Rapid screening of membrane protein activity: electrophysiological analysis of OmpF reconstituted in proteoliposomes

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Solvent-free planar lipid bilayers were formed in an automatic manner by bursting of giant unilamellar vesicles (GUVs) after gentle suction application through micron-sized apertures in a borosilicate glass substrate. Incubation of GUVs with the purified ion channel protein of interest yielded proteoliposomes. These proteoliposomes allow for immediate recording of channel activity after GUV sealing. This approach reduces the time-consuming, laborious and sometimes difficult protein reconstitution processes normally performed after bilayer formation. Bilayer recordings are attractive for investigations of membrane proteins not accessible to patch clamp analysis, like *e.g.* proteins from organelles. In the presented work, we show the example of the outer membrane protein OmpF from *Escherichia coli.* We reconstituted OmpF in proteoliposomes and observed the characteristic trimeric conductance levels and the typical gating induced by pH and transmembrane voltage. Moreover, OmpF is the main entrance for beta-lactam antibiotics and we investigated translocation processes of antibiotics and modulation of OmpF by spermine. We suggest that the rapid formation of porin containing lipid bilayers is of potential for the efficient electrophysiological characterization of the OmpF protein, for studying membrane permeation processes and for the rapid screening of antibiotics.

Introduction

Patch clamping is the state of the art for high fidelity ion channel analysis under voltage control and high temporal resolution. Although mostly used for cell recording, the patch clamp technique may also be used to characterize the conductance of artificial lipid bilayers, (also called black lipid membrane or BLM).[1] Lipid bilayers allow the reconstitution of ion channels and recordings under non-physiological conditions as well as the analysis of otherwise inaccessible ion channels, *e.g.* from intracellular membranes.

Recently, new approaches have been developed, using microstructured planar chip devices for patch clamp recordings instead of the common glass-pipette. With these patch clamp chips recordings from whole cells[2–5] and single channels in cell-attached mode[6] have been published. Moreover, artificial lipid membranes using painted or folded bilayers[2,7,8] as well as bilayers produced from fusion of giant unilamellar vesicles (GUVs) on apertures in a chip[9,10] have been described. For a detailed discussion we refer to a historical perspective of the development of the planar patch clamp technique.[11]

The development of chip-based patch clamping has enormously facilitated cell-based ion channel screening[12] by the possibility for automation and parallelization. In contrast the advantage has not yet been fully explored for membrane protein recordings from lipid bilayers, like ion channels, porins and transporters from organelles or bacteria.

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Many techniques have been developed to investigate the pore properties and to study the material flux through channels. Available techniques allow for the determination of ion channel properties such as selectivity, voltage dependence (voltage gating), conductance and permeation of, for example, antibiotics. However, these techniques have a very low throughput with regards to the number of compounds that can be screened, and are not suitable for automation, which would be required for efficient screening in the drug discovery process.

Different approaches have been pursued to facilitate the process of bilayer formation with different techniques and using different substrates such as Teflon, silicon or glass.[7–10,13,14] Clearly, an automated procedure for the formation of stable planar lipid bilayers with the option of fast solution exchange would greatly increase the efficiency of the characterization of reconstituted proteins.

Here, we study as an example of a porin, the outer membrane protein F (OmpF) from *Escherichia coli.* OmpF is a non-specific transport channel present in the outer membrane of *E. coli.* While the outer membrane protects and separates the cell from the environment, OmpF is used for passive translocation of vital molecules and ions between the cell and the environment and allows the diffusion of molecules with molecular weights up to 600–700 Da.

The OmpF protein is constituted by three monomers forming a trimer. Each monomer consists of 16 β-strands anti-parallel that span the outer membrane and form a barrel with short turns at the periplasmic side and large loops at the outside of the cell.[15]

The porins have been shown to play a major role in the bacterial resistance against antibiotics.

Several studies have described permeation processes in porins by antibiotics, such as ampicillin and amoxicillin.[16–18,21] The protein considered as the major porin in the antibiotics pathway has been identified as the outer membrane protein F, OmpF.

We present a novel approach to reconstitute proteins into GUVs to form proteoliposomes, which are fused onto the aperture of a microstructured chip to form a planar lipid bilayer containing the protein of interest. The fast and reliable formation of long-lasting solvent free bilayers allows for the rapid and efficient analysis of ion channel proteins in lipid bilayers and specifically antibiotic translocation processes under controlled electrophysiological conditions.

Materials and methods

Chemicals. In all experiments 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) from Avanti polar lipids, Inc. (Alabaster, AL), was used. Cholesterol, Sorbitol, KCl, polyamine spermine and β-lactam antibiotic (Ampicillin) were obtained from Sigma (Sigma, Steinheim, Germany). Chloroform, HEPES and KOH was obtained from Roth (Carl Roth GmbH, Karlsruhe, Germany). Bio-Beads were obtained from Bio-Rad (Bio-Rad Laboratories GmbH, Munich, Germany). Purified OmpF was a kind gift from Dr Helge Weingart (Jacobs University Bremen, Germany).

