

The effect of high hydrostatic pressure and TMAO on gating of MscS of *E. coli*

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The activity of MscS, the bacterial mechanosensitive ion channel of small conductance, has been investigated under high hydrostatic pressure (HHP) using a “flying-patch” patch-clamp technique. In inside-out excised patches of giant spheroplasts of *E. coli*, MscS was activated by negative pipette voltage to allow for open probability measurement at different levels of HHP up to 90 MPa. MscS open probability was found to gradually decrease on increasing HHP. To determine the extent that the cytoplasmic and transmembrane domains of the channel may contribute to this effect, the osmolyte methylamine N-oxide (TMAO) was applied to the cytoplasmic side of the excised spheroplast membrane patches. In the presence of TMAO the inhibitory effect of HHP on MscS activity was suppressed at pressures of up to 50 MPa. Above 50 MPa, channel open probability decreased similarly in absence or in presence of TMAO indicating that at pressures higher than 50 MPa, TMAO at concentrations used in this study could not counteract the effect of HHP on the MscS channel activity. The change in the reaction volume calculated in the presence of TMAO differs significantly from the reaction volume calculated in absence of TMAO. Our study suggests that TMAO can stabilize the open state of the MscS channel at HHP, most likely by interacting with the cytoplasmic domain of MscS.

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