# Characteristics of Baculovirus-Expressed rClC-1

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# Summary

The baculovirus/ Sf cell system was established and used to express rat skeletal muscle chloride channel ClC-1. Modified *clc*-1 reading frames were produced and the mutant proteins encoded thereby successfully expressed. Recombinant PCR techniques were employed to produce a 5'-histidine tagged protein for use in future protein purification and reconstitution experiments. Site-directed mutagenesis was also applied to produce a protein in which the highly conserved arginine at position 304 was replaced by a glutamic acid residue (R304E). Factors influencing protein yield were investigated, the data obtained being used to optimise expression in Sf cells. Removal of the upstream untranslated sequence of the cDNA was found to increase the level of expression suggesting the presence of regulatory sequences in this region. Wild type and modified forms of ClC-1 were expressed at high levels in Sf cells, protein being easily visualised on coomassie-stained polyacrylamide gels.

Whole-cell patch-clamping was used directly on ClC-1-expressing Sf9 cells to assess the biophysical properties of the channel in this system. Current kinetics were investigated and found to be similar to those reported by others using heterologously expressed rat and human ClC-1 protein in other cell lines and were in keeping with those expected for the channel responsible for the large chloride flux in skeletal muscle. Distinctive features of whole-cell currents included deactivation at hyperpolarising potentials and rectification at positive potentials. Under the conditions used here, the deactivating current was found to be made up of three components, two exponentially decaying and one constant. The time constants of the two exponential components, designated fast and slow, were around 6 and 25ms (test potential -120mV) respectively. On a small number of occasions when longer lasting voltage pulses were employed, a third exponentially deacying component could be

extracted with a time constant of several hundred milliseconds (Astill et al., 1995a). No attempt has been made, at this stage, to characterise this component in any detail. In contrast, the two faster components were studied in detail and their time constants were found to be voltage dependent, the fast component becoming faster and the slow component slower as the test potential became less negative. Increases in total instantaneous and quasi-steady state currents along with a slowing of the deactivation process were also noted during the first 10 - 20 minutes of current recording.

The effects of various compounds known to interfere with chloride permeation in muscle and other tissues were also assessed. Anthracene-9-carboxylate was found to be the most potent blocker exhibiting an $IC_{50}$ of around 20μM. Most other compounds tested displayed potencies in the millimolar range with the exception of niflumate for which preliminary results indicated a potency in the 50 - 100μM range. Different compounds were found to alter channel behaviour in varying ways suggesting several, discrete sites of interaction with the channel protein. A number of compounds, including 2-(4-chlorophenoxy)-propionate (clofibric acid) and its enantiomers, induced changes in current kinetics remarkably similar to those observed by other investigators working with *in vitro* expressed ClC-1 incorporating mutations found in the human myotonic muscle disease dominant myotonia congenita (Thomsen's disease).

Results obtained with the point mutant produced in our laboratories (R304E), in particular its differing response to $Zn^{++}$ at various external pHs, shed some doubt on the current proposed topological model of this protein and indicate the possible involvement of histidine residues in normal channel operation.