Preparation of liposomes. Planar lipid bilayers were obtained from GUVs prepared by using the electroformation method[19] in an indium tin oxide (ITO) coated glass chamber connected to the Nanion Vesicle Prep Pro setup (Nanion Technologies GmbH, Munich, Germany). The ITO-layers on the two glass slides are electrically conductive, and therefore serve as electrodes. As the ITO-layer is transparent, the whole process of forming and growing the vesicles could be visually monitored under phase contrast microscopy. Lipid-containing solution, 5 or 10 mM of DPhPC with 10% cholesterol, dissolved in chloroform, was deposited on the ITO-coated glass surface. After total evaporation of the solvent the lipids are assembled in a perfectly dehydrated lamellate phase. An O-ring was placed around the dried lipid film and 300 µl of a non-ionic intracellular solution, sorbitol with a concentration equal to 210 mM or 1 M, was carefully added to the lipid film. Then, the second ITO-slide was placed on the top of the ring, with the ITO-layer facing downwards. The process of electroformation was controlled by the Vesicle Prep Pro setup and all parameters (amplitude, frequency, duration, *etc.*) for the electroformation were programmed in the *VesicleControl* software (Nanion Technologies GmbH, Munich, Germany). The amplitude and the frequency could also be controlled manually. Generally, an alternating voltage of 3 V peak to peak was applied with a progressive increase for the rise time and a decrease for the fall time to avoid abrupt changes, which otherwise might rupture the formed GUVs. The frequency of the alternating current was 5 Hz and was applied to the ITO-slides over a period of 2 h at room temperature. After successful swelling, the vesicles were used directly for the reconstitution of the proteins.

Reconstitution of OmpF in giant unilamellar vesicles. In contrast to conventional bilayer recordings, where reconstitution of proteins is achieved by adding the protein after bilayer formation in the presence of detergent, we insert the porin into the GUVs directly after the electroformation. Purified OmpF (0.3 mg ml^{-1}) in solution containing the detergent octyl-polyoxyethylene (octyl-POE) (3%) and 50 mM NaCl was reconstituted into GUVs by mixing the solution containing GUVs (300 µl) and OmpF with a final concentration of protein between 0.8–1.7 µg ml^{-1} and a final concentration of detergent between 0.01% to 0.02% in the GUVs solution. The mixture of GUVs and protein was incubated for 1 h at room temperature, followed by the addition of Bio-Beads® SM-2 (Bio-Rad) at 40 mg ml^{-1} in GUVs solution. The mixture was incubated with the Bio-Beads for 1 h at room temperature and then overnight at 4 °C to remove the detergent. Bio-Beads were discarded after centrifugation and the protein containing GUVs could be used immediately. When kept at 4 °C, storage of the proteoliposomes was possible for weeks in a standard Eppendorf cup in 1 M sorbitol.

Planar lipid bilayers formation. For formation of a planar lipid bilayer containing the OmpF, 1 to 3 µl of the proteoliposomes solution was pipetted onto the patch clamp chip The microstructured chip, which is commonly used in patch clamp experiments with cells, contains an aperture approximately 1 micron in diameter. The GUVs were positioned onto the aperture in the chip by application of a slight negative pressure. Typically, (−)10 to (−)40 mbars were sufficient for reliable positioning within a few seconds after GUV addition. When the GUVs touch the glass surface of the chip, they burst and form planar bilayers[12] with a seal resistance of tens to hundreds of GΩ (Table 1). A schematic of the bilayer formation process and an image of the mounted glass chip is shown in Fig. 1. When proteoliposomes containing OmpF were used, we also obtained high resistance seals, but the apparent seal resistances could be as low as 300 MΩ. The lowered apparent seal resistance was due to reconstituted proteins conducting already at bilayer formation. In these cases, the seal resistance was well within the high GΩ range, once the OmpF switched to the fully closed state.

Data recording and analysis. Patch clamp experiments were performed with the Port-a-Patch automated patch clamp system (Nanion Technologies GmbH, Munich, Germany), using borosilicate glass chips with an aperture diameter of approximately 1 µm. Based on the aperture diameter of the chip and a specific capacitance of DPhPC of 0.5 µF cm^{-2},[12,20] the membrane capacitance could be estimated to be in the order of a few fF. Experiments were done in symmetric solutions of 1 M KCl, 10 mM HEPES at pH 5.4 and pH 7. Application of all

Table 1 Distribution of seal resistances of planar lipid bilayers formed with DPhPC in 10% cholesterol GUVs ($n = 161$)

Seal resistance	No seal	<1 GΩ	1–10 GΩ	10–100 GΩ	100–500 GΩ
GUVs (DPhPC 10 mM)	5.6%	2.5%	34.8%	44.7%	12.4%

