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Bilayer lipid membranes supported on Teflon filters: A functional environment for ion channels

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ABSTRACT

Many ion channel proteins have binding sites for toxins and pharmaceutical drugs and therefore have much promise as the sensing entity in high throughput technologies and biosensor devices. Measurement of ionic conductance changes through ion channels requires a robust biological membrane with sufficient longevity for practical applications. The conventional planar BLM is 100–300 μm in diameter and typically contains fewer than a dozen channels whereas pharmaceutical screening methods in cells use current recordings for many ion channels. We present a new, simple method for the fabrication of a disposable porous-supported bilayer lipid membrane (BLM) ion channel biosensor using hydrated Teflon (polytetrafluoroethylene, PTFE) filter material (pore size 5 μm, filter diameter = 1 mm). The lipid layer was monitored for its thickness and mechanical stability by electrical impedance spectroscopy. The results showed membrane capacitances of 1.8 ± 0.2 nF and membrane resistances of 25.9 ± 4.1 GΩ, indicating the formation of lipid bilayers. The current level increased upon addition of the pore-forming peptide gramicidin. Following addition of liposomes containing voltage-gated sodium channels, small macroscopic sodium currents (1–80 pA) could be recorded. By preloading the porous Teflon with sodium channel proteoliposomes, prior to BLM formation, currents of 1–10 nA could be recorded in the presence of the activator veratridine that increased with time, and were inhibited by tetrodotoxin. A lack of rectification suggests that the channels incorporated in both orientations. This work demonstrates that PTFE filters can support BLMs that provide an environment in which ion channels can maintain their functional activity relevant for applications in drug discovery, toxin detection, and odour sensing.

1. Introduction

Biological membranes are fundamental to life. Proteins embedded in them carry out a wide range of physiological and biochemical processes including the capture and transformation of energy, transport and exchange of nutrients and metabolites, and recognition and signaling. Mutations in membrane proteins can cause malfunction which may result in disease, such as muscle function disorders (Ashcroft, 2006). Substances which interact with membrane proteins can affect their function with either toxic results or therapeutic benefits. It is estimated that approximately a third of pharmaceutical drugs and many toxins act on membrane proteins (Meunier et al., 2009; Mouhat et al., 2004; Wang, 2008). Among these, ion channels are well recognised as important therapeutic targets for treating a range of pathophysiological diseases (Kaczorowski et al., 2008).

The sensitivity and specificity of many membrane proteins to ligands, together with their important roles in physiology, has stimulated considerable interest in the development of sensors and analytical devices in which membrane proteins are the central detection element. Potential applications for such technologies include the screening of compounds for pharmaceutical action and potential side effects (Fang et al., 2006; Matsuno et al., 2004), sensors for detecting biowarfare agents (Cox et al., 2006), environmental monitoring of marine biotoxins and sensors for odour detection (Rossini, 2005; Spehr and Munger, 2009; Vélez et al., 2001). Practical devices will require a reliable source of functional protein and a robust analog of the biological membrane. This paper focuses on the latter of these two requirements.

Research on biological membrane integrity has greatly benefited from the development of the planar bilayer lipid membrane (BLM) technique (Mueller et al., 1962) and the incorporation of ion channels into bilayers (Tien and Ottova, 2001). However the original planar BLM can be difficult to work with. They are thin fragile meta-stable structures, two lipid monolayers thick, under tension from the surrounding ring of solvent, and subjected to substantial electrical fields imposed by experimental conditions. Consequently planar BLM are short-lived as they frequently rupture before the

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experiment is completed. To circumvent these problems a number of researchers have developed methods to increase the robustness and longevity of BLM by forming them on, or tethering them to, some form of support (Cornell et al., 1997; Jeon et al., 2008; Reimhult and Kumar, 2008; Tiefenauer and Studer, 2008).

Recent examples of BLMs tethered to a metal surface through a thiolated hydrophilic spacer are based on the use of the DPTL thiolipid tethered to mercury (Becucci and Guidelli, 2007; Becucci et al., 2005, 2007, 2009) and to gold (He et al., 2005; Naumann et al., 2003; Schiller et al., 2003). Non-tethered or "true" BLMs have been formed directly on a variety of materials that include: nascent metal surfaces (Tien and Salamon, 1989), filters (Dhoke et al., 2005; Thompson et al., 1982), hydrophilic gels of biological and chemical origin (Baumgart and Offenhauser, 2003; Maurer et al., 2007), and custom micro-fabricated materials (Mayer et al., 2003). Biomimmetic membranes fabricated as arrays of BLMs spanning the holes of a microporous support are known as "nano-BLMs", some of which have been reported to support ion channel function (Favero et al., 2005; Romer and Steinem, 2004; Schmitt et al., 2006).

To create a membrane environment for a functioning ion channel, a further requirement for supported BLM is that both sides of the membrane are exposed to aqueous solutions. This approximates the physiological condition for a cell plasma membrane in that it allows an ionic reservoir for ion movements as well as space for incorporated membrane proteins. An earlier study (Thompson et al., 1982) in which a variety of different filter materials were examined including: PTFE, cellulose ester and polycarbonate, for their ability to support BLM found that filter-lipid membranes are resistant to mechanical shock and vibration. PTFE filter material is an irregular, highly porous structure that is chemically inert, and would therefore have potential for biological use *in vivo*.

The objective of this work was to produce a membrane system that is robust and stable over time, with a suitably high membrane resistance to record ion channel activity and effects of inhibitors. The voltage-gated sodium channel (VGSC) was used as a model ion channel, since this receptor is modulated by marine biotoxins and is an important pharmaceutical target. This study advances earlier work by hydrating hydrophobic PTFE filters with an aqueous solution prior to BLM formation. This was necessary to provide an aqueous environment on both sides of the BLM for ion channel function. A new technique for enhanced efficiency of ion channel-proteoliposome incorporation into the PTFE-supported BLM is described. This resulted in macroscopic current recordings from multiple ion channels and inhibition by a specific VGSC inhibitor. The PTFE-assembly was fabricated from commercially available materials and data can be collected on voltage-clamp recording equipment (pA to nA range), providing a straightforward method that is easily accessible to other researchers.

