Application Note

Channel: Ompf
Cells: GUVs
Tools: Port-a-Patch

Lipid Bilayer recordings of OmpF reconstituted in Proteoliposomes

The electrophysiology team at Nanion Technologies GmbH, Munich. OmpF was kindly provided by Helge Weingart, Group of Prof Winterhalter, Jacobs University Bremen.

Summary

Solvent-free planar lipid bilayers were formed in an automatic manner by bursting of giant unilamellar vesicles (GUVs) positioned by suction on the apertures of our patch clamp chips made from borosilicate glass substrate. Incubation of GUVs with purifi ed 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 diffi cult protein reconstitution processes normally performed after bilayer formation.

The mixture of GUVs and protein was incubated for 1 hour at room temperature, followed by the addition of Bio-Beads® SM-2 (Bio-Rad) at 40 mg/ml in GUVs solution. The mixture was incubated with the Bio-Beads for 1 hour at room temperature and then over-night 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.

Results

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 µm in diameter. The GUVs were positioned onto the aperture in the chip by application of a slight negative pressure. Typically, -10 to -40 mbar were suffi cient 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 with a seal resistance of tens to hundreds of GΩ (Table 1). A schematic of the bilayer formation process is shown in Figure 1.

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.

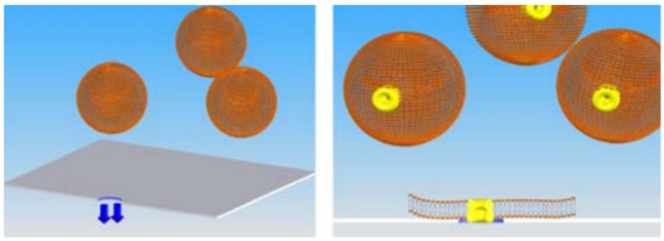

Figure 1: *The formation of a planar lipid bilayer by vesicle fusion onto a microperforated glass chip is schematically shown.*

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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 %

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

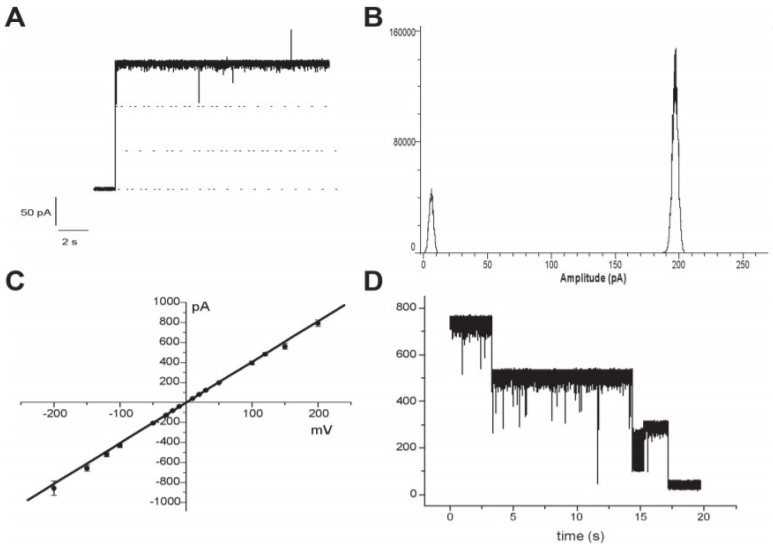

Figure 2: *(a) Single channel opening of OmpF channel in 1 M KCl at a transmembrane potential of +50 mV. (b) Current–amplitude frequency histogram constructed from 30 sec of single-channel data shown above. The main open channel conductance level is 4,01 nS. (c) I–V relation of single OmpF channels. (d) Typical recordings of ionic currents through a single trimeric OmpF channel at 200 mV.*

In conclusion, we have demonstrated that the Port-a-Patch platform enables automated formation of planar lipid bilayers from lipid vesicles. The use of proteoliposomes for bilayer formation allows for direct recordings from the protein of inerest without need for further reconstitution steps.

References

1. M. Kreier, C. Farre, M. Beckler, M.George and N. Fertig. 2008 Rapid Screening of Membrane Protein Activity: Electrophysiological Analysis of OmpF Reconstituted in Proteoliposomes. Lab Chip, 2008, DOI: 10.1039/b713982a.

Methods

GUVs

Planar lipid bilayers were obtained from Giant Unilamellar Vesicles (GUVs) prepared by using the electroformation method in an indium tin oxide (ITO) coated glass chamber connected to the Nanion Vescile Prep Pro setup.

Proteoliposomes Preparation

Purified OmpF (0,3 mg/ml) 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 and a final concentration of detergent between 0.01% to 0.02% in the GUVs solution.

Electrophysiology

Patch clamp experiments were performed with the Port-a-Patch, using borosilicate glass chips with an aperture diameter of approximately 1 µm. Experiments were done in symmetric solutions of 1 M KCl, 10 mM HEPES at pH 5,4 and pH 7.

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