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Abstract

The chloride intracellular channel CLIC1 is an anion channel protein that has been implicated in a number of physiological processes. It is fascinating in that it is synthesised as a soluble monomer that is able to reversibly bind membranes without the aid of a membrane-targeting tag or receptor. CLIC1 membrane binding is promoted by low pH and involves separation of the N- and C-domains and subsequent refolding of the N-domain, which traverses the membrane as an α-helix. At the low pH of a membrane surface, pH 5.5, soluble CLIC1 demonstrates decreased conformational stability and forms a partially unfolded intermediate state under mild denaturing conditions. In this study, these pH-effects are proposed to occur as a result of low pH-induced protonation of two conserved glutamate residues, Glu85 and Glu228. Both are involved in domain-maintaining interactions and are proposed to form part of an electrostatic network of pH-sensitive residues. At low pH, protonation of these glutamates would break their electrostatic interactions, allowing separation of the domains. To investigate this possibility, Glu228 and Glu85 were mutated to leucine residues. Each variant protein was then investigated at pH 7.0 and pH 5.5 and results were compared to the wild-type. Secondary and tertiary structures were examined using far-UV circular dichroism and fluorescence spectroscopy, respectively. Conformational flexibility was investigated with limited thermolysin proteolysis. Stability was studied using thermal and urea-induced equilibrium unfolding. The unfolding intermediate state was detected using ANS binding and its structure was characterised. While neither residue substitution caused global structural perturbations, both destabilised the structure and promoted intermediate formation at pH 5.5. This was particularly evident for the E85L variant, which also formed a significant intermediate population at pH 7.0. It was concluded that the interactions of Glu228 and Glu85 are involved in maintaining the CLIC1 native state. Additionally, the lack of pH-dependence of intermediate formation in the E85L variant suggested that Glu85 is likely to function as a pH-sensor. It is thus involved in the 'priming' of the CLIC1 structure for the conformational changes that may lead to membrane binding.