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Polydopamine Coating To Stabilize a Free-Standing Lipid Bilayer for Channel Sensing

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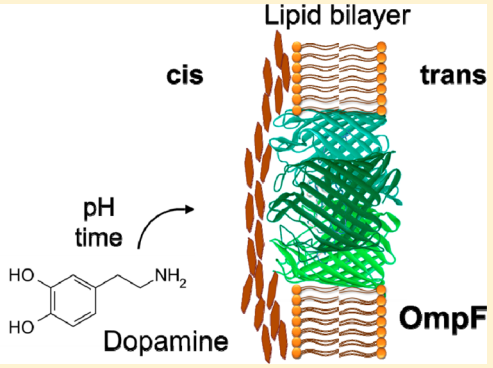
ABSTRACT: An appropriate method to study the function of membrane channels is to insert them into free-standing lipid bilayers and to record the ion conductance across the membrane. The insulating property of a free-standing lipid bilayer versus the single-channel conductivity provides sufficient sensitivity to detect minor changes in the pathway of ions along the channel. A potential application is to use membrane channels as label-free sensors for molecules, with DNA sequencing as its most prominent application. However, the inherent instability of free-standing bilayers limits broader use as a biosensor. Here we report on a possible stabilization of free-standing lipid bilayers using polydopamine deposition from dopamine-containing solutions in the presence of an oxidant. This stabilization treatment can be initiated after protein reconstitution and is compatible with most reconstitution protocols.



INTRODUCTION

Lipid membranes are fragile thin films that can be ruptured under controlled conditions through application of electric field pulses, causing irreversible pore formation.[1,2] This has been used to study the kinetic of defect formation or membrane stabilization strategies and was summarized in a recent review.[3] For example, polymerization of hydrophobic monomers sandwiched between the aliphatic chains of the lipid bilayer was attempted, but this approach appeared technically very demanding. A gentle and successful stabilization method is surface polymerization of an actin network. A further biomimetic approach was the precipitation and subsequent formation of an S-layer protein network on lipid membranes, leading to brittle membranes. A widely used method is to use the spontaneous self-assembly of triblock copolymers in bilayers, allowing the reconstitution of membrane channels in a functional state.[4,5] An obvious approach to stabilize free-standing lipid membranes is to reduce the area of the free-standing lipid bilayer.[6] Reduction in area is particularly interesting for single-channel characterization, and an easy to use solution is commercially available.[7−9] Smaller area reduces the capacity and thus allows faster recording.[6,9−14] A different approach is to form the lipid bilayer on a water permeable gel.[15−18] An elegant way to combine both has been shown by Ide and co-workers, who combined hydrogels with polypropylene tubes.[15] Here in this work we grow a polymer film on a lipid bilayer containing reconstituted channel-forming proteins. This polymer film allows later further functionalization of the surface. The availability of a simple procedure able to stabilize lipid bilayers would allow their wider application as a membrane channel in biosensing.[19−24]

Recently, polydopamine (PDA) films have been suggested as a platform for the functionalization of a broad spectra of materials,[25] among them biomaterials.[26] The high content of catechol and quinone groups provides multifunctional properties, like allowing the reduction of cations into metallic nanoparticles[25,27] or binding proteins in a covalent manner,[28] and results in their unique mechanical properties.[29] The mechanism leading to such eumelanin-like coatings from 5,6-dihydroxyindole or dopamine is not yet well-known, mostly because of the heterogeneous nature of the obtained material.[30] The most probable structure of PDA is either that of a linear polymer of 5,6-indolequinone[28] or that of a heterogeneous polymer containing small noncovalent aggregates[29] or that of an amorphous self-assembled material made from a heterogeneous mixture of small oligomers of DHI.[30,31] PDA presents some local graphitic domains with an interlayer spacing of 0.34 nm,[32,33] which is a strong argument in favor of the heterogeneous self-assembled aggregate model. In all cases, PDA presents many compositional and structural analogies with eumelanin, the brown-black pigment of the skin and hair. In particular it is strongly adhesive, absorbs light over all the UV−vis spectral range, and is biocompatible,[34] which is of

