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based on a combination of liposome extrusion techniques and continuous sucrose gradient centrifugation. We also demonstrate that MscL reconstituted into these liposomes may be used as a nanovalve for controlled release of small molecules including the self-quenching fluorescent dye 5,6-carboxyfluorescein (CF). CF release is regulated by the MscL-activating amphipath L-α-Lysophosphatidylcholine and exhibits a dependence on liposome size, amphipath concentration and protein-to-lipid ratio (see Figure).
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1513-Pos Board B423

Hydrogen Bond Formation Between the Gate and Water Molecules Accelerates Channel Opening of the Bacterial Mechanosensitive Channel Mscl

Yasuyuki Sawada, Masahiro Sokabe.

The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer (TM1, TM2) α-helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the membrane. To address this question, molecular dynamics (MD) studies have been performed, however, as they do not include MscL-lipid interactions, it remains unclear which amino acids sense membrane tension and how the sensed force induces channel opening. Thus we performed MD simulations for the opening of MscL embedded in the lipid bilayer. Among amino acids in TM2 facing the bilayer, Phe78 showed exceptionally strong interaction with lipids. Upon membrane stretch, Phe78 was dragged by lipids, leading to an opening of MscL. Thus Phe78 was concluded to be the major tension sensor. Neighboring TM1 inner helices are tilted and crossed each other near the cytoplasmic side, forming the most constricted hydrophobic part of the pore called gate. Upon membrane stretch, the helices are dragged by lipids at Phe78 and tilted, accompanied by the outward sliding of the crossings. This led to a slight expansion of the gate associated with an exposure of oxygen atoms of the backbone to the inner surface of the gate. This allows water penetration in the gate and formation of hydrogen bonds between water and the exposed oxygen, which in turn weakened the hydrophobic interaction at the crossings, causing a further opening of the gate and water permeation. To support this idea, we performed MD simulations of the GOF mutant G22N with almost the same pore size, and found that G22N mutant MscL permeated water molecules earlier than WT during opening.

1514-Pos Board B424

Lipid and Lyso-Lipid Effects on the Mechanosensitivity of Liposome Co-Reconstituted Mscs and Mscl

Takeshi Nomura, Andrew R. Battle, Boris Martinac.

Bacterial _m_echano_s_ensitive _c_hannels of _s_mall (MscS) and _l_arge (MscL) conductance have been proposed to play a major role in the protection of bacterial cells against hypo-osmotic shock. Although the genes of both channels have been cloned and X-ray crystallographic analysis has revealed their 3D structure, much less is known about how lipids surrounding the channels in the bacterial cell membrane may influence mechanosensitivity of both channels. Here, we utilize co-reconstitution system to examine the effects of different types of phospholipids, acyl chain length and lyso-lipids on mechanosensitivity of MscS and MscL by the patch-clamp technique. Co-reconstitution into liposomes of different lipid composition such as phosphatidylethanolamine (PE18), phosphatidylcholine (PC18), phosphatidylglycerol (PG), and cardiolipin (CL) did not affect the pressure-threshold activation ratio of the channels. However, reconstitution into liposomes made of shorter phospholipids, i.e. phosphatidylethanolamine and phosphatidylcholne having 16 instead of 18 hydrocarbons (PE16:PC16), dramatically decreased the threshold activation ratio. Addition of 5-30% cholesterol, which is known to affect the bilayer thickness, led to a decrease of the threshold activation ratio. In contrast, application of micromolar concentrations of lysophosphatidylcholine (LPC), which has been known as a mechanosensitive channel activator, led to an increase of the threshold activation ratio. These findings suggest that the length of acyl chain and cholesterol induced difference in membrane thickness (hydrophobic mismatch) on one side, and the change in intrinsic lipid bilayer curvature induced by LPC on the other, affect mechanosensitivity of both channels to a different extent and by a different mechanism.
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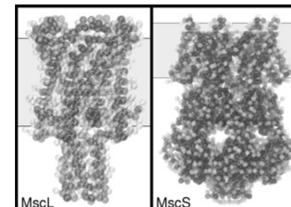
1515-Pos Board B425

Sensitivity of Coarse-Grained Mechano-Sensation

Martti Louhivuori, Siewert Jan Marrink.