2. Materials and methods

2.1. Filters and cuvettes

Polystyrene semi-micro spectrophotometric cuvettes (LP Italiana SPA, Cat. No. LPI122117) were cut down to a height of 23 mm. A 1 mm diameter hole was drilled through the centre of a smooth face of the cuvette at height of 4 mm from the base. A piece of PTFE filter (Millipore Mitex cat. no. LSWP01300) 5.0 μm mean pore diameter, 125 μm mean thickness, and 60% porosity was cut out to 3 mm diameter using a hole punch. The PTFE disc was then securely sealed over the hole in the polystyrene cuvette from the external side using a heated tube, 2 mm internal diameter, 0.2 mm wall thickness, to melt the underlying polystyrene and press the filter onto the melted material. A lever press was constructed that provided reproducible positioning and heating to improve uniformity in fabrication. A tight connection between the PTFE filter and the cuvette was found to be critical for BLM formation. Ensuring that sufficient PTFE material remained after trimming during manufacture increased the number of successful BLMs formed. The cuvettes were inspected optically with a stereo-microscope and those where the seal between the filter and cuvette appeared to be poorly joined were rejected. A photograph of the cuvette and a diagram of the experimental set-up are given in Fig. 1.

2.2. Infiltration

The highly hydrophobic nature of the Teflon filters prevents aqueous solutions from spontaneously entering the filter's pores and in this condition the resistance across the filter is effectively an open circuit. To infiltrate the pores with an aqueous solution the cuvettes were filled with the solution and placed in a beaker containing the same solution. The beaker containing the cuvette was placed in a vacuum desiccator which was then evacuated with a water pump to −75 kPa. Following evacuation for approximately 2 h, the pressure in the desiccator was allowed to equilibrate with the atmosphere which resulted in the solution being drawn into the filter matrix.

2.3. BLM formation

A BLM-forming solution containing 5% (w/w) of phosphatidyl choline and 2% (w/w) cholesterol in *n*-octane was centrifuged at 10,000 rpm for 1 min and the supernatant collected. 10–20 μl of this solution was "painted" onto the outer surface of the PTFE filter (from the beaker) using a piece of plastic tubing slipped onto the end of a needle attached to a 10 μl syringe.

2.4. Electrophysiology

2.4.1. Electrical impedance spectroscopy

Electrical impedance spectra (EIS) were obtained using PCI14/300 Potentiostat and FAS2 Femtostat manufactured by Gamry Instruments operating under Gamry Framework, Echem Analyst™ and EIS300 analysis software (Gamry Instruments, Warminster, PA, USA). Spectra were recorded for frequencies between 1 mHz and 100 kHz at 0 V potential with an AC modulation amplitude of 10 mV. Nine sample points were taken per 10-fold increase in frequency. For impedance recordings the measuring (trans) and reference (cis) electrodes were silver/silver chloride wires, and the counter wire (cis) was platinum.

2.4.2. Voltage-clamp

Voltage-clamp recordings of ion channel activity were made with two different sets of experimental equipment. The first used HEKA instruments, either an EPC7 amplifier with an Instrutech ITC16 digital interface or an EPC10 amplifier with an integrated interface controlled by HEKA Pulse software and data analysed using HEKA PulseFit and PulseTools v8.8 (HEKA, Lambrecht/Pfalz Germany). The second setup was an Axon 200B amplifier and Axon Digidata 1440A digital interface using pClamp 10.2 software (Molecular Devices, Sunnyvale, USA). Silver/silver chloride electrodes connected the cell to the amplifier to record ionic currents. In some experiments EIS and voltage-clamp measurements were made on the same preparation but in such cases only one set of electrodes, either EIS or voltage-clamp, were inserted into the cell at any given time. For both types of measurement the cell was enclosed in a Faraday cage, supported on a vibration isolation table, in which the pre-amplifiers for both EIS and voltage-clamp were also placed.