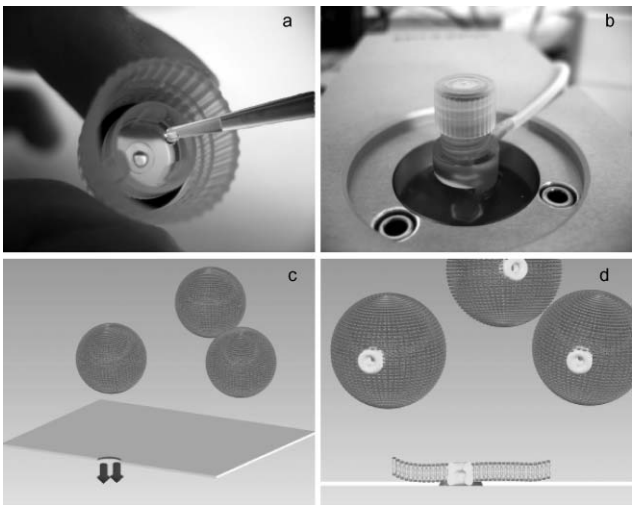

Fig. 1 Images of the microstructured glass chip being backfilled with solution (a) and on the holder for carrying out the recording (b). The chip is glued onto a twist cap, which can be simply screwed onto the holder (b), which contains the reference electrode. After mounting the chip, a droplet of recording solution is added to the top (glued glass chip), a Faraday ring placed on top and the ground electrode inserted into the solution droplet. 5 µl of a sterile filtered solution on each side is adequate to wet the glass chip and liposomes or compounds can be applied directly from the accessible top side of the chip. This design allows for fast exchange of chips after each experiment and enables experiments with low volume consumption. In (c) and (d), the formation of a planar lipid bilayer by vesicle fusion onto a microperforated glass chip is schematically shown. Initially, a suspension of GUVs is applied onto the chip (c) and a slight negative pressure is used to position a single vesicle on the aperture in the chip. When the vesicle touches the glass surface, it bursts open and forms a planar lipid bilayer on the substrate, which is suspended across the hole (d). This procedure works very efficiently and reliably, taking only a few seconds to form a gigaseal after application of suction. When proteoliposomes are used, a planar lipid bilayer is immediately obtained, with the reconstituted protein present so that the patch clamp recording can start right away.

solutions could be achieved manually with standard pipette or by perfusing the chip with a laminar flow chamber. Due to the small volumes required, a fast perfusion could be achieved.

Currents were amplified with an EPC-10 patch clamp amplifier (HEKA Electronics, Germany) and recorded to the computer hard disk using the Patchmaster software (HEKA Electronics, Germany). The data were filtered at 3 kHz or 10 kHz (Bessel filter, HEKA amplifier) digitized at a sampling rate of 50 kHz and analyzed with Clampfit (Axon instruments, part of Molecular Devices, USA). The bilayer formation process was computer controlled by the PatchControl software (Nanion).

Results

The stability of membranes is essential for the study of reconstituted proteins in bilayers and for automated screening purposes. The planar lipid bilayer formation by vesicle fusion onto a microperforated glass chip was easily obtained when using pure lipid GUVs without proteins. 92% ($n = 161$) of the experiments had seal resistances of at least 1 GΩ (Table 1).

The success rate to obtain a seal in the case of the proteoliposomes was 78% ($n = 152$). The decrease of the success rate with the proteoliposomes as compared with pure lipid vesicles was due to the residual presence of detergent remaining from the process of proteoliposome formation. A very small amount of detergent can destabilize the bilayers but the original concentration of detergent was indispensable to avoid the aggregation or denaturation of the proteins. Even after using Bio-Beads several times to remove the detergent, there was still a small amount of detergent present in the solution. The control was done with solution containing only detergent micelles without protein and showed a similar decrease of the success rate in gigaseal formation as compared to pure GUV derived lipid bilayers.

Once the seal is obtained, we perfused the buffer solution to remove the remaining vesicles and to stabilize the membrane by avoiding evaporation. The recording of OmpF in the lipid bilayer was stable for long periods allowing a thorough investigation of the properties and pharmacological aspects of the porin. In 60% of the experiments functional OmpF (1.6 ± 1.5 protein insertion per seal) was successfully inserted.

As shown in Fig. 2(a), we have analyzed the properties of a single OmpF channel in 1 M KCl, 10 mM HEPES at pH 5.4. For all recordings, the maximal filtering frequencies and sampling rates were used to achieve high temporal resolution. For example we used a filtering frequency of 10 kHz and sampling rate of 20 µs (50 kHz). The noise level for the current recordings was 2 pA rms using the settings described above.

Fig. 2(a) shows a single OmpF channel recorded at a membrane holding potential of 50 mV. Histogram analysis of the current traces was made to determine the conductance levels corresponding to those of the complete trimeric protein (Fig. 2(b)). In addition, the single channel currents were measured at different transmembrane potentials in order to construct an I/V-curve. The average single channel conductance was calculated from the slope of the fitted line and was determined as 4.06 ± 0.04 nS (Fig. 2(c)). This conductance value reported in 1 M KCl is in agreement with the value observed from others studies using the same purification protocol.[19] The trimeric conductance allowed us to deduce a single monomer conductance of ~1.35 nS. At high voltage, the porin exhibits the characteristic conductance levels of the monomers by gating. A typical trimeric gating is shown in Fig. 2(d).

It is interesting to note that as reported in ref. 22 there exist subconductance states which are much smaller than the monomer conductance. These subconductance states have been reported in experiments using conventional patch clamp and also reported in Nano-BLMs experiments,[44] but have never been shown previously in traditional BLM recordings. In our experiments, the occurrence of the subconductance states increases with higher voltage (Fig. 3) and with the presence of compounds like polyamines.

