

https://dspace.org/

Membrane interaction of the CLIC1 transmembrane domain

Peter, Bradley

2015-01-30
http://hdl.handle.net/10539/16836

Downloaded from a DSpace repository. https://dspace.org/

Abstract

The chloride intracellular channel protein 1 (CLIC1) is a dual-state protein that can exist either as a soluble monomer or in an integral membrane form. Dysfunction in membrane insertion has been implicated in several pathologies including apoptosis, cancer and homeostatic imbalance. The transmembrane domain (TMD) is implicated in membrane penetration and pore formation and is therefore a key target for understanding amphitropism in CLIC1. The mechanism by which the TMD binds, inserts and oligomerises in membranes to form a functional chloride channel is unknown. Here the secondary, tertiary and quaternary structural changes of the CLIC1 TMD and several TMD mutants are reported in an attempt to elucidate the membrane insertion mechanism. A synthetic 30-mer peptide comprising the TMD was examined in 2,2,2-trifluoroethanol (TFE), SDS micelles and POPC liposomes using far-UV CD, fluorescence and UV absorbance spectroscopy. The results suggest a four-step mechanism whereby the TMD, which is unfolded in buffer, refolds into a helix which partitions onto the membrane, followed by insertion and dimerisation to form a membrane-competent protopore complex. These helices associate via a Lys37-mediated cation-π interaction to form weakly active dimers. The complex is then tethered to the membrane by a cationic motif acting as an electrostatic plug. Thus, electrostatic interactions provide both a strong thermodynamic driving force for helix-helix association as well as structural integrity within the membrane. This represents an important step towards understanding how amphitropism occurs in CLIC1 and offers a unique insight into how CLIC1 and other proteins defy the 'one-sequence one-fold' hypothesis.