Investigating the mechanism of proton transfer through the bacterial ClC transporter

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The ClC chloride channel family is a ubiquitous, yet highly unique family of ion channels, involved in a diverse range of physiological functions. Accardi & Miller (2004) showed that the bacterial ClC channel, ClC-ec1, is not a simple chloride channel, but a chloride / proton exchange transporter, exchanging two chloride ions for every one proton. More recent experimental studies have shown that two eukaryotic members of the family, ClC-4 and ClC-5, are also chloride / proton exchange transporters (Picollo & Pusch, 2005; Scheel *et al.*, 2005). Computational investigations have provided a detailed description of the mechanism of chloride permeation through several ClC isoforms (Cohen & Schulten, 2004; Corry *et al.*, 2004). However, there is very little information describing the transport of protons through ClC-ec1, or the location of the proton translocation pathway. It is known, however, that Glu148, Ser107 and Tyr455 are involved in the translocation pathways of both chloride and protons (Accardi & Miller, 2004).

Here we use computational techniques to probe ClC-ec1 to determine the most energetically favourable translocation pathway for protons. First we ran multiple searches using the HOLE program (Smart *et al.*, 1993) to identify every continuous pathway through the protein with a radius greater than 0.6Å. Our results converged on four possible pathways through each protein dimer. We then used a Poisson-Boltzmann calculation to determine which of these pathways was energetically favourable for protons. Our investigations reveal a narrow fissure through each dimer, 0.75 Å in radius, close to the dimer interface. The protein surrounding these fissures is relatively rich in polar and ionizable amino acids, creating an environment favourable for protons. In support of the experimental evidence, we find that Glu148, Ser107 and Tyr455 are pore-lining residues of our proposed proton translocation pathway, as well as the chloride translocation pathway.

Electrostatic calculations of the unoccupied ClC-ec1 transporter show that our proposed proton translocation pathway contains an electrostatic potential barrier to proton permeation, in the intracellular region of the pathway, effectively barring proton permeation. However, when two chloride ions occupy the chloride pathway, the potential energy barrier in the proton translocation pathway is converted to an electrostatic potential energy well of approximately 18 kT, deep enough to hold one proton in a stable configuration. This occupancy pattern, confirmed by Brownian dynamics simulations, supports the experimentally predicted exchange rate of one proton for every two chloride ions (Accardi & Miller, 2004).

Accardi, A. & Miller, C. (2004) *Nature* **427**, 803-807.
Cohen, J. & Schulten, K. (2004) *Biophysical Journal* **86**, 836-845.
Corry, B., O'Mara, M. & Chung, S.H. (2004) *Biophysical Journal* **86**, 846-860.
Picollo, A. & Pusch, M. (2005) *Nature* **436**, 420-423.
Scheel, O., Zdebik, A.A., Lourdel, S. & Jentsch, T.J. (2005) *Nature* **436**, 424-427.
Smart, O.S., Goodfellow, J.M. & Wallace. B.A. (1993) *Biophysical Journal* **65**, 2455-2460.