2397-Pos Board B416

The Selectivity of MscS is Determined by the Cytoplasmic Domain

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The mechanosensitive channel of small conductance (MscS) is a heptameric pressure sensitive channel expressed in the inner membrane of *E. coli*. This channel possesses three transmembrane helices and a large water-filled cytoplasmic cage which comprises more than 50% of the total protein. This cytoplasmic domain is conserved throughout the MscS channel family and in MscS has been shown to be a dynamic structure with an active role in channel gating. It has also been suggested that the cytoplasmic domain determines the weak anion selectivity exhibited by MscS. In order to address this question this study reconstituted wild type MscS and single residue mutants of the cytoplasmic vestibule in azolectin liposomes (protein:lipid 1:10000). Patch clamp recordings of these channels were then performed in the presence of asymmetric solutions of KCl (600/200 mM) and $BaCl_2$ (50/200 mM). The MscS mutants studied were R184E, R185E, E187R and E227A. Both E187R and E227A mutants show reduced selectivity characterised by a lower anion-cation permeability ratio. These residues are likely to determine selectivity by binding cations. From these data it is clear that charged residues in the cytoplasmic domain of MscS determine its selectivity. This is interesting because unlike K^+, Na^+ and Ca^{2+} channels the selectivity of MscS is not determined by residues in the pore region but residues situated in the large water-filled cytoplasmic domain.

2398-Pos Board B417

The Right-Side-Out Orientation of MscS in Liposomal Membranes

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The bacterial mechanosensitive channel MscS plays a crucial role in the protection of bacterial cells against hypo-osmotic shock. MscS functional characteristics have extensively been studied in both giant spheroplasts and liposomes. Despite many studies of MscS reconstituted into liposomes the channel orientation in liposomal membranes is still unknown. We examined the orientation of MscS in liposomes by patch-clamp and confocal microscopy. using its previously determined electrophysiological and pharmacological properties we were able to determine that in liposomes MscS retains the right-side-out orientation as in giant spheroplasts based on the following evidence: (i) I-V curves recorded in both spheroplast and liposome preparations exhibited strong outward rectification at both negative and positive pipette pressures. (ii) MscS activation ratio in liposome patches at positive relative to negative pipette voltages and *vice versa* showed positive correlation at both positive and negative pipette pressures similar to MscS in inside-out excised spheroplast patches. (iii) MscS exhibited a voltage-dependent hysteresis upon application of sawtooth pressure ramps in both spheroplasts and liposomes. In both spheroplasts and liposomes the hysteresis was more pronounced upon positive pipette voltages compared to negative voltages. (iv) 2.5% of 2,2,2-trifluoroethanol (TFE) caused MscS inactivation in liposome patches when added to the cytoplasmic side of MscS, whereas addition of TFE to the periplasmic side did not inactivate the channel, although it caused a shift of the channel activation towards lower pipette pressures. We obtained a similar result when applying TFE to MscS in spheroplast patches. In conclusion, our findings strongly indicate that the cytoplasmic domain of MscS in liposome membrane patches faces the bath solution as in spheroplast patches. Consequently, upon liposome reconstitution MscS channels preserve their right-side-out orientation comparable to what was previously reported for the MscL channels.

Supported by the NH & MRC.

2399-Pos Board B418

Modulation of the G22E MscL Mutant Channel Gating by Lipid Bilayer Constituents and Gadolinium

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Bacteria respond to hypoosmotic changes through the mechanosensitive (MS) channels of Large (MscL) and Small (MscS) conductance, MscS responds first to pressure, i.e. bilayer tension changes, followed by MscL[1]. The lipid environment, lyso lipids and cholesterol have been shown to significantly influence the ratio of the opening of MscL to MscS[2,3]. Furthermore, introduction of the highly negatively charged cardiolipin to both azolectin and POPE/POPC lipid membranes causes rapid gating of MscS[4]. Here we report an expanded study using the spontaneously active G22E MscL mutant which, although spontaneously active, is still mechanosensitive. Addition of sub-millimolar amounts of the metal ion Gadolinium(III) reversibly inhibits spontaneous channel activity, but upon application of pressure, the channel exhibits mechanosensitivity similar to the wild-type MscL. Our results are consistent with the previous studies showing that Gd(III) inhibits MscL mechanosensitivity by binding to the lipid bilayer[5,6]

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Supported by the National Health & Medical Research Council of Australia.

2400-Pos Board B419

A Novel Approach to follow Helical Movements of an Ion Channel in Real-Time

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Mechanosensitive channel of large conductance (MscL) is one of the best-studied mechanosensitive channels in bacteria (Sukharev et al., 1994, Blount et al., 2007). It acts as a safety valve in response to hyperosmotic shock. High-resolution structure of Mycobacterium tuberculosis MscL revealed that it forms a homopentamer with two transmembrane helices per subunit. Although in nature the channel opens in response to tension, breaking the hydrophobic interactions at its pore region leads to the spontaneous opening of the channel. Here, by using this principle, we modified the hydrophobic gate of the channel with designed chemical switches and we gained external control over its activation. We followed the resulting structural changes on the protein by following the Electron Paramagnetic Resonance signal from a spin label on different positions at the pore forming helices. We developed a method in which we could control the number of switches and EPR spin labels per pentamer. By this approach, we start following the gradual activation of the channel in real time.

2401-Pos Board B420

MscL as a Triggered Nanovalve: New Modifications to Improve Design

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MscL is a small homopentameric bacterial protein that has, among other characteristics, an incredible pore size greater than 30Å and the ability to gate in response to mechanical tension in the membrane. Because of its amenability, *E.coli* MscL has been the most studied mechanosensitive channel, serving as a paradigm of how a protein can sense and transduce mechanical force. Early on in the study of the channel a critical domain for MscL gating was revealed by forward genetic experiments screening for mutations that led to a gain-of-function (slowed- or no-growth) phenotypes: mutations at residue G22, within the pore, led to severe gain-of-function phenotypes. This residue is thought to form part of a ''hydrophobic lock'' that stabilizes the closed state of the channel. If this hydrophobic lock is disrupted by the insertion of a charge, the transition energy barrier for MscL gating is destabilized and the channel gates even in the absence of membrane tension. using this observation, researchers have successfully changed the modality of MscL to be sensitive to stimuli such as light and pH simply by chemically modifying the G22 site within the MscL channel. Due to its ability to be triggered by different stimuli and the large pore size, MscL has been proposed as a triggered nanovalve for its use in nanodevices such as a liposome drug delivery system. Here, by utilizing *in vivo,* flux and patch clamp assays, we characterize other neighboring residue that also form part of the hydrophobic lock and we show that the G22 site may not be the best choice for all modifications that change channel modality.

2402-Pos Board B421

Mechanosensor and Gate is Tightly Coupled in the Bacterial Mechanosensitive Channel MscL

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