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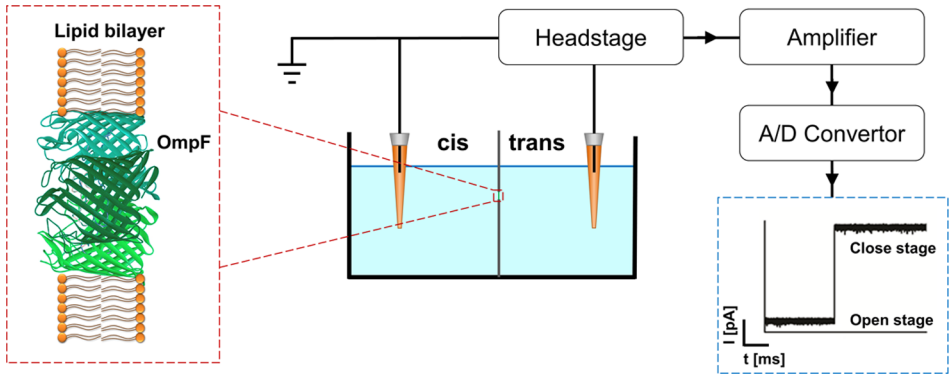

Figure 1. Schematic view of a typical setup of a planar lipid bilayer. Two compartments (cis and trans) are separated by a small aperture that is 50–100 μm in diameter, onto which the bilayer lipid membrane is formed. The compartments are each filled with an electrolytic solution and connected to electrodes via salt bridges. A membrane potential is applied, and the current is amplified using a voltage clamp amplifier and then converted into digital signals by an analog-to-digital convertor.

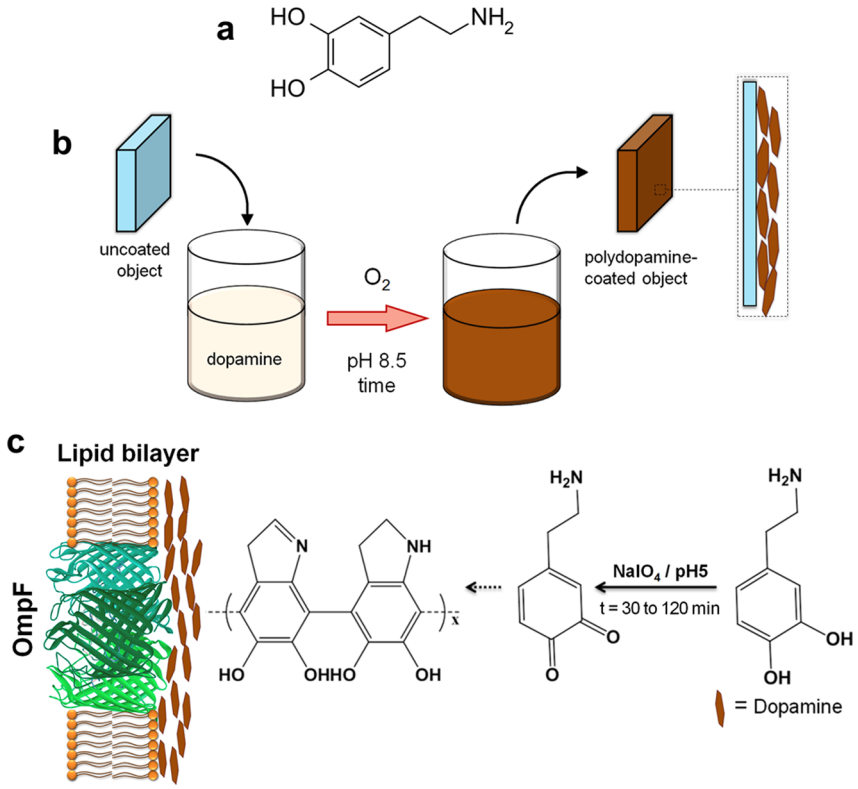

Figure 2. (a) Structure of dopamine, the bioinspired building block for versatile surface coatings. Schematic illustration of thin film deposition of PDA by spontaneous oxidation (b) and with the strong oxidant $NaIO_4$ (c).

upmost importance in our investigation, where PDA has to interact with lipid bilayer and membrane proteins.