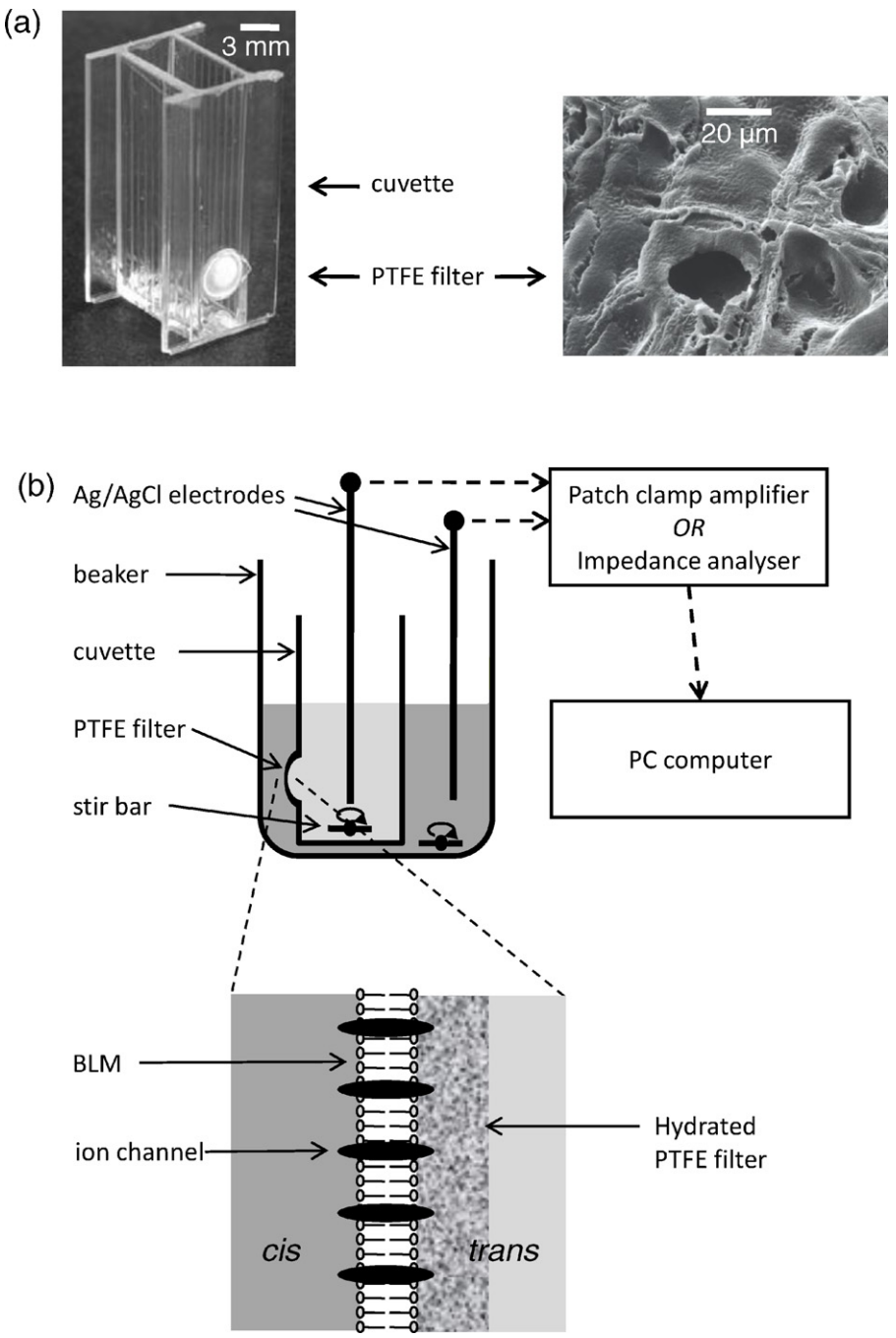

Fig. 1. Porous PTFE support for BLMs. (a) Photo of PTFE filter embedded in a polystyrene spectrophotometric cuvette. (b) Diagram of the support assembly for measurement of currents through ion channels on porous PTFE support and experimental setup, with supported membrane region enlarged. For EIS measurements there was also a platinum counter electrode on the cis side (not shown in the diagram).

2.5. Ion channels

Gramicidin D peptide from *Bacillus brevis* (Sigma) in an ethanol stock solution was added to both chambers at a final concentration of 60 μg/ml.

Voltage-gated sodium ion channel (VGSC) was expressed and purified in our laboratory by methods that have been fully described previously (Zhang et al., 2007, 2008). A poly-histidine tag was added to the C-terminus of human VGSC cDNA (*hSkM1*) (George et al., 1992) by sub-cloning from a mammalian vector into the baculovirus transfer vector, pFastBac. These recombinant bacmids were expressed in *E. coli* using the Bac-to-Bac system. Purified bacmid DNA was used to infect *Sf*9 cell cultures. Protein from these virus-infected cells was obtained by centrifuging the cultures, sonicating the cells, and isolating the membrane fraction by further centrifugation. These membranes were solubilised in detergent and his-tagged VGSC isolated by ion metal affinity chromatography. The detergent was removed using a detergent affinity resin, Gel D (Pierce, Rockford, IL, USA) and the protein was reconstituted into liposomes using a phospholipid solution containing 50 mg phosphatidyl ethanolamine, 10 mg of phosphatidyl choline and 10 mg of cholesterol in 9 ml of buffer solution and using freezing and thawing to form proteoliposomes. The liposomes were collected by centrifugation. Aliquots of the resuspended liposomes were frozen and stored at −80 °C.

Table 1
Values determined from fit of equivalent model circuit to the data.

Parameter	Model values
R_Ω	11.1 ± 2.5 kΩ
R_{Sup}	2.1 ± 0.4 GΩ
W_{Sup}	102 ± 25 pS/s
R_{bl}	25.9 ± 4.1 GΩ
C_{bl}	1.8 ± 0.2 nF
C_{Stray}	13.4 ± 0.6 pF
Goodness of fit[a]	0.012 ± 0.003

$n = 20$, mean ± S.E.M.
R_Ω: resistance of the solution (between the working and reference electrodes), R_{Sup}: resistance of the support, W_{Sup}: Warburg impedance element for the support, R_{bl}: membrane resistance, C_{bl}: capacitance of the BLM, C_{Stray}: stray capacitance.
[a] Non-linear least squares regression.

2.6. Solutions

For experiments with either VGSC or gramicidin solutions contained 300 mM NaCl and 10 mM HEPES (N-2-Hydroxylethylpiperazine-N-2-ethane sulphonic acid) buffer, adjusted to pH 7.4 with potassium hydroxide. Veratridine (VTD) was purchased from Sigma and tetrodotoxin (TTX) from Alomone Labs (Jerusalem, Israel).

2.7. Statistical analysis

Results are expressed as mean ± S.E.M. Statistical comparisons were made using a one-way anova nested design in Genstat v12 (VSN International Limited, Hemel Hempstead, UK).

3. Results

3.1. Evaluation of electrical impedance spectra

Measuring the electrical impedance of an object over a broad range of frequencies allowed a model circuit of its electrical properties to be constructed. This provided information on the integrity and area of the bilayer membrane (Diao et al., 1999; Gao et al., 2001; Romer and Steinem, 2004; Steinem et al., 1996; Vallejo and Gervasi, 2002). EIS spectra for PTFE supports were recorded over the frequency range 1 mHz to 100 kHz, before and after the formation of a BLM. The Bode plots for the resistive and capacitive components of the impedance of a typical spectrum are shown in Fig. 2(a) and (b). The painting of the surface of the support with BLM forming solution resulted in an increase in resistance from kilo-Ohms to giga-Ohms (25.9 ± 4.1 GΩ) and a capacitance of 1.8 ± 0.2 nF. These changes in electrical properties suggest the formation of lipid bilayers. Model circuits consistent with expected electrical properties of the support and bilayer were fitted to the data. Diagrams of these circuits are shown for PTFE supports before (Fig. 2(c)) and after (Fig. 2(d)) the formation of BLM, and the computed Bode plots are shown in Fig. 2(a) and (b). The model circuit for the support alone (Fig. 2(c)) includes resistive elements for the measuring electrode and the support plus a Warburg element to account for the constricted nature of diffusion through the porous support (Jacobsen and West, 1995; Vallejo and Gervasi, 2002). For supports with BLM (Fig. 2(d)), additional elements for the resistance and capacitance of the BLM were added (Diao et al., 1999; Gao et al., 2001; Romer and Steinem, 2004; Steinem et al., 1996; Vallejo and Gervasi, 2002). The values for the membrane resistance and capacitance are shown in Table 1. The computed spectra for the fitted models agree well with the experimental data. Goodness of fit is 0.012 ± 0.003 using a non-linear least squares fitting algorithm (Echem Analyst™ EIS300 analysis package) (Sgura and Bozzini, 2005).

