Orientation of the bacterial mechanosensitive channel MscS in liposomal membranes

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The bacterial mechanosensitive channel of small conductance (MscS) has been shown to play a crucial role in the protection of bacterial cells against hypo-osmotic shock. Shortly after its gene was cloned (Levina *et al.*, 1999) the X-ray crystallographic analysis revealed that the MscS channel was a homoheptamer (Bass *et al.*, 2002). The functional characteristics of the channel have extensively been studied in both giant spheroplasts and liposomes (Akitake *et al.*, 2005; Sukharev, 2002). Despite many studies performed on the MscS channel proteins reconstituted into liposomes (Sukharev, 2002; Nomura *et al.*, 2012) the orientation of MscS in liposomal membranes is still unknown.

In this study we examined the orientation of MscS reconstituted into liposomes by the patch-clamp technique and confocal microscopy. Using several previously determined electrophysiological and pharmacological characteristics of the channel, we have been able to determine that in liposomal patches MscS retains the same orientation as in giant spheroplast patches based on the following evidence: (i) the current-voltage relationship (I-V curves) obtained from the MscS activity recorded in both spheroplast and liposome preparations exhibited strong outward rectification between −100 and +100 mV at both negative and positive pressures applied to patch pipettes; (ii) the data obtained for the MscS activation ratio in liposome patches at positive relative to negative pipette voltages (+20 to −20 mV, +100 to −100 mV) and *vice versa* (−100 to +100 mV) showed positive correlation at both positive and negative pipette pressures (*i.e.* membrane tension) - similar result was obtained with MscS in spheroplast patches; (iii) A voltage-dependent hysteresis in MscS activity upon application of saw-tooth pressure ramps was observed in both spheroplasts and liposomes, although the hysteresis observed in liposome patches was much less pronounced compared to spheroplast patches - importantly, in both spheroplasts and liposomes the hysteresis was more pronounced upon positive pipette voltages compared to negative pipette voltages; (iv) addition of 2.5% 2,2,2-trifluoroethanol (TFE), which was reported to perturb lipid-lipid interactions and promote dissociation between TM2 and TM3 transmembrane helices of MscS, caused inactivation of MscS in liposome patches when added to the bath solution (*i.e.* cytoplasmic side of MscS). In contrast, addition of 2.5% TFE to the patch pipette (*i.e* periplasmic side of MscS) did not cause inactivation of 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. These pharmacological results are consistent with the results of a previous independent study obtained with MscS in giant spheroplasts (Akitake *et al.*, 2007).

In conclusion, our findings strongly indicate that the cytoplasmic domain of MscS in liposome membrane patches faces the bath solution as in the native membrane of spheroplast patches. Consequently, upon liposome reconstitution MscS channels preserve their right-side out orientation similar to what was previously reported for the MscL channels (Ajouz *et al.*, 2000).

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