The formation of thin PDA films on almost all kinds of materials can be initiated and modulated by changing the pH of the reaction solution[35] and also by playing on the nature of the used oxidant ($NaIO_4$, Cu^{2+}).[36−40] The interaction between lipid bilayers and polydopamine films has been investigated in an indirect manner by fusion of dimyristoylphosphatidylcholine and dioleoylphosphatidylcholine vesicles on polydopamine films.[41] Similarly, aquaporin containing vesicles were fused with polydopamine films with the goal of forming a stable filter for water purification purposes.[42] Here we aim to compare two deposition methods of polydopamine using different oxidants to find out the most efficient method to form an electrical density polydopamine coating able to stabilize the lipid bilayer in the shortest possible amount of time. Indeed the polymerization of monomers in the lipid bilayer and the deposition of actin filaments provides an important membrane stabilization in a time frame of a few minutes.

EXPERIMENTAL SECTION

Materials. We used 1,2-diphytanoyl-*sn*-glycero-3-phosphatidylcholine (DPhPC) lipids from Avanti Polar Lipids (Alabaster, AL), and dopamine hydrochloride (Sigma-Aldrich, ref H8502), potassium chloride, sodium chloride, sodium acetate, sodium periodate ($NaIO_4$), 2-amino-2-(hydroxymethyl)propane-1,3-diol (TRIS), *n*-pentane, hexadecane, and agarose were purchased from Sigma-Aldrich. Doubly distilled and deionized water was used to prepare all solutions. The protein OmpF from the outer membrane of *Escherichia coli* was

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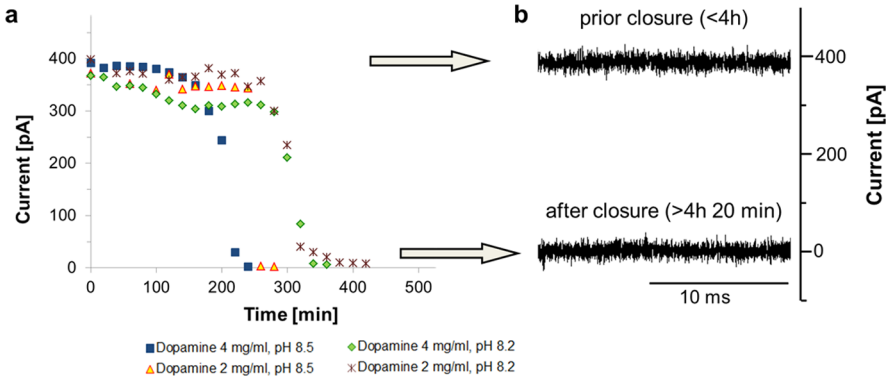

Figure 3. (a) Dependence of ionic current through the single OmpF trimeric channel versus time during the formation of PDA coatings under condition 1 and in the presence of dopamine on the cis side. (b) Closeup of a typical trace in the early stage (<4 h) and after 4 h 20 min of polymerization. The buffer contained 1 M KCl, 10 mM TRIS (pH 8.5 or 8.2) and dopamine at 4 or 2 mg/mL. The applied voltage was 100 mV.

from our in-house stock solution prepared as described previously.[5,43] OmpF is stable over several years at 4 °C if stored at 1 mg/mL in 1% octyl-POE detergent (Alexis, Lauchringen, Switzerland).

Free-Standing Planar Lipid Bilayer Formation. Planar lipid bilayers were formed using DPhPC according to the Montal–Mueller technique.[43,44] As schematically shown in Figure 1 and described in detail,[43] a Teflon partition with an approximately 50–100 μm diameter aperture in the 25 μm thick Teflon film (Goodfellow, Cambridge, UK) was placed between the two homemade chambers of the cuvette each containing 2.5 mL of an aqueous solution. The surrounding of the hole in the Teflon septa was prepainted with 1 μL of a 1% hexane/hexadecane solution and dried for 20 min.[43] Bilayers are formed by spreading 3 μL of a 5 mg/mL stock solution of DPhPC dissolved in *n*-pentane on the buffer surface on each side of the measurement cell (about 1 cm^2 surface on each side). After allowing the solvent to evaporate for about 15 min, the bilayer is formed by lowering and raising the buffer containing a lipid monolayer deposited at the water/air interface. By passing the lipid monolayer twice across the prepainted hole, a bilayer is formed.