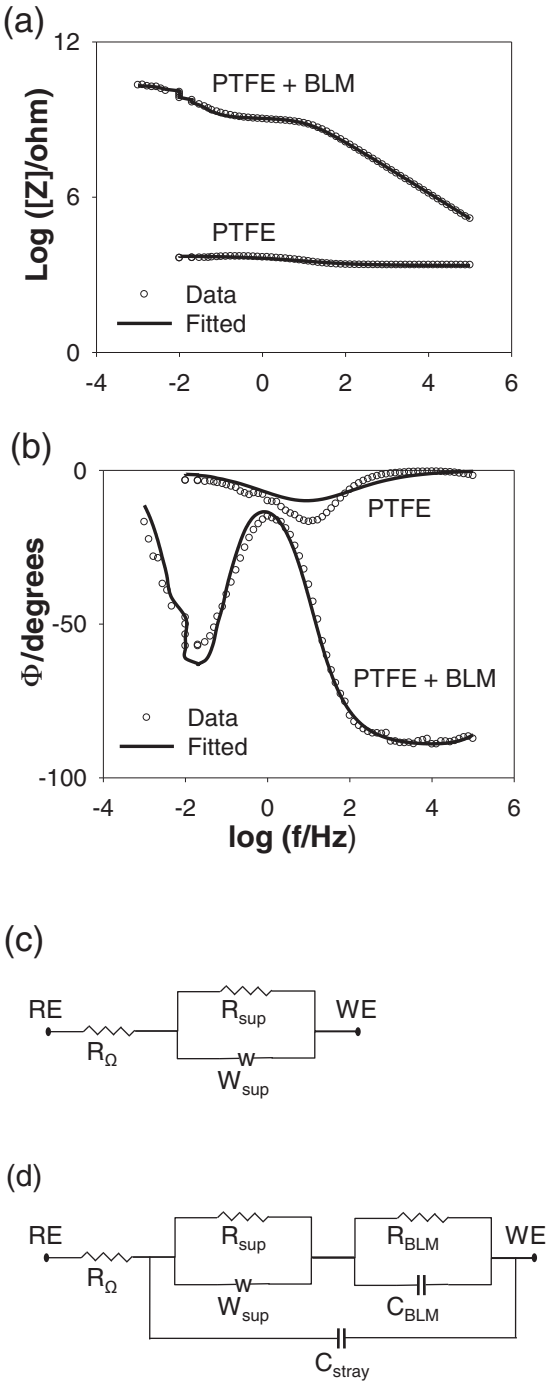

Fig. 2. Electrical impedance of BLMs. (a and b) Bode plots show impedance spectra of porous PTFE before and after spreading of a lipid droplet across the surface. Models of equivalent electrical circuit composed of support (c) and support plus BLM (d). R_Ω: resistance of the solution between the working and reference electrodes, R_{Sup}: resistance of the support, W_{Sup}: Warburg impedance element for the support, R_{bl}: membrane resistance, C_{bl}: capacitance of the BLM, C_{Stray}: stray capacitance.

3.2. Gramicidin currents

To determine whether these changes in electrical properties on applying BLM forming solution to the surface of the support were due to the formation of BLM, the function of an ion channel pore in the membrane was assessed. Gramicidin, an antibiotic which inserts itself into membranes to form ion channels, only functions in a BLM. The length of a gramicidin peptide is only sufficient to span half the width of a bilayer. The formation of the gramicidin

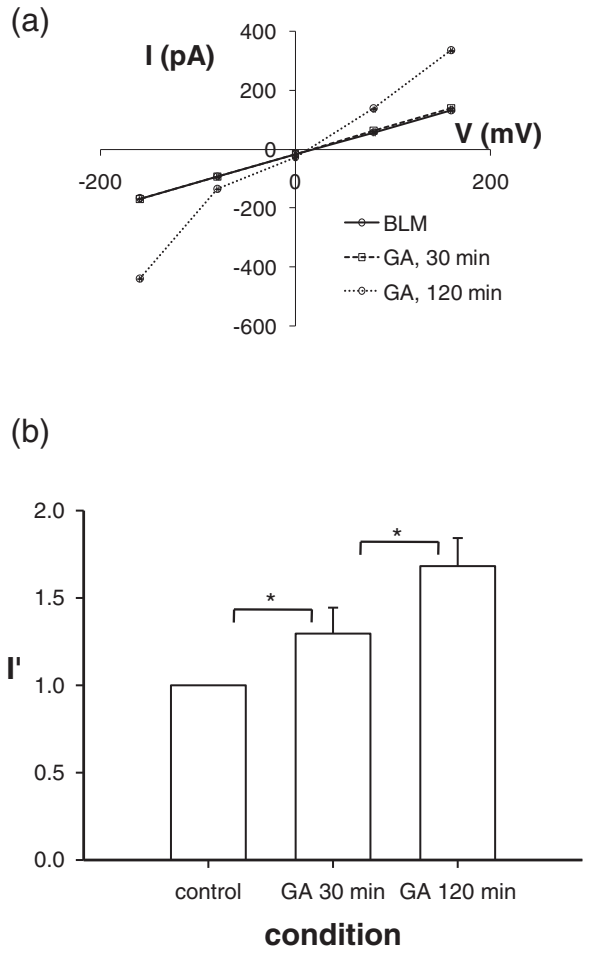

Fig. 3. Gramicidin in PTFE supported BLMs. Current–voltage relationship of BLMs prior to addition of gramicidin compared with that at 30 min and 2 h time points for (a) a single experiment, and (b) average of four experiments where I' is the current following addition of gramicidin relative to controls, $^{*}p < 0.05$.

ion channel therefore, requires a molecule spanning the plane of one half of the BLM to align with a molecule in the other half. A BLM was formed on the PTFE support and then gramicidin was added to both chambers. The solutions contained 300 mM NaCl in both chambers. The current–voltage relationship was recorded from +80 mV to −80 mV in 20 mV increments for 1 min, after 30 min and 2 h (Fig. 3(a)). Although an increase in current was only seen at 2 h in this example, some experiments showed an increase at 30 min as seen in the pooled data in which the current response at +80 mV had increased by 30% at 30 min, and 68% at 2 h, $n = 4$, $p < 0.05$ (Fig. 3(b)). The increased current upon addition of gramicidin indicates that the lipid membrane on the PTFE is in a bilayer form.

