Ligand-Gated Ion Channel Opening and Closing Mechanism from Molecular Simulations

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Pentameric Ligand Gated Ion Channels comprise key receptors for neurotransmitters including acetylcholine, GABA, and serotonin. They are thus targets for many anesthetics, alcohol, and other drugs such as antipsychotics and antidepressants. The channels typically open after physiologic ligand binding to a site in the extracellular domain, but the channels are also highly susceptible to allosteric modulation at sites in the transmembrane domain, the mechanism utilized by many drugs. To better understand ligand and drug action, we are studying ion channel gating using Gloeobacter violaceus (GLIC) channels. GLIC is a prokaryotic homologue believed to share all the important characteristics of metazoan channels, but with several structures available. We have previously(1) shown a single closing event for GLIC at neutral pH in molecular simulations. Here, we have employed ensemble molecular dynamics simulations to systematically explore the conformational dynamics of the channel, starting from both open and locally-closed conformations. We observe a large number of both opening and closing events. We have also simulated multiple functional mutants of the GLIC channel and observe shifts in opening or closing propensity that agree well with the functional data. Mutants strongly biased towards opening remained open for greater than one microsecond in our simulations. Based on our results, we generate a structural model for which portions of the channel can close sufficiently to restrict water and ion flow.


Sodium Absorption by epithelial sodium channel (ENaC) is main driving force of lung liquid clearance at birth and lung edema clearance in adulthood. We investigated the molecular mechanism underlying the modulation of ENaC current by TNF-z and TNF-z lectin-like domain derived (TIP) peptides. With the help of the patch-clamp technique we show that TIP peptides caused a substantial increase in amiloride sensitive sodium current through ENaC in human alveolar adenoacinaroma cells (A549), in both whole cells as well as single channel configurations. ENaC in A549 cells are proteolytically cleaved. This model cell line mimics the ENaC as in edema conditions. We next analyze the effect of TIP peptide in heterologous expression systems. To do so, we transiently transfected ENaC into CHO cells and studied the effect of TIP peptide. Our results show that TIP peptide has direct interaction with ENaC and can modulate the amiloride sensitive sodium current through these channels. In contrast, we barely observe an effect of TIP peptide when applied to the extracellular side of the HNC cell line RPMI2650. It is widely accepted that two different populations of ENaC are expressed in cells. First, proteolytically cleaved with high open probability called active ENaCs and the second naive with low open probability silent/near silent ENaCs. In RPMI2650 cells ENaC are near silent. To activate these near silent ENaC in RPMI cells we applied Trypsin to the extracellular side and subsequently demonstrate that TIP peptide modulates the sodium current considerably. Our results strongly support a model where modulation of ENaC with TIP peptide AP301 happens in proteolytically active channels and that assaying proteolytic cleavage of ENaC could report on the benefit of therapeutic interventions.


Fast Photoswitching of Tethered Ligands to Study Ionotropic Glutamate Receptor Activation and Desensitization

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Binding of glutamate triggers both the activation and subsequent desensitization of ionotropic glutamate receptors (iGluRs). Matching their role in excitatory neurotransmission, iGluR activation and desensitization are fast processes occurring on the submilliseconds and millisecond timescale, respectively. However, little is known about how ligand binding to the four subunits of the tetrameric channel assemblies mediates these processes. Here we address this question using photo-switchable ligands (MAGs) that can be tethered to individual subunits via a cysteine-reactive maleimide group. An azobenzene group serves as photo-switch that allows binding of the glutamate head group in its cis, but not its trans state [1]. This allows us to control ligand binding and...