FRET study of C-terminal movements of the cytoplasmic tail of human skeletal muscle chloride channel, hClC-1, during gating

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Like other members of the CLC channel and transporter family, ClC-1 is a homodimeric protein with individual chloride permeation pathways through each subunit. It exhibits quite complex transport/gating characteristics with two distinct gating processes being identified, so far. One is called protopore gating (or fast gating), which regulates each individual pore independently and the other is common gating (or slow gating), which closes both pores simultaneously. An understanding of these gating processes is still far from complete, especially the common gating, which, because of its high temperature coefficient, is presumed to involve large conformational rearrangements of the protein. Some basis for this has recently been found using fluorescence resonance energy transfer (FRET) by Bykova *et al*. (2006) in the related chloride channel, ClC-0, from electroplaque cells of *Torpedo* where large displacements of the C-termini were shown to accompany common gating. Encouraged by this work, by known effects of site-directed mutations in the cytoplasmic tail on gating and by knowledge of the characteristics of naturally-occurring myotonic mutants in this domain of human ClC-1 (hClC-1), we decided to analyse its common gating, also using FRET. We first prepared a variety of full-length, truncated and split hClC-1 constructs with and without fluorescent tags (Cerulean and Enhanced Yellow Fluorescent Protein, eYFP) to determine whether these would affect channel function as determined by patch-clamp when heterologously expressed in HEK293 cells. As for ClC-0, using both full-length hClC-1 (M_1-L_{988}) and a shorter version (M_1-F_{887}, truncated just after the second CBS domain of the carboxyl tail), we then observed changes in FRET suggesting a significant movement of the C-termini that was functionally associated with common gating. Closure of the common gate was accompanied by a physical separation of the C-termini of the two subunits, whereas, on opening the two C-termini moved closer to each other. These movements and, presumably, associated conformational changes in hClC-1 during common gating are considerably smaller than in ClC-0, and this might then underlie the differing properties of common gating in these two channels, such as, their different temperature dependence of relaxation and their very different time scales. Interestingly, although the voltage dependence of common gating is opposite in the two channels (apparent open probability of the common gates increasing on depolarization for ClC-1), C-terminal movement is congruent. A comparison of single mutants E232Q and C277S, and double mutants E232QA272E and E232QC277S in our FRET study strongly suggests that fast gating and common gating are closely linked in hClC-1. It seems that in the absence of the carboxyl side chain of E232, neither fast nor common gating can occur and the channel remains open. On the other hand, FRET shows that the conformational rearrangements of the C-terminals, normally associated with common gating, are retained in E232Q even though chloride current is unimpeded by any gating. Positive FRET between split channel fragments Cerulean-M_1-H_{451} and Y_{380}-L_{988}-eYFP provides answers to several further questions. It shows that these two channel components, split within the membrane resident domain of the protein, but with overlap including helices K and L, must, indeed, combine appropriately to form the functional channel. Secondly, the putative crystal structure for CLC membrane resident domains is supported because the Cerulean and eYFP must be located sufficiently close to each other across the membrane for FRET to occur. Thirdly, and perhaps more importantly, this construct forms the basis for future studies of relative movements of different regions of the channel vertically through the membrane during gating.

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