Application Note

Channel:	MscL
Type:	GUVs
Tools:	Port-a-Patch®
	Vesicle Prep Pro®

Lipid bilayer recordings of a mechanosensitive channel, MscL, using Nanion's pressure clamp

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The MscL protein samples were kindly provided by Dr. Chris Gandhi, CalTech, California

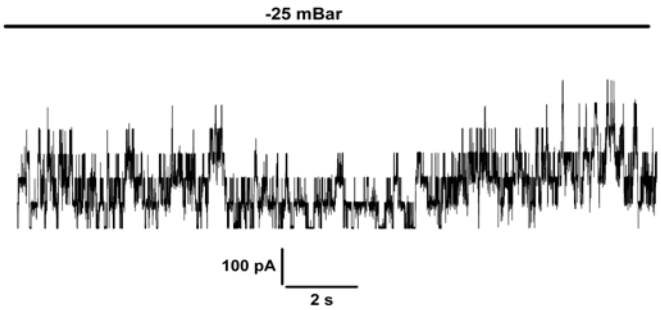
Summary

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 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 archaebacteria (Martinac et al., 1992) (Pivetti, Yen et al. 2003). In response to mechanical stimuli, the MS ion channels open and convert the mechanical signal into electrical and/or biochemical signals. This suggests their role in physiological functions in different types of cells such as osmoregulation in bacteria, turgor control in plant cells as well as mechanosensation like touch, hearing, proprioception and cell division in eukaryotic cells. The MS channels have been extensively studied in bacteria (gram negative and positive) where three types of MS channels were found: MscL (large conductance), MscS (small conductance, known as being 50 % of the conductance of MscL), and MscM (mini conductance). For example, MscL, from E. coli, is a large non selective channel with a conductance of 2.5 to 3 nS (Sukharev 1999). The structure of MscL has been resolved to high resolution. The alpha helices span the membrane twice and contain an inter-TMS-loop that connects the two transmembrane domains. The channel has also an amino-terminal alpha helix and a short carboxy-terminal helix. MscL from Mycobacterium tuberculosis has been resolved to 3.5 Å resolution giving the residues information of MscL (Chang, Spencer et al. 1998).



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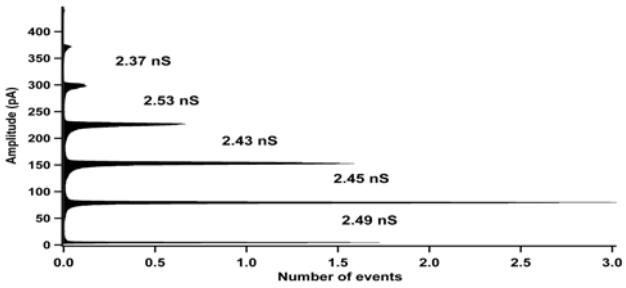

Fig. 1. MscL EC trace. MscL EC gating recorded at 30mV and the indicated negative hydrostatic pressure, in symmetrical recording solution (200mM KCl, 40mM MgCl2, 5mM Hepes, pH 7.2/KOH). The histogram shows the conductances of each opening level (100 s recording). The conductance of a single channel is ~2.45 nS.

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.

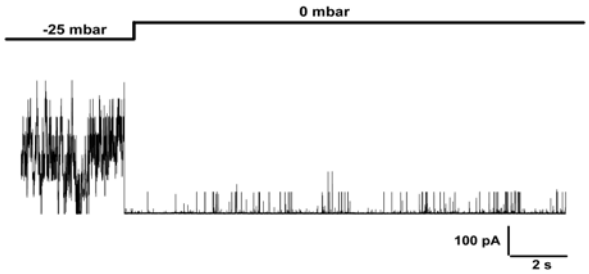

Fig. 2. MscL EC gating recorded at 30mV and the indicated negative hydrostatic pressure.

When the suction was turn off, from -25 to 0 mBar the current was reduced, indicated that the channel is only responding to the suction. The voltage was applied. Some channels were still activated at 0 mBar, with a low open probability. When the suction was on again, the current was increasing right away (figure 3).

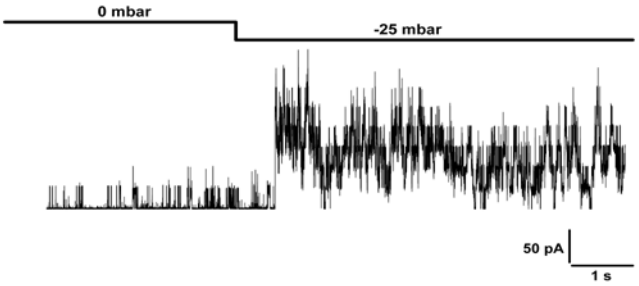

Fig. 3. MscL EC gating recorded at 30mV and the indicated negative hydrostatic pressure.

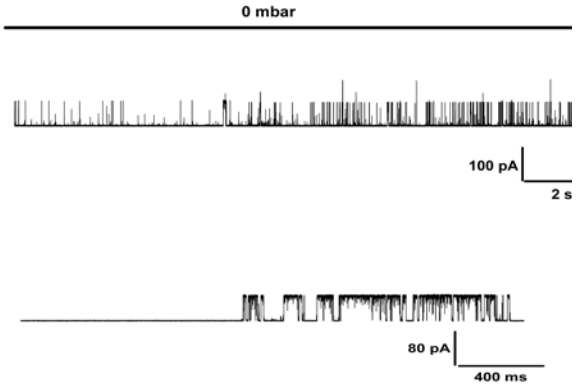

Fig. 4. MscL EC gating recorded at 30mV and the indicated negative hydrostatic pressure

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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

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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. 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/cm2, the membrane capacitance could be estimated to be in the order of a few fF. Experiments were done in 200 mM KCl, 40mM MgCl2, 5mM 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).

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