ELUCIDATING THE MOLECULAR BASIS OF CHARGE SELECTIVITY IN PENTAMERIC LIGAND-GATED ION CHANNELS

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EXECUTIVE SUMMARY

Pentameric ligand-gated ion channels (pLGICs) are a unique superfamily of ion channels containing both cation- and anion-selective channels. While charge selectivity has been studied in pLGICs for many years experimentally, the minimal number of mutations needed to alter charge selectivity does not appear to be consistent among all members of the superfamily. In recent years, high-resolution structures of both cation- and anion-selective pLGICs in multiple conformational states have become available. Therefore, we set out to understand the molecular mechanisms that underlie anion selectivity. We tested experimentally generated hypotheses about the origin of charge selectivity with the goal of providing an encompassing model that can explain charge selectivity in a wide variety of pLGICs. To do this, we used Blue Waters to calculate free energy profiles of ions moving through the transmembrane region of various pLGICs. We found that charge selectivity is determined by the electrostatics of the transmembrane pore domain.

INTRODUCTION

Ion channels passively allow ions to diffuse in and out of a cell. However, many ion channels allow only specific ions to cross the membrane. One way an ion channel can discriminate among ions is based on the formal charge of an ionic species, known as charge-selectivity. Cation-selective ion channels allow only positively charged cations to move across the membrane, whereas anion-selective ion channels allow only negatively charged ions to cross the membrane. Charge-selectivity is highly important for ion channel biophysics because in adult neurons, when cations are allowed to pass through the cell, the electrical signal is said to be excitatory because it enhances the likelihood of an action potential by depolarizing the cell and therefore the propagation of the neuronal signal. However, when anions are allowed to pass, the electrical signal is inhibitory because it hyperpolarizes the cell, making it more difficult for an action potential to occur.

The pentameric ligand-gated ion channel (pLGIC) superfamily is particularly well-characterized. These channels open in response to neurotransmitters such as acetylcholine, serotonin, and GABA and are important for regulating neuronal signals in the body. These channels are also targets for important classes of drugs such as anesthetics and benzodiazepines. Furthermore, they are the only superfamily of neurotransmitter-gated ion channels that contains members that are highly cation-selective, such as the serotonin and acetylcholine receptor, and other members that are highly anion-selective, such as the glycine and GABA receptor. This feature makes pLGICs a model system for understanding the molecular basis for how ion channels are able to selectively catalyze the conduction of ions based on the formal charge of the ion.

METHODS AND RESULTS

Ion-selectivity has been studied experimentally for years using patch-clamp electrophysiology. While these experiments are able to observe patterns in ion-selectivity and elucidate mutations needed to change selectivity of a particular channel, electrophysiology does not have the resolution necessary to explain the molecular underpinnings of charge-selectivity. To understand charge-selectivity at atomic and molecular scales, computer simulations are necessary. To accomplish this task, free energy calculations are performed on a variety of member of the pLGIC superfamily. Utilizing NAMD, umbrella sampling simulations are performed on anion- and cation-selective ion channels in order to compute the free energy profiles for both cations and anions. In doing this, one can ascertain differences in ion conduction between the cation- and anion-selective channels. Furthermore, mutations to these channels can be done *in silico* to observe how the energy landscape for both types of ion changes, and these observations can be directly compared to experimental results. The comparison with experimental results is powerful because the two techniques are highly complementary. Experimental evidence provides verification for the observations seen in simulations, while simulations provide a highly detailed atomistic picture of the ion permeation process.

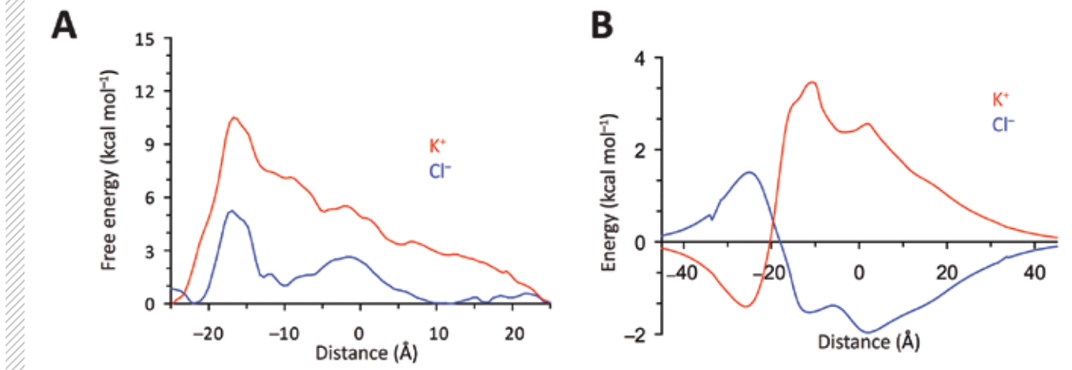

FIGURE 1: Energetics of ion permeation through GlyR: (A) PMF of potassium and chloride permeation through GlyR (PDB 3JAF) M1-M3 transmembrane segment using umbrella sampling molecular dynamics simulations. The transmembrane segments are centered about 0; positive numbers are toward the extracellular end of the protein and negative numbers are toward the intracellular portion. (B) Electrostatic ion-protein solvation energies for potassium and chloride, calculated using APBSmem. The distance axis corresponds to the same axis as in (A).

Figure 1A shows a potential of mean force plot generated using umbrella sampling for an anion-selective member of the pLGIC superfamily. This channel is clearly anion selective because the major barrier is much larger when the cation, potassium, is permeating through the channels relative to the anion, chloride. Figure 1B shows an electrostatic calculation for both a potassium and chloride along the axis of permeation. The electrostatic calculation shows the channel stabilizing the passing anion by creating an energetic well and destabilizing the passing cation by creating a barrier. Together, these calculations show that the charge selectivity of pLGICs is due to the electrostatics of the protein.

WHY BLUE WATERS

Ion permeation, especially in absence of a membrane potential, can be a slow process. Therefore, computational methods such as umbrella sampling have been devised in order to make the process of computing the energy landscape of permeation much more efficient. To do free energy calculations for ion permeation using all-atom molecular dynamics simulations, a large computational resource is needed. To make the calculation computationally efficient, umbrella sampling is used where the reaction coordinate that describes the permeation of an ion through the channel is discretized into many small windows that can be run independently. Umbrella sampling allows each umbrella window to be run as an independent simulation, which speeds the process of calculating free energies but requires a large number of processors to run each simulation efficiently.