3.3. Reconstitution of ion channel protein into supported BLM (not preloaded)

To assess the ability of the PTFE-supported BLM to provide a functional environment for a eukaryotic membrane protein, the activity of a human VGSC reconstituted into proteoliposomes was examined. Following the formation of a BLM on the support VGSC proteoliposomes were added to the cis chamber in close contact with the BLM and the solution was stirred for up to 15 min to facilitate incorporation into the BLM. The initial current after BLM formation, after addition of VGSC proteoliposomes together with the VGSC activator veratridine (VTD, 100 μM), and following addition of the inhibitor tetrodotoxin (TTX, 100 nM) are shown in Fig. 4(a). The initial current level was 7 pA after BLM formation and increased to 71 pA 10 min after addition of VGSC proteoliposomes and VTD, and was inhibited to 14 pA after 30 min exposure to TTX. The BLM resistance remained consistent throughout the experiment (Fig. 4(a)). Averaged current data from six experiments showed that 10–30 min following addition of VGSC proteoliposomes and VTD, the current level had increased 8.1-fold. Upon addition of the VGSC inhibitor TTX (100 nM) to both chambers, the current level returned close to control levels (Fig. 4(b)). The current level produced by liposomes without added protein in response to VTD (53.38 ± 7.80 pA at +80 mV, $n = 6$) was similar to that of the BLM alone (56.36 ± 7.11 pA at +80 mV, $n = 6$).

3.4. Preloading of ion channel protein into PTFE-supports

It was found that if a broken BLM was reformed, channel activity was seen immediately. This finding raised the possibility that proteoliposomes had gained access to the porous PTFE matrix and suggested prior loading of proteoliposomes into the PTFE-support as a means to facilitate incorporation into the BLM. To examine whether the presence of proteoliposomes in the PTFE-support would increase incorporation into the BLM and therefore produce macroscopic sized currents (nanoampere), the proteoliposomes were loaded into the PTFE-support before lipid was painted onto the outer surface of the PTFE-support. Current–voltage recordings were then carried out.

An example of the current–voltage relationship for VGSC currents obtained using a support preloaded with VGSC protein is shown in Fig. 5(a). Addition of 100 μM VTD resulted in a large increase in current. The subsequent addition of the blocker 100 nM TTX caused a reduction in current to the control level over 15 min. The ion channel currents did not show the rectification typical of VGSC currents seen *in vivo*. A probable explanation is that the protein inserts into the BLM in both directions resulting in the partial reduction or elimination of rectification. The mean current level from four experiments was 10 nA. Pooled data results show that TTX inhibited by $53 \pm 16\%$ at +80 mV, and was similar at −80 mV, $n = 4$, $p < 0.001$ (Fig. 5(b)). These results demonstrate that at least half of the current is through VGSC and that the inhibited current amplitude for preloaded proteoliposomes is 100-times greater than when added to the chamber.

3.5. Re-used cuvettes

To determine whether cuvettes could be reused from previous VGSC preloading experiments, they were soaked in 70% ethanol overnight, rinsed in water, dried at 45 °C overnight, rehydrated and pre-loaded with proteoliposomes, and fresh solutions used in a clean chamber. Following infiltration with proteoliposomes, BLMs were formed on re-used cuvettes and had an initial BLM resistance of 1.4 ± 0.3 GΩ and capacitance of 0.47 ± 0.06 nF ($n = 14$), 64% of which lasted for 30 min, and 21% lasted for 3 h. The lower capacitance in the re-used cuvettes compared to new ones suggests that the bilayer may have been thicker in the re-used cuvettes (Moran et al., 1980; Thompson et al., 1982). An alternative or additional factor may have been that some of filter's surface was rendered unsuitable for BLM formation by the prior formation of BLM and subsequent washing. Currents were recorded in the presence of VTD that increased by 7.0-fold compared to the control, $n = 6$ (Fig. S1). The current responses varied in amplitude, but all were inhibited by TTX.

