A KcsA/MloK1 chimeric ion channel has lipid-dependent ligand-binding energetics

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Cyclic nucleotide-modulated ion channels play crucial roles in signal transduction in eukaryotes. The molecular mechanism by which ligand binding leads to channel opening remains poorly understood, due in part to the lack of a robust method for preparing sufficient amounts of purified, stable protein required for structural and biochemical characterization. To overcome this limitation, we designed a stable, highly expressed chimeric ion channel consisting of the transmembrane domains of the well characterized potassium channel KcsA and the cyclic nucleotide-binding domains of the prokaryotic cyclic nucleotide-modulated channel MloK1. This chimera demonstrates KcsA-like pH-sensitive activity which is modulated by cAMP, reminiscent of the dual modulation in hyperpolarization-activated and cyclic nucleotide-gated channels that display voltage-dependent activity that is also modulated by cAMP. Using this chimeric construct, we were able to measure for the first time the binding thermodynamics of cAMP to an intact cyclic nucleotide-modulated ion channel using isothermal titration calorimetry. The energetics of ligand binding to channels reconstituted in lipid bilayers are substantially different from those observed in detergent micelles, suggesting that the conformation of the chimera's transmembrane domain is sensitive to its (lipid or lipid-mimetic) environment and that ligand binding induces conformational changes in the transmembrane domain. Nevertheless, because cAMP on its own does not activate these chimeric channels, cAMP binding likely has a smaller energetic contribution to gating than proton binding suggesting that there is only a small difference in cAMP binding energy between the open and closed states of the channel.

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