) BLMs, single channel recordings were obtained daily, with minimal effects on α-HL activity, for an average of *1 week* ($n = 4$ pipettes, with lifetimes of 4, 6, 7, and 10 days). An example of this unprecedented long-term stability is shown in Figure 2. In all cases, after α-HL activity was no longer observed, *the BLM remained intact*. Thus for poly(bis-DenPC) BLMs, loss of α-HL activity (not membrane rupture) is the cause of failure.

In conclusion, we have prepared polymeric BLMs that exhibit greatly enhanced long-term stability compared to unpolymerized BLMs. The functional lifetime of ion channels incorporated in poly(bis-DenPC) BLMs is measured in days, whereas it is at most a few hours for unpolymerized BLMs. These polymerized BLMs may represent an enabling technology for development of robust biosensors and drug screening devices.

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Supporting Information Available: Additional figures, tables, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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