Application Note

Channel: KcsA
Cells: GUVs
Tools: Port-a-Patch®
Vesicle Prep Pro®

Lipid bilayer recordings of KcsA channels reconstituted in proteliposomes

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The KcsA protein samples were kindly provided by Prof. O. Pongs, Hamburg.

Summary

Solvent-free planar lipid bilayers were formed in an automated manner using suction to attract a giant unilamellar vesicle (GUV) to the patch clamp chip which subsequently bursts across the aperture. Incubation of GUVs with purified KcsA channel protein yielded proteoliposomes. These proteoliposomes allow for immediate recording of channel activity after GUV sealing. The rapid formation of protein-containing planar lipid bilayers is of potential use for the efficient electrophysiological characterization of KcsA as shown here (Fig. 2) and also other ion channel proteins of interest.

Results

For formation of a planar lipid bilayer containing the KcsA protein, 1 to 3 µl of the proteoliposome 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. 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. The GUVs were positioned onto the aperture of the chip by application of a little negative pressure. Typically, (-)10 to (-)40 mbar were sufficient for reliable positioning of a vesicle within a few seconds after GUV addition. When the GUVs touch the glass surface of the chip, they burst and form planar bilayers (1) with a seal resistance of tens to hundreds of GΩ (Table 1). A schematic of the KcsA protein and the bilayer formation process is shown in Figure 1.

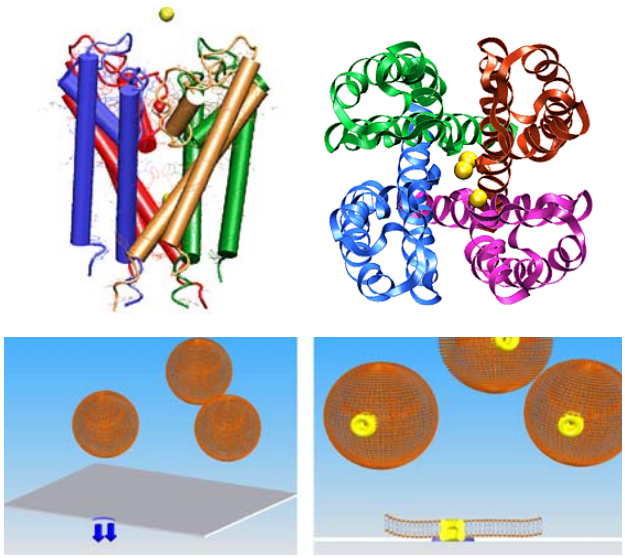

Figure 1:
Top: Schematic of the KcsA potassium channel, which has 4 identical subunits and each subunit contains two alpha-helices connected by an approximately 30 amino acids long loop proof-reading into the pore region. Bottom: The formation of a planar lipid bilayer by vesicle fusion onto a microperforated 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 planar lipid bilayers formed with DPhPC in 10 % cholesterol GUVs. (n=161)

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 protein into the GUVs directly after the electroformation step. When kept at 4°C, storage of the proteoliposomes was possible for weeks in a standard Eppendorf tube in 1 M sorbitol.

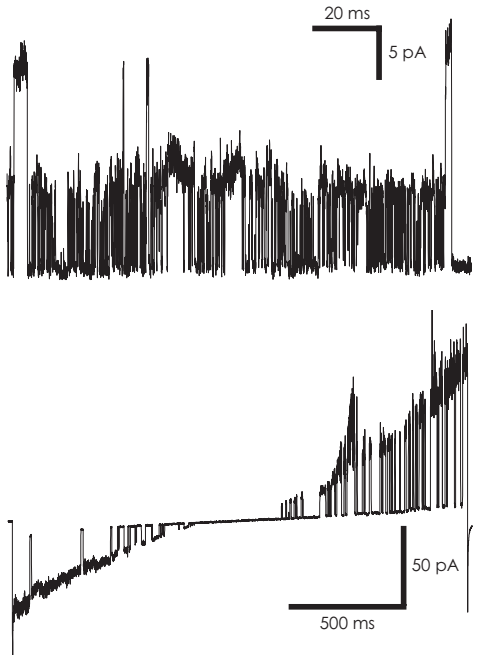

Figure 2:
Measurements of KcsA conductance in 100 mM KCl. Top: Representative current trace of the KcsA channel at a transmembrane potential of +150 mV. Bottom: Ramp from -200 mV to 200 mV.

References

1. M. Kreir, 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 Vesicle Prep Pro setup.

Proteoliposome preparation

KcsA solubilized in 400 nM Imidazol, 200 mM $NaPO_4$, 150 mM KCl, pH 7.8 was added to the solution containing GUVs at concentration of 1-1.5 mg/ml in 100 mM sorbitol. 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 overnight at 4°C to remove the detergent. Bio-Beads were discarded after centrifugation and the protein containing GUVs could be used immediately.

Electrophysiology

Patch clamp experiments were performed with the Port-a-Patch®, 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, the membrane capacitance could be estimated to be in the order of a few fF. Experiments were done in internal solution: 100 mM KCl, 10 mM HEPES pH 7 and external solution: 100 mM KCl, 10 mM MES pH 4. 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). The bilayer formation process was computer controlled by the PatchControl software (Nanion).

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