Metamorphic chloride intracellular channel proteins: evidence for transmembrane extension and membrane induced oligomerisation of CLIC1

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Classically, the study of protein structure is based on the assumption that the native protein fold is unique, with at most small structural alterations to facilitate function. However, the existence of several proteins capable of independently interchanging between two or more vastly different but stable folds arising from the same amino acid sequence have been shown. These proteins have collectively been termed "metamorphic" (Murzin, 2008).

The highly conserved Chloride Intracellular Channel (CLIC) protein family is an example of the metamorphic protein class. While the function of the CLIC proteins is not well understood, the CLICs are expressed as soluble proteins but can also reversibly auto-insert into the membrane to form active ion channels. This conformational transition has previously been shown to involve a large-scale unfolding between the C- and N-domains for CLIC1 (Goodchild *et al.*, 2010). The CLIC1 homologue also displays the unique ability to undergo a dramatic structural metamorphosis from a monomeric state, displaying a classic glutathione-S-transferase fold, to a soluble all α-helical dimer solution upon oxidation (Littler *et al.*, 2009). Furthermore, in the presence of membranes, the effect of oxidation has been shown to increase the interaction of CLIC1 with the lipid bilayer (Goodchild *et al.*, 2009). However to date, experimental evidence characterising the dramatic structural rearrangements that must occur within CLIC1 to confer favourable interactions with the membrane and enable formation of an ion channel pore are lacking.

In the current study, site-directed fluorescence labeling of a series of single cysteine residues (T44C, T45C, K49C, C89) within the vicinity of the single putative transmembrane domain (aa24-46) of CLIC1 was performed using a novel labeling strategy described recently (Goodchild *et al.*, 2010). Fluorescence Resonance Energy Transfer (FRET) was used to monitor for changes in the distance from a single native Trp35, located within the transmembrane, to each of the 1,5-IAEDANS acceptor labeled cysteine residues. This was performed in both the soluble CLIC1 form and upon the addition of lipid bilayers. The FRET changes observed indicate that an extension of residues 24-46 occurs upon interaction with the membrane. This result is consistent with the current model of a single extended helical transmembrane region. To test the hypothesis that the CLIC1 forms an oligomeric channel structure in the presence of membranes, a population of CLIC1 labeled with a donor fluorescent label (1,5-IAEDANS) was mixed with a population of CLIC1 labeled with an acceptor fluorescent label (5-IAF). Appreciable FRET interaction and thus evidence for oligomerisation was only detected upon oxidation of the CLIC1 in the presence of the membrane. Together, these two FRET results reinforce the notion of the CLIC protein family as dynamic and metamorphic entities and challenge many accepted views of protein structure. Currently, our labeling scheme is being extended to further refine our model for the structural transitions and environmental triggers of CLIC1 membrane-induced metamorphosis.

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