In another series of experiments we investigated the effect of polyamines on OmpF. In several studies the effect on reconstituted OmpF was investigated using BLM electrophysiological recordings or by patch clamp recordings with OmpF reconstituted into liposomes.[21] It has been shown that polyamines inhibit processes like chemotaxis and flux of β-lactam through the outer membrane[24] by interaction with residues of the L3 loop and the barrel of OmpF.[25] It has been speculated for several years, that bacteria could use polyamines, such as cadaverine, to prevent

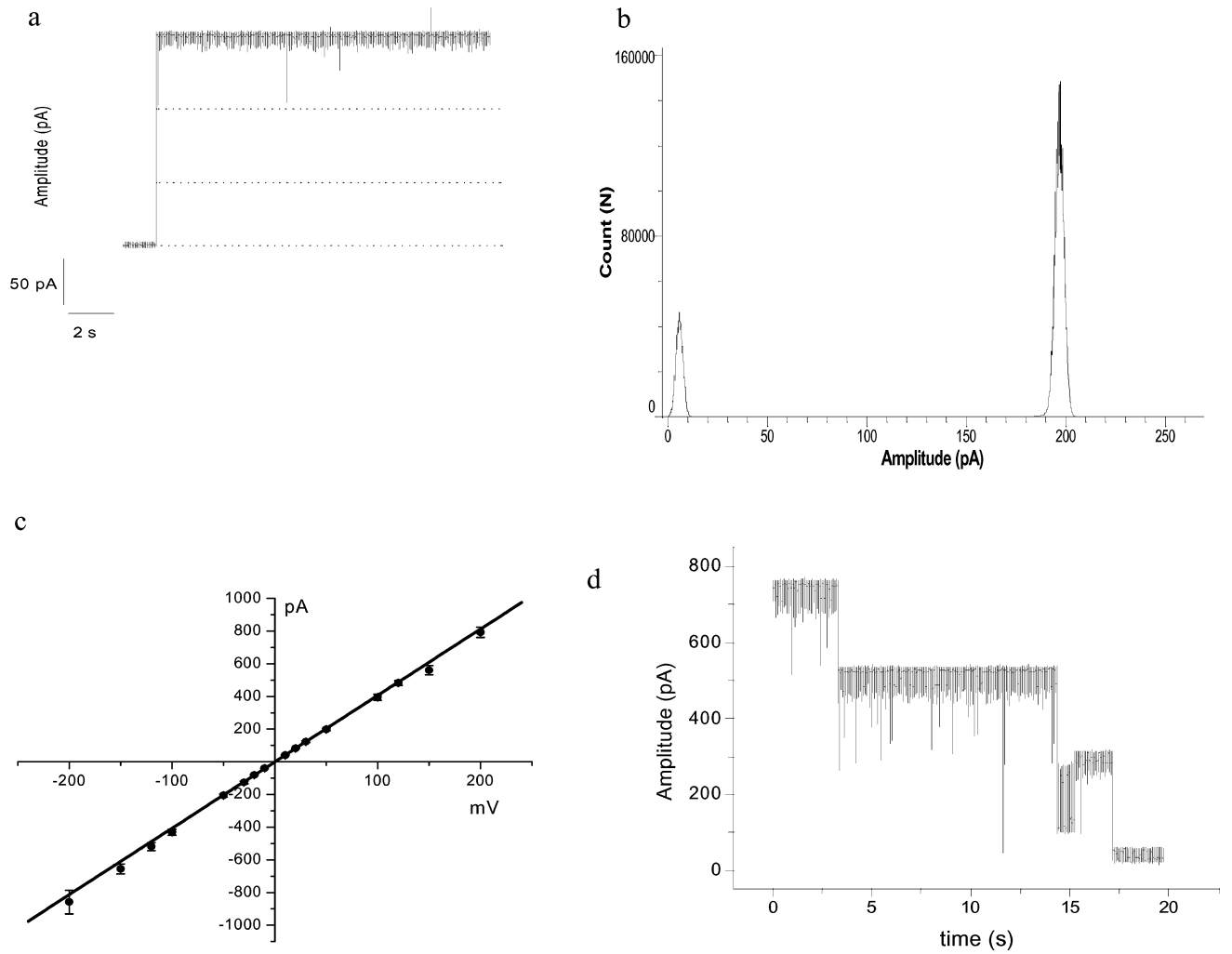

Fig. 2 Measurements of OmpF conductance in 1 M KCl, and 10 mM HEPES, pH = 5.4. (a) Representative current traces of the OmpF channel in 1 M KCl at a transmembrane potential of +50 mV after fully closed state. (b) Current–amplitude frequency histogram constructed from 30 s of single-channel data shown above. The main open channel conductance level is 4.01 nS. (c) I–V relation of single OmpF channels. Single-channel conductance was calculated from the slope of the fitted line and was determined as 4.06 ± 0.04 nS. (d) Typical recordings of ionic currents through a single trimeric OmpF channel at 200 mV. Application of higher voltages closes the channel in three steps revealing its trimeric organization and the monomer conductance.

the effect of antibiotics. Cadaverine is produced by bacteria and the production is regulated by external factors such as pH,[42] presumably to escape the toxic effects of antibiotics, such as β-lactams. Indeed, the viability of bacteria increase in the presence of polyamines when antibiotics are applied.[34]

One example is spermine, which is a polyamine that blocks the OmpF porin at submicromolar concentrations.[23] We used spermine to determine the blocking of OmpF with the chip-based approach mentioned above. Fig. 4 shows representative traces of porin activity with and without application of different concentrations of spermine. It is apparent that the application of spermine changes the kinetics of the opening and closing events as reported previously.[22,23] Knowing the monomeric conductance under the experimental conditions applied, we can interpret the increased activity as increased monomeric gating of OmpF (confirmed by current–amplitude frequency histogram of the trace presented in Fig. 4c) and also increased gating of the subconductance states.

