Structure function studies of muscle-type ClC chloride channels

Thesis submitted for the degree of Doctor of Philosophy on May 22, 2006.

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Declaration

This work contains no material which has been accepted for the award of any other dgree or diploma in any other tertiary institution and, to te best of my knowledge, contains no material previously published or written by another person, except where due reference has been made in the text.

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Summary

ClC proteins are chloride channels and transporters that are found in a wide variety of prokaryotic and eukaryotic cell-types. The mammalian chloride channel ClC-1 is an important modulator of the electrical excitability of skeletal muscle. The *Torpedo* electric-organ chloride channel, ClC-0 is structurally and functionally similar to ClC-1. These proteins are referred to as the muscle-type ClC channels. The present work identifies several functional differences between the muscle type channels, and explores the structural basis of these and other previously reported differences.

First the temperature dependence of ClC-1 channels was quantified. These calculations revealed distinct contrasts to previously published measurements of ClC-0 temperature sensitivity, indicating differences between the channels in the structural rearrangements associated with channel gating. Next the effect of extracellular ion substitution on ClC-0 function was examined. These measurements suggested that occupancy of an anion binding-site on the extracellular side of the selectivity-filter stabilises the open state of the channel, and that the diameter of the channel pore increases during channel opening. Three-dimensional models of the muscle-type channels were constructed based on the atomic coordinates of prokaryotic homologues. Differences in selectivity between ClC-0 and ClC-1 could be rationalised, in part, by differences in the chemistry of the narrow constriction of the channel pore. The major structural divergence between the muscle-type channels occurs in the expansive intracellular carboxy terminus. Replacing this region of ClC-1 with the corresponding region from ClC-0 resulted in distinct changes in common gating of the channel. These experiments rigorously characterise the dependence of ClC-1 function on temperature and the effect of foreign anionic-substrates on ClC-0

function. The results identify important residues involved in ionic selectivity of the channels, and validate the use of high-resolution prokaryotic channel structures as a predictive tool for studying the muscle-type channels. They also demonstrate that the carboxy-terminal of the channels is an important determinant of common gating.