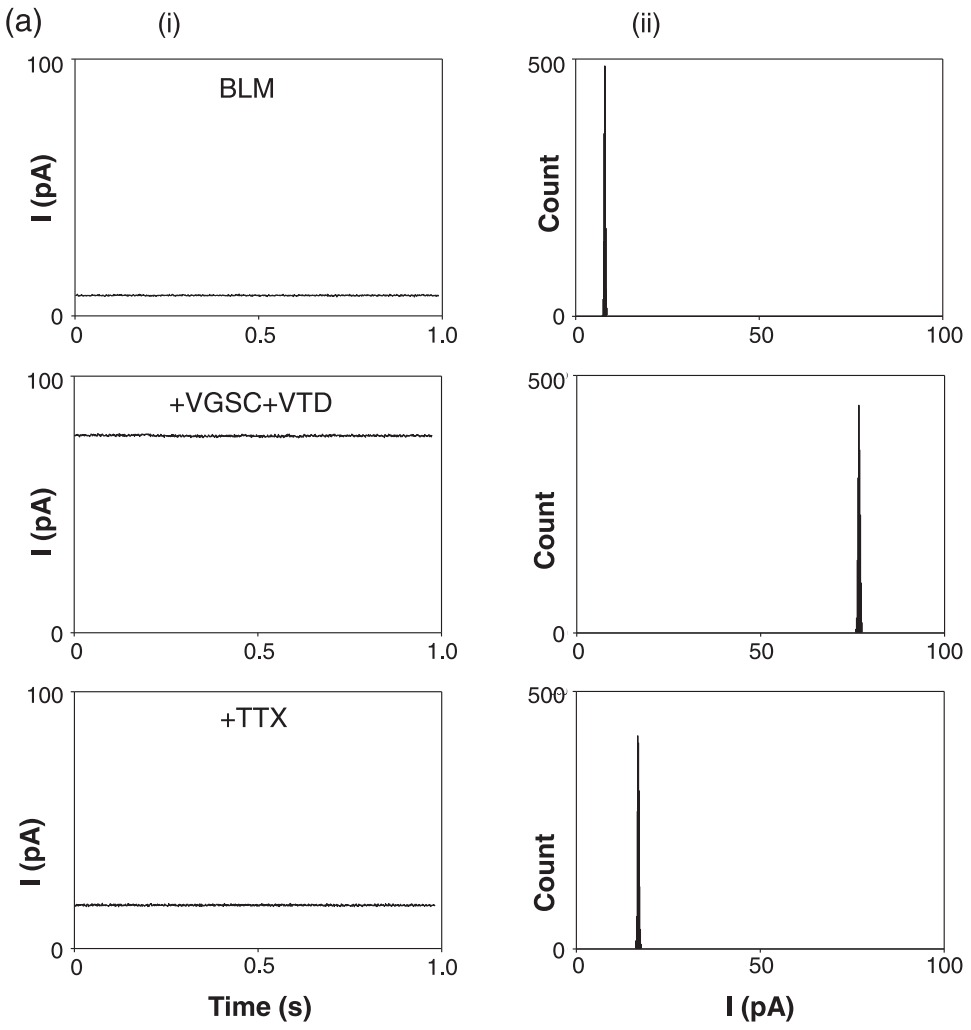

Fig. 4. VGSC currents in PTFE supported BLMs. (a) (i) Current recordings from a single experiment are shown for a BLM pulsed to −120 mV. A BLM was formed on the PTFE filter and the control current level recorded. The current level is shown 10 min after addition of VGSC liposomes and VTD (100 μM) to the cis side, then 30 min after addition of TTX (100 nM). (ii) Corresponding histograms show the current amplitude distribution over the period of the recording. The recorded BLM resistance was 900 MΩ after BLM formation, 850 MΩ before addition of VTD, 700 MΩ after addition of VTD, and 750 MΩ after addition of TTX. (b) Histogram showing mean current in control (BLM only), compared to that recorded 10–30 min following addition of VGSC proteoliposomes, and 30 min after addition of TTX, $n = 6$; $^{*}p < 0.05$, $^{**}p < 0.01$.

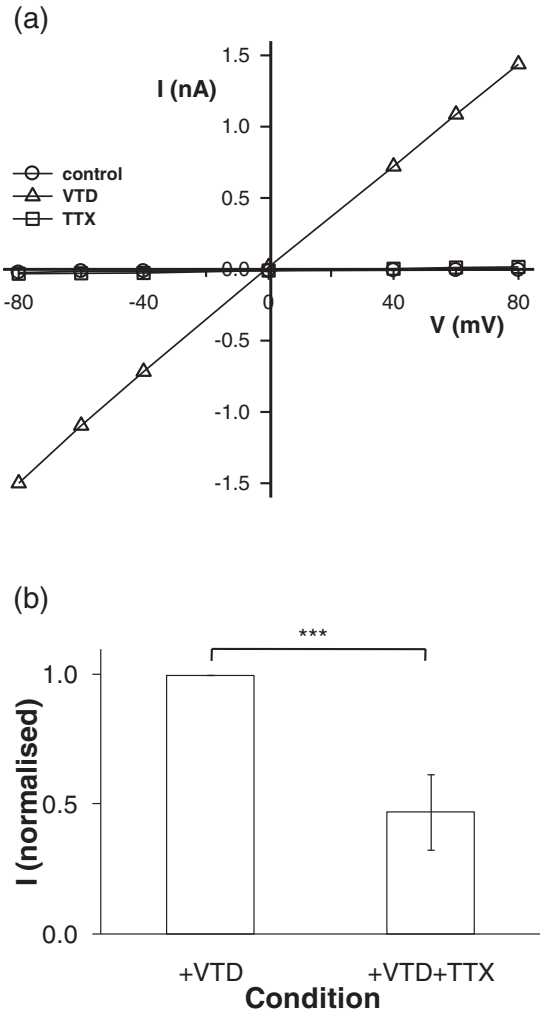

Fig. 5. PTFE supported BLMs preloaded with Na^+ channel protein. (a) Current–voltage relationship is shown for BLMs held at −80 to +80 mV, before and after addition of VTD (100 μM) and after addition of TTX (100 nM). (b) Histogram showing mean current normalised to the control at +80 mV (ii) recorded 10–30 min following addition of VGSC proteoliposomes, 30 min after addition of VTD, and 20–30 min after addition of TTX, $n = 4$; $^{***}p < 0.001$.

4. Discussion

4.1. Evidence for the formation of a BLM

This study provides three lines of evidence for the formation of BLM on hydrated porous PTFE. Firstly, the large increase in resistance and capacitance on application of BLM-forming solutions fit model circuits that are indicative of BLM formation and agree with that expected for relevant model circuits. Secondly, that cation currents were produced upon addition of gramicidin, and thirdly Na^+ currents produced when VGSC proteoliposomes were added.

EIS data that fit models in which the resistance and capacitance of the BLM connected in parallel is an important element in the equivalent circuit. Such an element is found in previous analysis of EIS spectra of supported BLM (Diao et al., 1999; Gao et al., 2001; Romer and Steinem, 2004; Steinem et al., 1996; Vallejo and Gervasi, 2002). Gao et al. (2001) studied BLM formed directly on stainless steel and did not include any other circuit elements. However the other studies, working with gold surfaces coated with thiol attached compounds, included a resistance identified as arising from the electrode. In the case of the study by Vallejo and Gervasi (2002), an additional Warburg impedance was included. The porous PTFE used in our study required a different arrangement of these circuit elements as it presented more restricted and complex electrical access to the surface of the BLM facing the support. The equivalent circuit which fitted the data for the support and electrodes in the absence of a BLM consisted of a resistance for the solution between the working and reference electrodes (R_Ω), and a resistance (R_{sup}) and Warburg impedance element (W_{sup}) in parallel for the support (Fig. 1(b)). Warburg elements account for diffusion phenomena and are appropriate for the convoluted and restrictive interstices of the support material (Jacobsen and West, 1995). These elements were connected in series with the components representing the BLM. It was found that the fit of the data was significantly improved by the addition of a stray capacitance connected in parallel with the components for the support and BLM. The values associated with R_Ω, $R_{sup,}$ and W_{sup} are small compared with the values obtained for $R_{bl,}$ and values obtained for C_{stray} were small compared with the values for C_{bl}.