Furthermore, we investigate the effect of antibiotics, in particular ampicillin. It is well known that antibiotics influence the channel activity of porins. Ampicillin is an antibiotic which modifies the gating of OmpF by transiently blocking the ion flow. The antibiotic permeates through the OmpF channel, thereby temporarily reducing the conductance of the porin. This translocation process is similar to a Coulter Counter event, *e.g.* a resistive sensing of the passage of a non-charged particle through a pore filled with conducting solution. Typical recordings of ion currents through OmpF trimers are shown in Fig. 5 in the presence (Fig. 5b) and in the absence (Fig. 5a) of ampicillin. The recording without ampicillin shows a stable current without gating. Addition on one side of the bilayer of ampicillin (5 mM) to the bath solution induces spontaneous blocking by the translocation of the antibiotic across the pore. The events are well characterized as one monomer in the channel trimer being temporarily blocked by the passage of an ampicillin molecule.

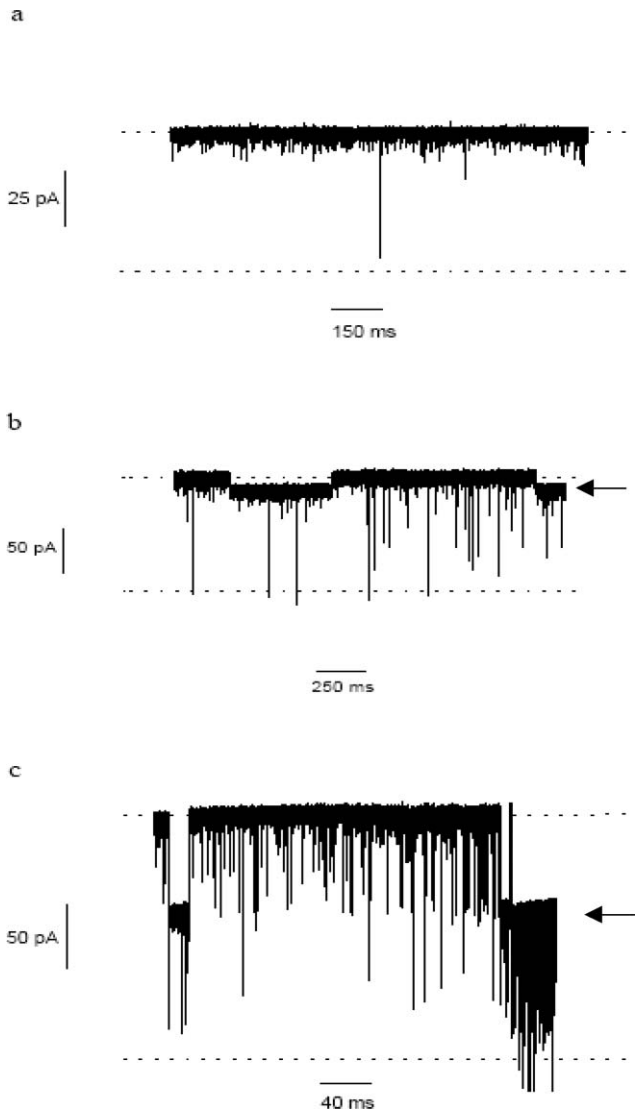

Fig. 3 Subconductance levels (arrows) at different voltages recorded with planar patch clamp technique. The current step corresponding to the monomeric conductance is indicated by the dotted line. The subconductance level activity increases with the applied transmembrane voltage. (a) 50 mV, (b) 100 mV, (c) 150 mV.

We also determined the drug-induced fluctuation by dwell time analysis for spermine and ampicillin (Fig. 6). The single exponential fitting of the blockage time histogram showed the time constants of compound binding or translocation process, which were $\tau = 14.53 \pm 0.28$ ms for spermine at 1 M and $\tau = 0.262 \pm 0.05$ ms for ampicillin at 2.5 mM. The characteristic residence time for ampicillin was also determined by power spectrum analysis, where $\tau = 0.224 \pm 0.012$ ms which compares well with the dwell time analysis. The residence time for an ampicillin molecule in the pore shows that the permeation process is fast compared to spermine binding. Furthermore, statistical analysis was carried out by the power-spectrum method (Fig. 7). Using Lorentzian fitting of the power spectral densities of current fluctuation recordings at +50 mV at four different concentrations of ampicillin were done to determine the residence time, τ, which shows that the residence time of the antibiotic is independent of the antibiotic concentration.