Mechano-sensitive channels are ubiquitous membrane proteins that activate in response to increasing tension in the lipid membrane. They facilitate a sudden, non-selective release of solutes and water that safe-guards the integrity of the cell in hypo- or hyper-osmotic shock conditions. Mechano-sensitive channels react to a sudden increase in membrane tension by forming trans-membrane pores that counteract the pressure gradient build-up by balancing the osmotic conditions on either side of the cell membrane. They have a crucial role in diverse biological functions from sensory feedback to the prevention of cell death by membrane rupture.
A varity of different mechano-sensitive channels exist with names such as MscL, MscS, and MscM describing their experimentally observed gating properties. Recent crystal structures of MscL (2OAR) and MscS (2OAU, 2VV5) have opened the way for computer simulation studies of the function, dynamics, and molecular mechanisms of these exciting channels. The coarse-grained MARTINI force-field, which has been build to reproduce accurately macroscopic and thermodynamic properties of biological systems, is ideally suited for the task. We have looked at the sensitivity of the coarse-grained channels to internal and external changes and their impact on the channel function.



1516-Pos Board B426

Outward Currents Through "low" Conductance Non-Mechanosensitive Channels in *Escherichia Coli* Membranes

Charles D. Cox, Anthony K. Campbell, Kenneth T. Wann.

Mechanosensitive high conductance channels in bacteria have received considerable attention (e.g.1,2) and have a presumed role against osmotic stress. In contrast non-mechanosensitive channels are less well described. In patch-clamp on-spheroplast recordings from *E. coli* (BW25113) with high K^+ (200 mM) in both the pipette and bath solution MscL, MscK, MscS and MscM channels are reproducibly seen, and appear to be the only channels present. In on-spheroplast recordings with high Ca^{2+} or Mg^{2+} (100 mM) in the pipette and bath solution both MscL and MscM channels are observed. In addition, lower conductance channels of < 30 pS (n = 12) and 80 - 100 pS (n = 12) respectively are observed at depolarised potentials (pipette potential - 55 mV and above) in the absence of applied pressure in these solutions. These openings are long duration, non-flickery (in the case of the < 30 pS channel) and shorter duration, flickery (in the case of the 80-100 pS channel). The I-V relationships appear linear over a limited voltage range (pipette potentials −55 to −90 mV). The ''low'' conductance channels are seen in the absence of Cl^- ions (sulphate as replacement) in the pipette and bath solutions. These channels are not seen in excised inside-out patches (n = 6). The identity, ionic selectivity and role of these channels remain to be characterised. An anion preferring 100 pS conductance channel in the absence of applied pressure has been reported previously in excised patches at both depolarised and hyperpolarised potentials in high K^+ (150) solutions (3).

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Modeling of the Open-Channel Structure of Mscl Using Restrained Simulations

Evelyne Deplazes, Martti Louhivuori, Siewert J. Marrink, Ben Corry.

Mechanosensitive channels are membrane proteins that act as safety valves to protect bacterial cells from sudden osmotic shock. The gating is induced by tension in the surrounding lipid bilayer and results in a a large conformational change. The structure of the mechanosensitive channel of large conductance (MscL) in the closed state has been solved by XRD [1]. The protein has been characterized using EPR [3] and FRET [3] spectroscopy but a detailed structure of the open-channel structure is unknown.
In this study we present a method for incorporating structural data from EPR and FRET experiments into a coarse grained model of MscL. The simulations system was modelled using the MARTINI force field [4]. Restraints based on solvent accessibility from EPR data were implemented by altering the interactions of specific residues with water and lipid particles. Distance restraints between specific residues were implemented using harmonic potentials. A series of MD simulations with different combinations of restraints and membrane