MjClC Cl^- Channels Increase Their Open Probability at High Temperatures

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Membrane transport proteins carry out the selective transport of ions and non-electrolytes for many biological processes. Although most transport proteins operate only at physiological temperatures, the mjClC gene product from *methanococcus jannaschii,* a hyperthermophile, retains its function at 95ºC. The characteristics of this channel may lead to insights into engineering heat-resistant proteins for channel arrays and sensors. To determine how temperature affects the biophysical characteristics of the mjClC Cl^- channel, the protein was cloned from genomic DNA and expressed in HEK 293 cells. Plasma membrane vesicles were then prepared from these cells. Planar lipid bilayer experiments were conducted by forming a lipid bilayer with 20mg/mL 3:1 POPE:POPG across a 100μm diameter aperture between two chambers of 50% glycerol/800mM TEACl solution and incorporating membrane vesicles expressing the mjClC channel into the bilayer. Currents across the bilayer at different holding potentials were measured at 25ºC, 60ºC, and 80ºC to assess the flow of chloride ions at different temperatures. Current recordings and corresponding amplitude histograms showed that mjClC channels are mostly closed at 25˚C. At 60˚C, the channel is active and has higher open probability. At 80˚C, many channels are open and the open probability is higher than at 60˚C. In conclusion, higher temperatures significantly increase the open probability of mjClC Cl^- channels. Further experiments are needed to assess the effects of temperature on other mjClC channel characteristics and to relate channel function to structure. This study was supported by AFOSR FA9550-07-1-0257 and T35 DK 60444.