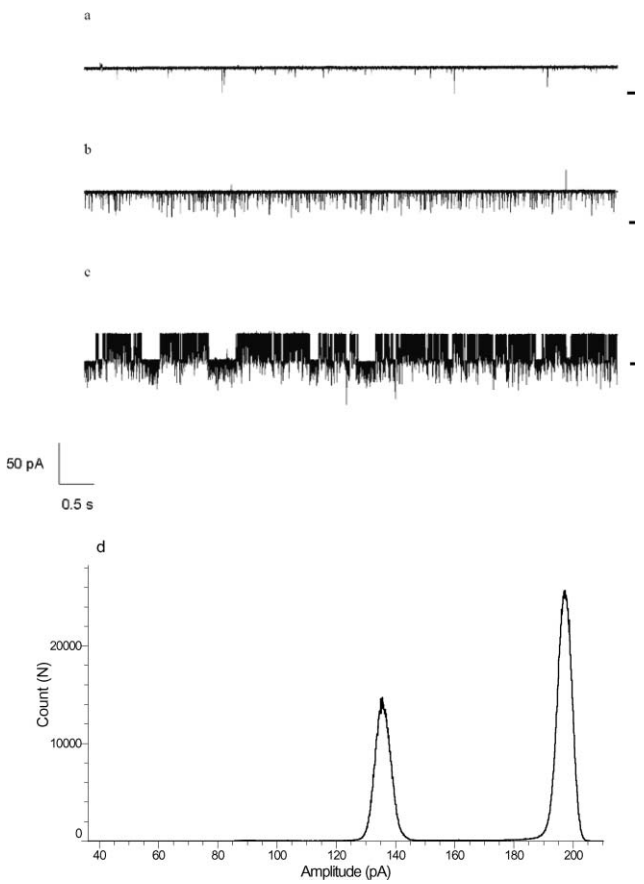

Fig. 4 Representative current traces of the modulation of OmpF channels by application of spermine. (a) Current of a single OmpF in buffer 1 M KCl, 5 mM HEPES, pH 5.4. The bar line indicates the closed monomer state. The activity of the same channel was recorded in 0.1 mM (b) and at 1 mM spermine (c) applied in the bath solution. The downward spikes represent the closure events of the effect of spermine. In (d), the current–amplitude frequency histogram of the trace presented in part (c) is shown to confirm the increased gating activity.

Discussion

We have shown that the reconstitution of OmpF into solvent free giant unilamellar vesicles and subsequent electrophysiological investigations are possible on microstructured glass substrates or planar patch clamp chips. OmpF activity and modulation could be measured in solvent free lipid bilayers in a semi automated and miniaturized setup. The OmpF porin that was used here as an example of protein reconstitution in solvent free lipid bilayers plays an important role in drug transport over the outer cell wall of gram-negative bacteria. More complex membrane proteins, especially ion channels or transporters with mainly α-helical structure, may be more fragile and also more difficult to reconstitute. While the actual measurements would be the same when working with other proteins, the reconstitution protocols would likely require modification. The detergent generally plays an important role in the reconstitution process. As a porin, OmpF remains stable and functional even at low concentrations of detergent, whereas some other proteins aggregate rapidly if the concentration of detergent decreases below the critical micelle concentration, thus making the reconstitution into GUVs much more difficult.