The deposition of polydopamine was carried out under two different conditions (see Figure 2): In condition 1, 1 M KCl, 10 mM TRIS buffer at pH 8.2–8.5 serves as an electrolyte using naturally dissolved O_2 as the oxidant. This is the most commonly used synthesis method of PDA; it yields 40 nm thick films after 16–20 h of dopamine oxidation.[26] In condition 2, 100 mM NaCl, 50 mM CH_3COONa at pH 5.0 is the electrolyte, with sodium periodate ($NaIO_4$) as the oxidant. The use of sodium periodate as an oxidant allows one to considerably accelerate the deposition of PDA films. In the case of $NaIO_4$ at 10 mM (dopamine being dissolved at 2 mg/mL, namely 10.6 mM), the PDA film is already 30–35 nm on a silicon substrate after 3 h of oxidation. However, the PDA synthesized in the presence of $NaIO_4$ as the oxidant and at pH 5.0 is much more hydrophilic than the compound synthesized using O_2 as the oxidant at pH 8.5. This originates from the dual role played by $NaIO_4$: it speeds up the oxidation of dopamine but it simultaneously degrades and oxidizes the obtained compound, increasing its content of carboxylic acid groups.[40] It is hence not excluded that even if $NaIO_4$ will speed up the deposition of PDA on lipid bilayers, it will also alter the conformation and functionality of membrane proteins enchased in the lipid bilayer.

To monitor the advancement of the film polymerization, we selected outer membrane protein F (OmpF), a well-characterized channel-forming membrane protein from *E. coli*. In addition, we are interested in using channels as sensors, and OmpF is a good candidate in particular for its thermal and chemical stability. For the latter application we need to characterize its channel behavior under polydopamine film formation. After formation of a lipid film, single OmpF channels are reconstituted into planar lipid bilayers. For single-channel measurements, small amounts of OmpF–porin were added to the cis side of the chamber (Figure 1). Spontaneous channel insertion was usually obtained by stirring under an applied voltage near −150 mV. On the basis of our previous experience, we have a strong indication that the OmpF–porin inserts in an oriented manner with its periplasmic loop first, that is, if the protein is added to the cis side, the periplasmic side is on the trans side and the extracellular side is on the cis side. In order to prevent insertion of more than one porin, the cis side of the chamber was carefully diluted with the same buffer to remove remaining noninserted OmpFs. It is interesting to note that after polydopamine film formation, protein reconstitution failed, even from the opposite site of film formation.

To measure the ion current, standard silver–silver chloride electrodes from WPI (World Precision Instruments, Sarasota, FL) were placed in each chamber via salt bridges containing 1 M KCl and 2% agarose for protection. The entire setup was shielded from external electromagnetic fields, as well as from vibrations, to minimize extra noise from other sources. The homemade half-cells are made of Delrine and are enclosed in an isolated Faraday cage connected with the signal ground and also with a homemade acoustically isolating closet placed on a stone table. All experiments were performed at room temperature (22 °C).

Conductance was recorded using an Axopatch 200B with a capacitive headstage, digitized by an Axon Digidata 1440A digitizer, computer controlled by Clampex 10.0 software (all by Axon Instruments, Foster City, CA) in the voltage clamp mode. Signals were filtered by an on-board low-pass Bessel filter at 10 kHz and recorded onto a computer hard drive with a sampling frequency of 50 kHz. Amplitude, probability, and noise analyses were performed using Origin (Microcal Software Inc.) and Clampfit 10.0 (Axon Instruments) software.

RESULTS

The goal of this investigation is to develop a protocol to stabilize free-standing lipid bilayer using PDA coatings, in the shortest possible amount of time without damaging the lipid bilayer and without altering the functionality of the inserted OmpF. We are aware that PDA will not only deposit on the lipid bilayer but also on the internal lumen of the trimeric OmpF, leading to a probable channel closure after a long reaction time. Although this might hinder particular functionalities, we use this as a quick indication to screen for optimal parameters for functionalization.