The specific capacitance of a wide variety of BLM approximate 1 $\mu F\,cm^{-2}$ (Tien and Ottova, 2003). Using this value, the area of PTFE suggests values of more than an order of magnitude greater than those obtained for C_{bl}. This discrepancy may be explained by proposing that BLM only form across pores at the surface of the PTFE support in a manner analogous to planar BLM formed across an aperture in a PTFE septum. The area of painted BLM, as studied here, is smaller than the aperture because of the presence of the Plateau-Gibbs Border of solvent containing phospholipids that surrounds the BLM. The pores are irregular rather than circular which will decrease the area of BLM relative to the Plateau-Gibbs Border. If this description is valid, the area of the BLM is likely to be less than 10% of the area of the PTFE support material thus explaining the low values for specific capacitance obtained by assuming that the area of BLM is the same as the support.

The ability of the antibiotic peptide gramicidin to produce cation currents further supports the presence of a BLM (Romer and Steinem, 2004; Woolley and Wallace, 1992). The length of one peptide is only sufficient to span half the thickness of a BLM. A channel only forms when two peptides in opposite lamella of the bilayer align to form a dimer (Vallejo and Gervasi, 2002). Such events are signalled by currents arising from the movement of monovalent cations through the channel formed by the dimer.

The reconstitution of VGSC channels in porous PTFE-supported BLM is the most significant demonstration that a functional analog of a biological membrane has been formed on this support material. Macroscopic currents are reported in which the resistance of the membrane layer approaches that of good patch-clamp seals. The presence of macroscopic currents indicates that the method has potential for a variety of sensing and screening applications. We have demonstrated that the environment provided by porous PTFE-supported BLM is sufficiently natural to allow the protein to respond to voltage and toxins as expected. The results demonstrate the effects of the channel activator VTD and the blocker TTX.

Reconstituted VGSC currents differed from those in their native environment in that they did not show the rectification observed *in vivo*. This most likely results from the protein being positioned in the supported bilayers in a mixed orientation with some molecules having the extracellular face exposed to the cis chamber and other exposed to the trans chamber. The consequence would be that each molecule would respond to the electrical potential gradient applied to it as normal. However cis-trans oriented molecules would experience the opposite gradient to that experienced by the trans-cis oriented molecules. This would tend to reduce the rectification observed.

4.2. Preloading of ion channel protein

The preloading of the supports by adding proteoliposomes to the solution used to infiltrate the porous PTFE prior to BLM formation produced currents that were greater in amplitude that those measured when BLM were formed and proteoliposomes then added to the *cis* chamber. From this we infer that liposome-BLM fusion efficiency was enhanced under infiltrated conditions. The phenomena that occur when protein is preloaded into the support are not known. It is possible that some liposomes form a monolayer of phospholipids on the internal walls of the PTFE with the hydrophobic tails oriented towards the PTFE walls of the interstices and the polar heads facing the interstitial spaces. This would tend to create a hydrophilic environment with the possibility that other proteoliposomes would be adsorbed to the hydrophilic surface with sufficient strength to retain them against the force of diffusion. The process of preloading would force some proteoliposomes through small apertures in the interstices of the PTFE resulting in them being resized to smaller dimensions. Another possibility is that interstices of the filter remain hydrophobic until the phospholipid containing BLM forming solution is painted onto it whereupon a monolayer would be formed. This work does not differentiate between these possibilities.

4.3. Usefulness of PTFE compared to other supported BLM

In the past decade there has been a rapid increase in the variety of supported BLM that provide a suitable environment for membrane protein function. These include solid supported BLM (Castellana and Cremer, 2006), nano- and microporous supports (Reimhult and Kumar, 2008), polymer supported BLM (Tanaka and Sackmann, 2005), membrane arrays (Groves, 2002), BLM formed on silicon nanowires (Misra et al., 2009), BLM formed by microfluidic circuits (Suzuki et al., 2007), and droplet interface bilayers (Hwang et al., 2008). Each of these membranes has useful characteristics in terms of resistance, stability, longevity and exposed membrane area, and ability to incorporate membrane proteins. The porous PTFE-supported BLM described in this study shares many of these characteristics – they are of high resistance, sufficiently robust over several hours to survive being bathed in stirred solutions and being moved. The support material is readily available and the construction of the cuvette-PTFE assembly requires only basic workshop equipment. As with some other types of supported BLM, ion channel proteins functioned and responded to toxins in porous PTFE-supported BLM, and both single channel and macroscopic currents could be observed. However the possibility of preloading ion channel proteoliposomes into the support is a unique advantage. The nanoampere ion channel currents produced by the preloading method, together with the possibility of reusing the PTFE-support, provides a convenient and practical platform for the next generation of HTS ion channel technologies.

4.4. Concluding perspective

This work demonstrates that PTFE filters can support BLM that are sufficiently long-lived and robust for research purposes. The technology involved is simple and readily accessible. These BLM provide an environment in which ion channels can maintain their functional activity. This makes a wide range of new biotechnologies conceivable with applications in drug discovery, toxin detection, and odour sensing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.12.013.