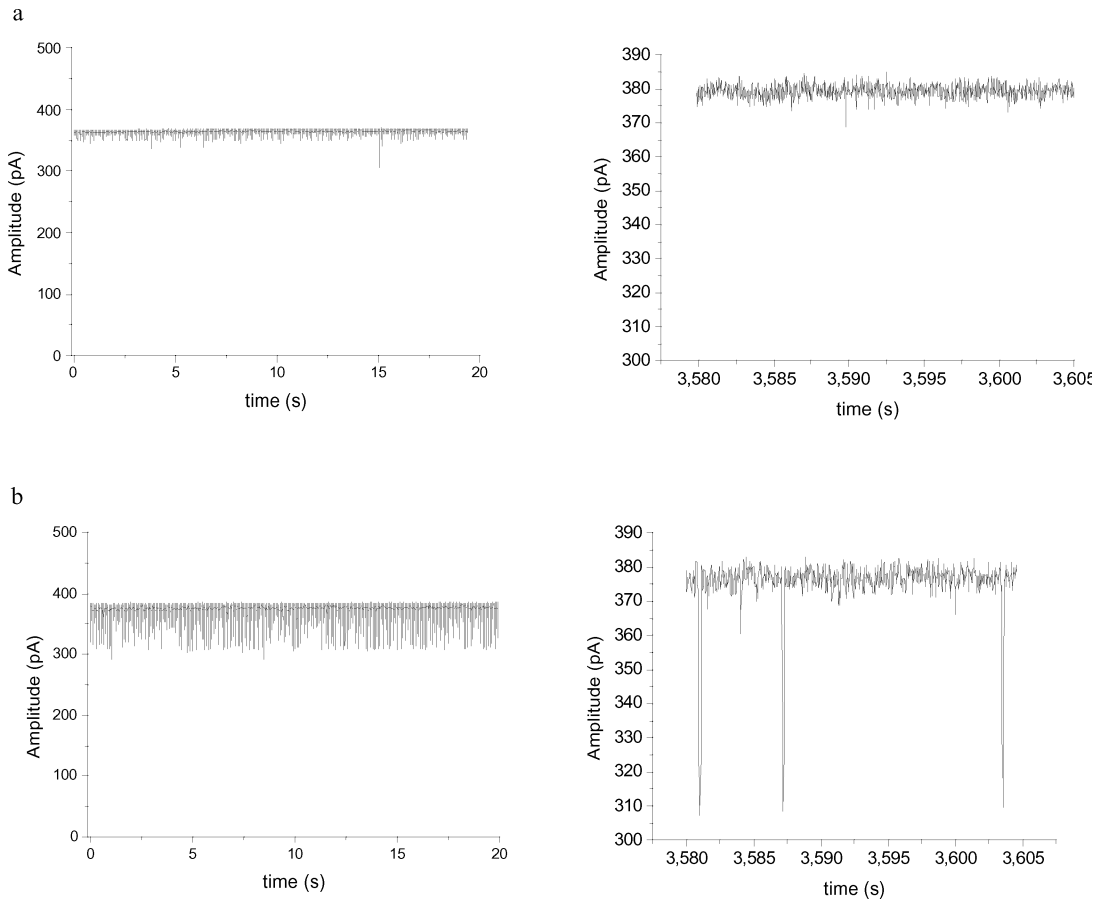

Fig. 5 Permeating ampicillin molecules modulate the ionic current through OmpF channel reconstituted in the planar lipid bilayer. (a) In the absence of antibiotics the ionic current is very stable; (b) in the presence of 5 mM ampicillin one of the three OmpF pores spontaneously is blocked due to the translocation process of the antibiotic. At higher time resolution (0.025 s) the blocking events of ampicillin can be seen as well defined, transient monomer conductance step flickering.

The characteristics of OmpF such as voltage sensitivity, gating kinetics, conductance *etc.* were compared to previously measured data with the conventional BLM and patch clamp experiments.[21,26,30]

Here, we compared state of the art knowledge on OmpF and antibiotic translocation from the literature with the results obtained by the presented approach of automated planar lipid bilayer formation by proteoliposome fusion on an aperture. First of all, we reconstituted the OmpF into the solvent-free lipid bilayers of giant unilamellar vesicles. The reconstitution into GUVs was done in the presence of detergent which has been a source of instability for the bilayers, introducing fluctuations and hence inconsistency in the recordings. Therefore, we developed a protocol for the reconstitution of the protein followed by the removal of detergent by application of Bio-Beads.[27,28,29] With proteoliposomes prepared in this manner, we successfully obtained long-term stable bilayer recordings. It was possible to apply different solutions to the bilayer-containing chip and to repeatedly perfuse it without breaking the giga-sealed membrane.

The recordings performed with the planar lipid bilayers containing OmpF agreed nicely with others using BLM electrophysiological recordings. The gating behaviour at higher voltage allowed us to determine the monomer conductance of OmpF.

In this article, we also showed the existence of substates in OmpF gating which has been mentioned in other publications.[22,44] These subconductance states seem to be more apparent in patch clamp experiments[31] rather than in classical BLM experiments, but also exist in BLMs under certain conditions.[22] The increased subconductance gating could be due to an overall higher rigidity of the lipid membrane on the glass chips as compared to classical BLMs. BLMs prepared by the painting method always have a lipid and solvent containing torus on the edge of the aperture. This torus and the fact the solvent is present, make the lipid membrane quite flexible and hence adaptive to any potential pressure gradients across the bilayer. Also the size of the BLM itself with a typical diameter of a few hundred microns makes the bilayer adaptive to mechanical stress. In contrast, the planar lipid bilayers on the chips as well as in the case of patch clamp pipettes are very small and do not have a lipid torus. Hence, they are more rigid and any mechanical stress posed on the bilayer can act more directly on any reconstituted protein, which could be the reason for the increased subconductance activity seen here. This explanation would also be in accordance with the subconductance activity described in ref. 44 where nano-BLMs were employed.

The subconductance states activity could be increased by the addition of polyamines.[23,32] Spermine is not endogenous

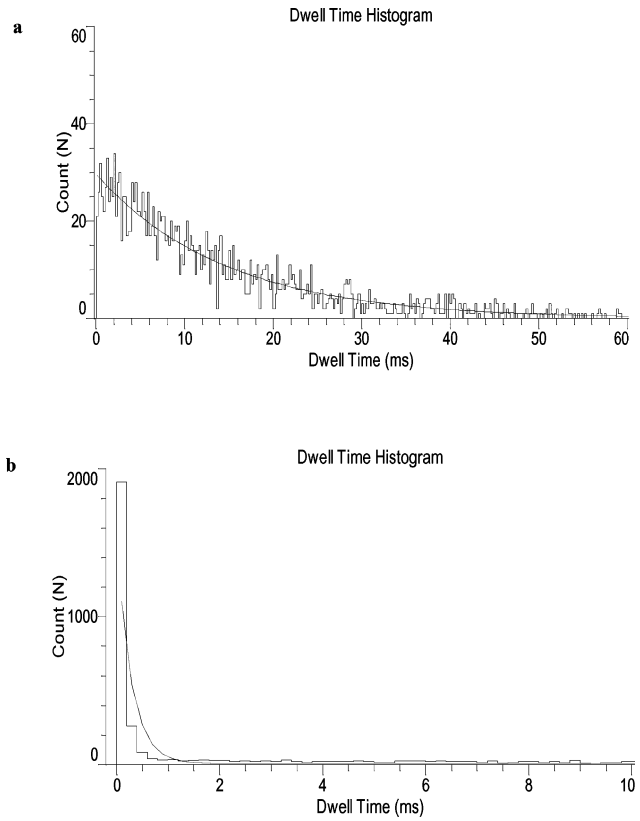

Fig. 6 Kinetics of interaction of ampicillin and spermine with OmpF at 50 mV. Dwell time histograms of 1 mM spermine–OmpF (a) and 2.5 mM ampicillin–OmpF (b) were fitted by an exponential with characteristic time of 14.53 ± 0.28 ms for spermine and 0.262 ± 0.05 ms for ampicillin.