In the first series of experiments we tested condition 1. To follow the time course of polymer film formation, spectroscopic methods are less suited due to the strong optical absorption of PDA. Here we follow indirectly the advancement of the polymerization through modification of single-channel conductance. We reconstituted a single trimeric OmpF channel in a black lipid membrane (condition 1) and recorded the conductance. A freshly prepared dopamine solution was then added to the cis side at a final concentration of either 2 or 4

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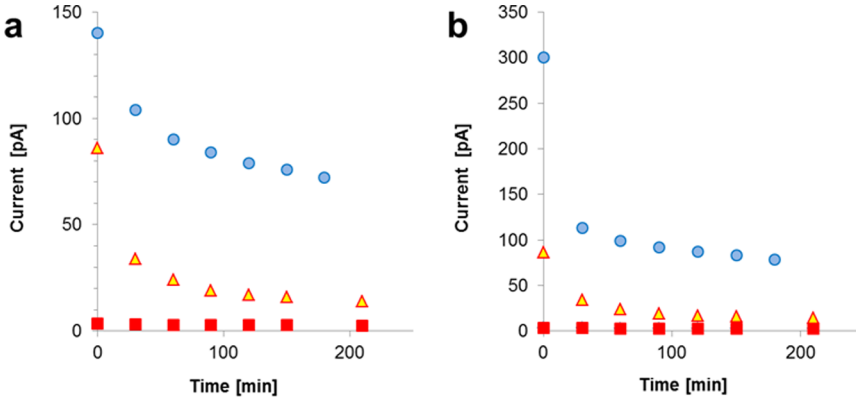

Figure 4. Relaxation of the conductance after an initial high-voltage pulse vs time under a 10 mV transmembrane negative (a) and positive (b) voltage for dopamine film formation under condition 1 (1 M KCl, 10 mM TRIS pH 8.5) (squares and circles) and condition 2 (100 mM NaCl, 50 mM CH_3COONa at pH 5.0, with $NaIO_4$) (triangles). Dopamine was added to the cis side only.

mg/mL. The addition of dopamine initialized the formation of a PDA coating on the cis side. The deposition of a PDA film is followed through single-channel conductance, as shown in Figure 3. Repeated measurement suggested that the PDA deposition in the cis chamber of the cuvette is reproducible. The deposition might start at various places on the membrane and as patches of PDA deposition, as was already demonstrated on silicon.[44] However, in all experiments, a sudden and fast reduction of the transmembrane current was found after about 4−5 h of dopamine oxidation, implying the closure of the OmpF channels. This process is slow and in agreement with related experiments lasting over hours.[15]

To compare the stability of the bilayer−PDA films from experiment to experiment, we apply a transmembrane electric potential to induce membrane rupture. The deposition of a PDA film on a planar lipid bilayer increased the bilayer stability measured by application of stepwise increasing transmembrane potentials, leading to final rupture. Using the complete closure of the OmpF channels as an indicator of sufficiently advanced film deposition, we tested the stability of the composite PDA−lipid film. Typically, we revealed stable membranes resisting even 1 V transmembrane potential, compared to about only 200 mV in the absence of dopamine. Continuous application of transmembrane voltages below 200 mV resulted in electrically dense membranes suitable for channel characterization.

In a second series of experiments (condition 2) we used a strong oxidant, sodium periodate ($NaIO_4$), to increase the rate of polydopamine formation.[39,40] In presence of $NaIO_4$, the color of solution immediately changes from colorless to orange and then to dark brown/black during the first minutes of oxidation. This color change to orange is a signature of the rapid formation of dopaminochrome.[40] After 2−6 h, a black precipitate appears at the bottom of the cuvette. Under these conditions, the stability of the bilayer lipid membrane (BLM) with respect to the transmembrane potential is increased in less than 2 h, compared to 4−6 h under condition 1 with O_2 being the oxidant. Lower concentrations in dopamine require longer reaction times, and below 1 mg/mL, the polymerization time was comparable to those found for condition 1. The simultaneous presence of dopamine in the cis and trans sides allowed one to decrease the time of PDA formation and it increased the membrane stability up to an applied voltage of 1 V. However, using higher field pulses (300−350 mV and above) to test the stability of the film, we observed an irreversible increase in conductance.

