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 MscL 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 MscL as shown here and also other ion channel proteins of interest.

In order to study the effect of pressure, the functional MscL purified was reconstituted in our system. The reconstitution was done in GUVs and then bilayers were formed on a chip (Kreir, Farre et al. 2008). The Port-a-Patch system has a pump controlled by a computer and could apply from +300 to -300 mBar and is controlled via software allowing accurate pressure control. All pressure applications could be visualized and recorded at the same time as the recordings.

Introduction

Mechanosensitive (MS) ion channels were first described in gram positive bacteria using either giant protoplasts of bacteria and patch clamping, or the fusion of membrane with liposomes. The MS channels have then been found in organisms of different phylogenetic origin including mammalians, plants, fungi, bacteria (Gram-positive and -negative) and recently archaeabacteria (Martinac et al., 1992) (Pivetti, Yen et al. 2003).
It forms a homopentameric channel and it is proposed that the carboxy-terminal helix form a bundle when the channel is closed. The phenomenon leading to the open state after mechanical stimuli (pressure) is the conformational change involving the amino-terminal helix. Despite the structural knowledge, the gating mechanisms via forces applied to membrane lipid bilayers are still poorly understood. Electrophysiology recordings shows that MscL, despite its large conductance and its non selective pore, have different subconductance states, as well and the studies of Sukharev (1999) which proposed a model of gating (Sukharev, Sigurdson et al. 1999). Recently, the structure of MscL in an expanded intermediate state has been resolved by X-ray diffraction (3.82 Å) (Liu, Gandhi et al. 2009) in comparison to the previous crystal structure in the closed state.

Results

For formation of a planar lipid bilayer, 1 to 3 µl of the proteoliposomes solution is 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 are positioned onto the aperture in the chip by application of a slight negative pressure. Typically, (~)10 to (~)40 mbars are 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 with a seal resistance of tens to hundreds of GΩ. Current in response of pressure and voltage is shown in Figure 1. The current was elicited using a continuous voltage of 30 mV and a suction of -25 mBar.
Summary
In summary, we used the Port-a-Patch and Vesicle Prep Pro to study MscL channel and successfully recorded and produced high quality single-multi channel data. The patch clamp method offers the possibility to investigate ion channels and their effectors, in real time, and with unparalleled sensitivity compared to other methods.

References

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
MscL solubilized in detergent was added to the solution containing GUVs in 1 M 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 2 hours at room temperature to remove the detergent. BioBeads were discarded after centrifugation and the protein containing GUVs could be used immediately.

Electrophysiology
Patch clamp experiments were performed with the Porta-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/cm², the membrane capacitance could be estimated to be in the order of a few fF. Experiments were done in 200 mM KCl, 40 mM MgCl2, 5 mM Hepes, pH 7.2/ KOH. 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).