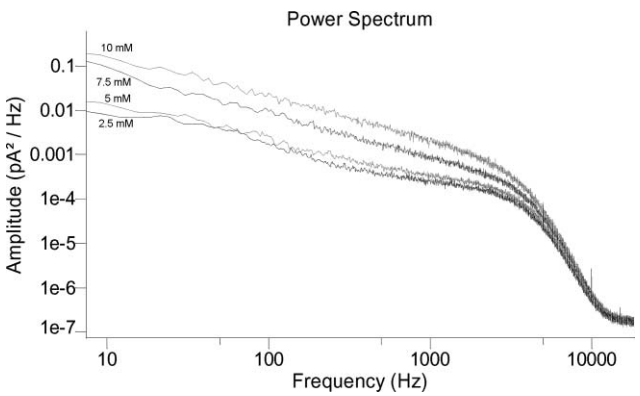

Fig. 7 Power spectral densities of current fluctuations at four different ampicillin concentrations at + 50 mV applied voltage, 1 M KCl, pH = 5.4. Each spectrum was analyzed by Lorentzian fitting with characteristic time of $\tau = 0.224 \pm 0.012$ ms.

to *Escherichia coli*[33] and is an effective compound eliciting inhibition of channels even at submicromolar concentrations. It has been shown that spermine enters and blocks *E. coli* OmpF channels.[41] Our results with spermine showed the blocking of the OmpF and suggest that antibiotics flux through the porin is indeed inhibited by polyamines as the studies of Iyer and Delcour[23] and Pagès *et al.*[34] suggest.

The diffusion of β-lactam antibiotics through the porin channels were shown by several studies.[36–40] It has previously been shown that the interaction between the permeating ampicillin and the channel pore is seen as short blocks of ion flux through the porins,[16,21] well resolved in high bandwidth electrophysiological analysis. We recorded the effects of ampicillin on the OmpF channel and obtained blocking events which were in accordance with the studies of Bezrukov *et al.*[16,21] and further computational studies.[35]

Furthermore, we analyzed the blocking events using dwell time analysis and compared the results obtained for ampicillin and spermine. The kinetics of events show the fast interaction of ampicillin with OmpF porin ($\tau = 0.262 \pm 0.05$ ms for 2.5 mM ampicillin) which suggests the translocation of ampicillin through OmpF.[21] The results obtained with spermine show longer blocking events, which correspond well with binding of spermine to the porin for inhibition of the conductance in accordance with the literature.[23,25,34,41]

The formation of planar lipid bilayers by the presented approach is very reliable and efficient. After the addition of GUVs, a bilayer forms within seconds. Once a lipid vesicle is positioned on the micro-aperture by suction and a giga-seal forms, the applied suction is automatically released to minimize stress on the bilayer. Bilayers formed in this manner offer low noise recordings, are stable even at high transmembrane voltages of a few hundred mV and enable long term recordings lasting several hours.

It is especially attractive to pre-incubate the GUVs with purified protein, so that direct preparation of lipid bilayers with functionally reconstituted ion channels is possible in a straight forward, basically automated manner. This eliminates the commonly critical and time-consuming step of reconstituting channel proteins in a classical black lipid membrane experiment.[43] To achieve reconstitution of *e.g.* a single ion channel rather than an ensemble of channels, as for example required in stochastic sensing experiments, one commonly applies protein to a BLM at low concentration to avoid multiple insertions and has often waiting times of tens of minutes before introduction into the bilayer occurs and the recording can start. Additionally, this needs to be repeated for every single bilayer experiment. With the GUV derived proteoliposomes on the other hand, a large batch of proteoliposomes can be prepared with an appropriate concentration of protein to obtain a suitable channel density in the vesicles, *e.g.* a single channel per vesicle. As only a few microliters of proteoliposome suspension are needed for the bilayer formation, one can use the proteoliposomes with predefined protein density for an extensive set (~100) of experiments in time periods stretching over weeks. Again it is important to note that reconstitution of different ion channels will probably require adjustments in the protocols and could be more demanding for the more complex ion channel proteins as compared to OmpF.

As the whole procedure of mounting a chip, pipetting the GUV containing solution and forming a gigaseal with a (proteo)-liposome takes only about a minute, this approach offers great advantages as compared to classical BLM type experiments with regards to efficiency and data quality. In conclusion, the work presented here greatly facilitates lipid bilayer recordings and makes them suitable for use in routine screening efforts. By offering a reliable, efficient and automated formation of ion channel containing planar lipid bilayers, the means for rapid electrophysiological analysis of ion channels, especially for those not easily accessible by the classical patch

clamp technique, is provided. The approach is naturally of great advantage for investigations on bacterial resistance and for screening of antibiotics activity, which has been validated by the analysis of translocation processes and the competitive action of antibiotics and polyamines on OmpF.

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