C-terminal charged cluster of the mechanosensitive channel MscL, RKKEE, functions as a pH sensor

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A highly conserved cluster of charged residues, RKKEE, located within the C-terminus of the bacterial mechanosensitive channel MscL is essential for the channel gating. The mutated protein lacking these amino acid residues is not functional (Häse *et al.*, 1997). This structural motif is a part of a cytoplasmic helix and is proposed to serve as a stabilizing element of the closed configuration of the MscL channel (Perozo *et al.*, 2001). The crystal structure of MscL was obtained at low pH showing the channel in its closed state (Chang *et al.*, 1998). In the crystal structure the charged residues are facing each other inside the C-terminal helical bundle. However an independent study of the channel closed structure (Perozo *et al.*, 2001) shows that at neutral pH these residues are outwardly oriented facing the aqueous medium. This suggests that the orientation of the C-terminal helices relative to the aqueous medium is pH dependent. Thus, it is possible that the RKKEE cluster functions as a pH sensor. In the present study we examined the effects of pH as well as of charge reversal and substitution within the RKKEE cluster on mechanosensitivity of *E. coli* MscL reconstituted into liposomes using the patch-clamp technique.

Charge reversal mutations did not affect the free energy of activation or activation pressure of the channel (ΔG_0 = 15.8 kT, $p_{1/2}$ = 76.3 mmHg and ΔG_0 = 16.4 kT, $p_{1/2}$ = 84.2 mmHg) for the RKKEE wild type and the EEKKR mutant respectively. Protonation of E107 and E108 residues, achieved by decreasing the experimental pH or replacement of negative charges by glutamine, significantly increased free energy of activation for the MscL channel due to an increase in activation pressure $p_{1/2}$ (ΔG_0 = 26.9 kT, $p_{1/2}$ = 120.2 mmHg for wild type MscL at pH 5.5 and ΔG_0 = 26.1 kT, $p_{1/2}$ = 130.3 mmHg for the RKKQQ mutant channel at pH 7.0). A similar increase in ΔG_0 was observed when positive charges were substituted by glutamine (ΔG_0 = 23.8 kT, $p_{1/2}$ = 118.6 mmHg at pH 7.0) or the overall charge of the cluster was neutralized by increasing experimental pH of the RKKQQ mutant (ΔG_0 = 25.7 kT, $p_{1/2}$ = 124.0 pH 9.5). Interestingly, protonation of the positively charged residues of the RKKQQ mutant by lowering the experimental pH to 5.5 resulted in $p_{1/2}$ (89.0 mmHg) and ΔG_0 (13.0 kT) comparable to the wild-type MscL at physiological pH of 7.0 suggesting the importance of the preservation of the total charge of the cluster. Our data indicate that the RKKEE charged cluster acts as a pH sensor that regulates the stability of the cytoplasmic helix. Our data further suggest that, in contrast to the gating model proposed by Anishkin *et al.* (2003) the cytoplasmic helix is not only functioning as a size-exclusion filter but also substantially influences channel gating.

Anishkin, A., Gendel, V., Sharifi, N.A., Chiang, C.S., Shirinian, L., Guy, H.R. & Sukharev, S. (2003) *Journal of General Physiology*, 121, 227-244.

Chang, G., Spencer, R.H., Lee, A.T., Barclay, M.T. & Rees, D.C. (1998) *Science*, 282, 2220-2226.

Häse, C.C., Ledain, A.C. & Martinac, B. (1997) *Journal of Membrane Biology*, 157, 17-25.

Perozo, E., Kloda, A., Cortes, D.M. & Martinac, B. (2001) *Journal of General Physiology*, 118, 193-205.