References

Ashcroft, F.M., 2006. Nature 440 (7083), 440–447.
Baumgart, T., Offenhauser, A., 2003. Langmuir 19 (5), 1730–1737.
Becucci, L., Guidelli, R., 2007. Langmuir 23 (10), 5601–5608.
Becucci, L., Guidelli, R., Karim, C.B., Thomas, D.D., Veglia, G., 2009. Biophys. J. 97 (10), 2693–2699.
Becucci, L., Moncelli, M.R., Naumann, R., Guidelli, R., 2005. J. Am. Chem. Soc. 127 (38), 13316–13323.
Becucci, L., Santucci, A., Guidelli, R., 2007. J. Phys. Chem. B 111 (33), 9814–9820.
Castellana, E.T., Cremer, P.S., 2006. Surf. Sci. Rep. 61 (10), 429–444.
Cornell, B.A., Braach-Maksvytis, V.L.B., King, L.G., Osman, P.D.J., Raguse, B., Wieczorek, L., Pace, R.J., 1997. Nature 387 (6633), 580–583.
Cox, T., Tattersall, J., Geddes, N., Evans, S., Nelson, A., 2006. J. Defence Sci. 10, 115–121.
Dhoke, M.A., Ladha, P.J., Boerio, F.J., Lessard, L.B., Malinowska, D.H., Cuppoletti, J., Wieczorek, D.S., 2005. Biochim. Biophys. Acta Biomembr. 1716 (2), 117–125.
Diao, P., Jiang, D., Cui, X., Gu, D., Tong, R., Zhong, B., 1999. Bioelectrochem. Bioenergy 48 (2), 469–475.
Fang, Y., Hong, Y., Webb, B., Lahiri, J., 2006. M. R. S. Bull. 31 (7), 541–545.
Favero, G., Campanella, L., Cavallo, S., D'Annibale, A., Perrella, M., Mattei, E., Ferri, T., 2005. J. Am. Chem. Soc. 127 (22), 8103–8111.
Gao, H., Feng, J., Luo, G.A., Ottova, A.L., Tien, H.T., 2001. Electroanalysis 13 (1), 49–53.
George, A.L., Komisarof, J., Kallen, R.G., Barchi, R.L., 1992. Ann. Neurol. 31 (2), 131–137.
Groves, J.T., 2002. Curr. Opin. Drug Discov. Dev. 5 (4), 606–612.
He, L., Robertson, J.W., Li, J., Kärcher, I., Schiller, S.M., Knoll, W., Naumann, R., 2005. Langmuir 21 (25), 11666–11672.
Hwang, W.L., Chen, M., Cronin, B., Holden, M.A., Bayley, H., 2008. J. Am. Chem. Soc. 130 (18), 5878–5879.
Jacobsen, T., West, K., 1995. Electrochim. Acta 40 (2), 255–262.
Jeon, T.J., Malmstadt, N., Poulos, J.L., Schmidt, J.J., 2008. Biointerphases 3 (2), FA96.
Kaczorowski, G.J., McManus, O.B., Priest, B.T., Garcia, M.L., 2008. J. Gen. Physiol. 131 (5), 399–405.
Matsuno, N., Murawsky, M., Ridgeway, J., Cuppoletti, J., 2004. Biochim. Biophys. Acta Biomembr. 1665 (1–2), 184–190.
Maurer, J.A., White, V.E., Dougherty, D.A., Nadeau, J.L., 2007. Biosens. Bioelectron. 22 (11), 2577–2584.
Mayer, M., Kriebel, J.K., Tosteson, M.T., Whitesides, G.M., 2003. Biophys. J. 85 (4), 2684–2695.
Meunier, F.A., Mattei, C., Molgo, J., 2009. Prog. Mol. Subcell. Biol. 46, 159–186.
Misra, N., Martineza, J.A., Huanga, S.C.J., Wanga, Y., Stroevec, P., Grigoropoulosb, C.P., Noya, A., 2009. Proc. Natl. Acad. Sci. U.S.A. 106 (33), 13780–13784.
Moran, A., Tal, E., Eytan, E., Nelson, N., 1980. FEBS Lett. 110 (1), 62–64.
Mouhat, S., Jouirou, B., Mosbah, A., De Waard, M., Sabatier, J.M., 2004. Biochem. J. 378 (3), 717–726.
Mueller, P., Rudin, D.O., Ti Tien, H., Wescott, W.C., 1962. Nature 194 (4832), 979–980.
Naumann, R., Schiller, S.M., Giess, F., Grohe, B., Hartman, K.B., Kärcher, I., Köper, I., Lübben, J., Vasilev, K., Knoll, W., 2003. Langmuir 19 (13), 5435–5443.
Reimhult, E., Kumar, K., 2008. Trends Biotechnol. 26 (2), 82–89.
Romer, W., Steinem, C., 2004. Biophys. J. 86 (2), 955–965.
Rossini, G.P., 2005. Toxicology 207 (3), 451–462.
Schiller, S.M., Naumann, R., Lovejoy, K., Kunz, H., Knoll, W., 2003. Angew. Chem. Int. Ed. Engl. 42 (2), 208–211.
Schmitt, E.K., Vrouenraets, M., Steinem, C., 2006. Biophys. J. 91 (6), 2163–2171.
Sgura, I., Bozzini, B., 2005. Int. J. Non Linear Mech. 40 (4), 557–570.
Spehr, M., Munger, S.D., 2009. J. Neurochem. 109 (6), 1570–1583.
Steinem, C., Janshoff, A., Ulrich, W.P., Sieber, M., Galla, H.J., 1996. Biochim. Biophys. Acta Biomembr. 1279 (2), 169–180.
Suzuki, H., Tabata, K.V., Noji, H., Takeuchi, S., 2007. Biosens. Bioelectron. 22 (6), 1111–1115.
Tanaka, M., Sackmann, E., 2005. Nature 437 (7059), 656–663.
Thompson, M., Lennox, R.B., McClelland, R.A., 1982. Anal. Chem. 54 (1), 76–81.
Tiefenauer, L.X., Studer, A., 2008. Biointerphases 3 (2), FA74.
Tien, H.T., Ottova, A., 2003. In: Tien, H.T., Ottova-Leitmannova, A. (Eds.), Planar Lipid Bilayers and their Applications. Elsevier, Amsterdam, pp. 1–73.
Tien, H.T., Ottova, A.L., 2001. J. Membr. Sci. 189 (1), 83–117.
Tien, H.T., Salamon, Z., 1989. Bioelectrochem. Bioenergy 22 (3), 211–218.

Vallejo, A.E., Gervasi, C.A., 2002. Bioelectrochemistry 57 (1), 1–7.
Vélez, P., Sierralta, J., Alcayaga, C., Fonseca, M., Loyola, H., Johns, D.C., Tomaselli, G.F., Marban, E., Suarez-Isla, B.A., 2001. Toxicon 39 (7), 929–935.
Wang, D.Z., 2008. Drugs 6 (March (2)), 349–371.
Woolley, G.A., Wallace, B.A., 1992. J. Membr. Biol. 129 (2), 109–136.
Zhang, Y.L., Dalziel, J.E., Dunlop, J., Leitmannova Liu, A., 2008. In: Leitmannova Liu, A. (Ed.), Advances in Planar Lipid Bilayers and Liposomes. Elsevier, Amsterdam, pp. 27–47.
Zhang, Y.L., Dunlop, J., Dalziel, J.E., 2007. Biosens. Bioelectron. 22 (6), 1006–1012.