To elucidate further this defect formation process, we applied a short high-voltage pulse followed by a lower constant holding voltage. Figure 4 shows, after an initial voltage pulse, the typical ion current relaxation to stable remaining ion current values under positive (Figure 4a) or negative (Figure 4b) 10 mV holding potential. Note that condition 1 contains higher ionic strength (about 1 M KCl), and thus, a similar conductance corresponds to less or smaller defects. In Figure 5 we summarize the final defect conductances under various polymerization conditions. In Figure 5a we show the final ion current under various holding potentials after 2 h of PDA formation under condition 2. Note that positive voltages (positive at the opposite side of dopamine addition) lead to a somewhat higher defect, but overall there was no clear trend for the various conditions. In Figure 5b we show defect formation using condition 1 (1 M KCl, 10 mM TRIS pH 8.5) causing lower defects compared to condition 2 (100 mM NaCl, 50 mM CH_3COONa at pH 5.0, with $NaIO_4$). To summarize, the defect current for the PDA under condition 1 of spontaneous oxidation is substantially less than for the oxidation by the sodium periodate, condition 2.

PDA−BLM films may resist high-voltage pulses and may recover within minutes. However, a larger voltage pulse toward the end of the polymerization induces permanent background conductance under an applied voltage of 300−350 mV. In contrast, such defects did not appear if the voltage was kept below 200 mV throughout the experiment. Previous publications suggested a PDA film structure with local domains containing graphitic fragments with a distance between stacked dopamine/melanin molecules being 3.4 Å on average.[36] It is possible that ions may penetrate across the PDA−BLM system by the free space between the dopamine/melanin molecules and defects in the membrane. This implies that using the fast polymerization approach with $NaIO_4$ reduces the potential application below 300 mV, before the appearance of defects in the membrane. Overall, even if the spontaneous oxidation of dopamine in the presence of dissolved O_2 at pH 8.2−8.5 allows for a slow stabilization of the lipid bilayers (4−6 h), it ensures a higher stability, up to 1 V of applied voltage, than the PDA film deposited in the presence of $NaIO_4$ at pH 5.0.

Detergent Stability. To elucidate the detergent resistance of the membranes, we added the detergent Triton-X100 at 1 μM on one side of the cuvette after dopamine polymerization. If the detergent is added on the opposite side of the PDA, the BLM−PDA ruptured within 3 min. In contrast, when the

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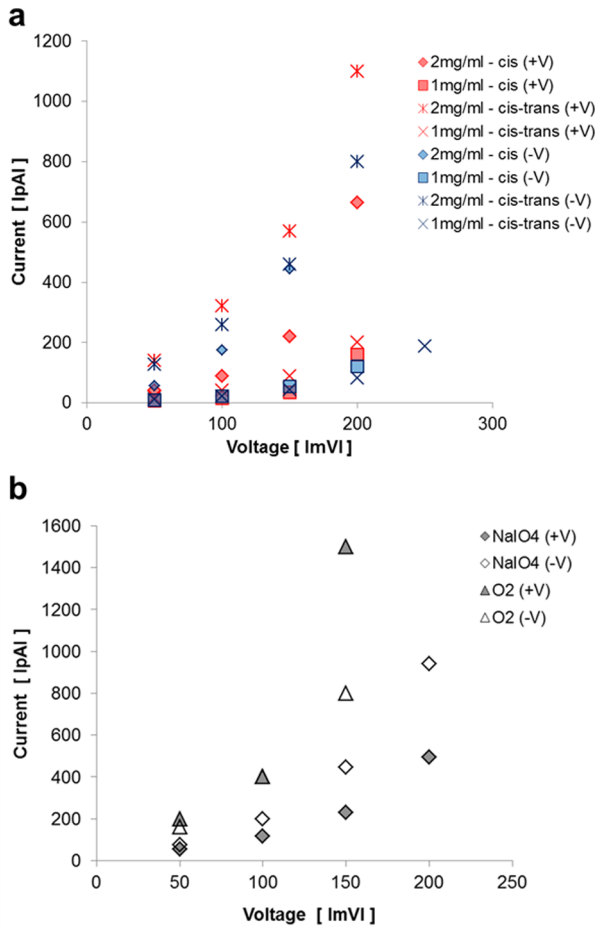

Figure 5. (a) Ion current across PDA–BLM film after 2 h under condition 2. A brief higher transmembrane voltage pulse was applied followed by lower constant voltage as indicated on the *x*-axis. The dopamine was given at two different concentrations (2 or 1 mg/mL) and on the cis side, the trans side, and both sides. (b) Comparing the ion conductance for both oxidation methods: condition 1 (1 M KCl, 10 mM TRIS pH 8.5) and condition 2 (100 mM NaCl, 50 mM CH_3COONa at pH 5.0, with $NaIO_4$).

detergent was added on the same side where the PDA film was formed (in the all compositions), the membrane was stable for more than 30 min. This indicated that the PDA layer has a finite but reduced permeability for Triton-X100.

