Electrostatic Effects on the Conformational Stability of the Intracellular Chloride Channel Protein CLIC1

Fanucchi, S. and Dirr, H. W.

Protein Structure-Function Research Programme, School of Molecular and Cell Biology, University of Witwatersrand, Johannesburg 2050, South Africa.

CLIC1 is unusual in that it can exist in both a soluble and a membrane bound form. The manner in which this protein inserts into membranes is unknown. This study focuses on the characterisation of reduced CLIC1 in terms of its secondary and tertiary structure, the determination of its conformational stability at equilibrium and the establishment of its unfolding kinetics, all under conditions of varying pH and ionic strength. Although a change in pH or ionic strength appears not to affect the structure of the native state, a decrease in ionic strength resulted in an increase in the hydrodynamic volume occupied by native CLIC1. At pH 7.0, the equilibrium unfolding of the protein follows a two-state transition with a $\Delta G(H_2O)$ of 10 kcal/mol and an m-value of 2.0 kcal/mol/M urea. As the pH is dropped to 5.5 these parameters drop by more than 40% and the fluorescence monitored unfolding curve has a pronounced inflection in the region of 3 M to 5 M urea. A blue shift in the fluorescence spectra and ANS binding were also detected under these conditions. Salt concentrations of 0.2 M NaCl were found to stabilise the protein at pH 5.5. CLIC1 unfolds 40 times slower at pH 7.0 than at pH 5.5 consistent with the drop in stability with a drop in pH. Electrostatic effects play a dominant role in determining CLIC1 structure and stability and are likely to be involved in the formation of a hydrophobic molten globule intermediate state at low pH, which could reduce the energy barrier to membrane insertion.