Effect on Channel Conductance for Future Sensor Applications. In the presence of dopamine, the conductance of OmpF decreased in time, likely due to the formation of the PDA film around the surface of the protein channel. The conductance of the protein channel is reduced to zero with prolonged reaction time. As shown in Figure 3, the current value decreases suddenly after the 4–6 h, within a few minutes. Decreasing the buffer pH value from 8.5 to 8.2 and the dopamine concentration from 4 until 2 mg/mL gives the longer oxidation time until 6 h, but this is not enough for the stable and partial channel blockage. We further investigated the effect of the PDA deposition procedure on the conductance of a single protein channel. For this we recorded the single-channel conductance in the absence of dopamine but in the presence of sodium periodate, as this strong oxidant (E° = +1.55 V vs NHE) could damage the membrane protein. Surprisingly, during the first 10 min under strong oxidative conditions we did not observe any effect of oxidation on the transport properties of the protein. The ionic current traces through the protein channels at an applied voltage of 75 mV without or with $NaIO_4$ in the buffer (100 mM NaCl, 50 mM CH_3COONa at pH 5.0) are presented in Figure 6a, and no significat difference is observed. This observation is supported by a time-resolved analysis using the power density spectra revealing no significant change (Figure 6b). However, in condition 2, the channel starts to close after 30 min and suddenly closed after 60 min in comparison with condition 1.

CONCLUSIONS

We showed the possibility to stabilize planar lipid bilayers by the oxidation of dopamine in solution. The PDA coatings obtained in the presence of $NaIO_4$ as the oxidation agent allows for a faster stabilization than in the case of the spontaneous dopamine oxidation by oxygen in solution/buffer. Substantial stabilization can be achieved in less than 30 min versus 3–6 h.

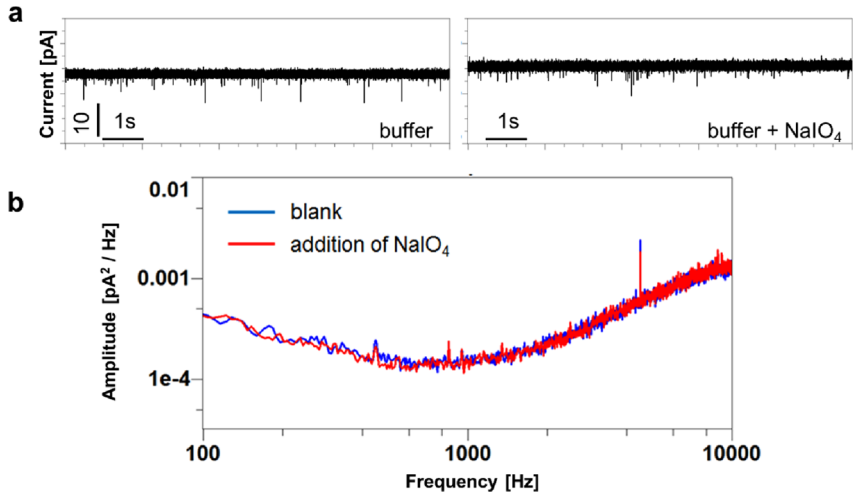

Figure 6. Dopamine polymerization on the bilayer surface as well as protein channels. (a) Snapshots of the ionic current traces show the protein channels without and with the $NaIO_4$ in the buffer 100 mM NaCl, 50 mM CH_3COONa at pH 5. Applied voltage is 50 mV. The current trace is filtered by a low-pass Bessel filter at 10 kHz and recorded onto a computer hard drive with a sampling frequency of 50 kHz. The data are filtered at 2 kHz to obtain the traces. (b) The power spectrum without (blue) and with the $NaIO_4$ (red) in the buffer 100 mM NaCl, 50 mM CH_3COONa at pH 5. Applied voltage is 50 mV.

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Overall, PDA−BLM films may resist high-voltage pulses, but the larger voltage pulse toward the end of the polymerization induces permanent background conductance under an applied voltage of 300−350 mV. In contrast, such defects did not appear if the voltage was kept below 200 mV throughout the experiment.

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Notes

The authors declare no competing financial interest.

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