Investigations of Ion Channel Structure-Function Relationships Using Molecular Modeling and Experimental Biochemistry

Thesis by

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Chapter 1: Introduction

Ion Channels

Ion channels are integral membrane proteins found in all cells that mediate the selective passage of specific ions or molecules across a cell membrane (Alberts et al., 1994). These channels are important in a diverse range of physiological processes, including signal transmission in the nervous system, sensory perception, and regulation of vital systems, such as circulation.

These ion channels can be considered selective in two ways. First, since channels can exist in open and closed conformations they are *temporally* selective. In an open conformation, a channel mediates the formation of a column of water across the membrane through which ions can pass, while in a closed conformation this column is blocked, preventing the flow of ions. Different channels are converted from their closed to open states—or "gated"—by different types of stimuli. Thus, channels are often divided into three general categories based on the type of stimulus to which they respond (Fig. 1.1).

Perhaps the simplest of these categories includes channels that respond to mechanical stress in the membrane (Fig. 1.1A). These mechanosensitive channels are gated by tension that they sense either through direct contact with membrane lipids or indirectly through forces applied through attached cytoskeletal elements. Although their gating stimulus appears relatively primitive, these types of channels are nonetheless very important physiologically, playing a role in touch and hearing in higher organisms and osmotic regulation in prokaryotes (Hamill and Martinac, 2001). Channels that are gated by changes in transmembrane voltage form the second class of channels (Fig. 1.1B). These voltage-gated channels that respond to membrane depolarization or

hyperpolarization are central to the transmission of electrical signals along nerve axons. The final category of channels are ligand-gated channels, or channels that are gated upon the binding of some small molecule ligand, such as acetylcholine, serotonin, or glycine (Fig. 1.1C). Some notable examples of ligand-gated channels occur at the synapses between nerves, where the electrical signal is transmitted from the end of an axon to an adjacent neuron through the passing of a neurotransmitter molecule—a ligand—through the synaptic gap.

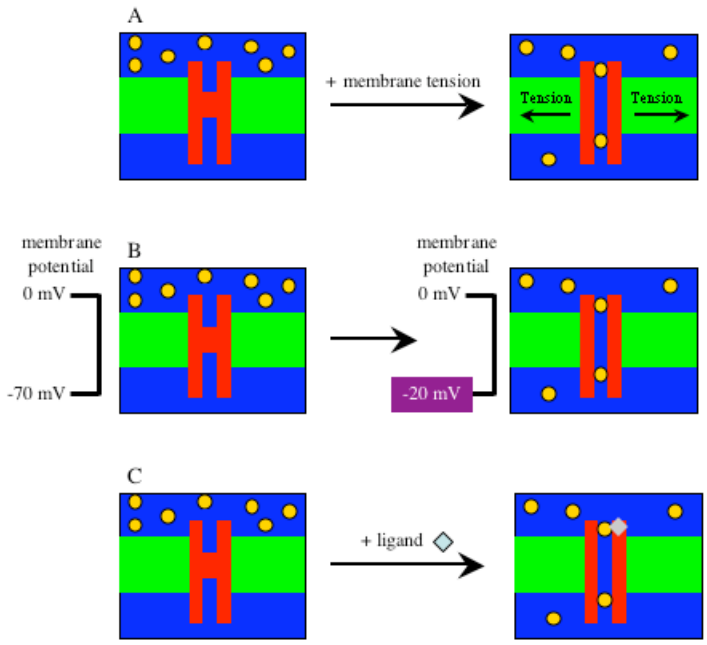

Figure 1.1: The three general categories of gating stimuli for ion channels. Ion channel proteins are shown in red with membrane, water, and ions in green, blue, and yellow, respectively. A) Mechanosensitive channels are gated by membrane tension sensed through the membrane or cytoskeletal elements. B) Voltage-sensitive channels are gated by changes in transmembrane voltage, such as the membrane depolarization depicted here. C) Ligand-gated channels are gated by the binding of some small molecule ligand, shown here as a light blue diamond.

Although channels are typically divided into these three groups, it is important to remember that some channels can respond to more than one type of stimulus. For example, the MscS channel discussed below appears to be modulated by transmembrane

voltage in addition to being gated by mechanical stress (Martinac et al., 1987). In fact, it has been hypothesized that all types of channels show at least some mechanosensitive modulation as they respond to stresses in the surrounding lipid environment (Gu et al., 2001).

In addition to temporal selectivity, channels are also selective for certain ions. For example, certain channels are highly selective for K^+ ions, while others selectively pass Na^+, Ca^{2+}, or Cl^-. This ion selectivity is particularly noteworthy since channels that allow a relatively rapid flow of ions also show an impressive selectivity between two very similar cations, such as K^+ and Na^+. Thus, ion selectivity has been, and continues to be, the focus of numerous studies of ion channels (Chung and Kuyucak, 2002).

Although ion channels are clearly an important class of molecules, they can also be quite difficult to study. Since the passage of ions through channels produces a current, the gating behavior, selectivity, and other characteristics of channels can be investigated by measuring the currents of open channels through electrophysiological techniques. The activity of a single ion channel can even be measured through patch-clamp electrophysiology. Although electrophysiological studies have provided detailed information about many channels, they provide limited structural information. However, it is quite difficult to produce sufficient quantities of most eukaryotic ion channels for biochemical and spectroscopic studies or for efforts towards direct structure determination. As well, the lack of detailed structural information on ion channels has severely limited the application of molecular modeling techniques. Thus, it would be useful to have ion channel systems that could be studied with a wider range of techniques.

Bacterial Ion Channels

Until relatively recently, many researchers believed that bacteria did not necessarily have ion channels like more complicated organisms (Koprowski and Kubalski, 2001). However, over the past few decades people have come to realize not only that bacteria contain these channels, but that their channels also provide particularly useful models of ion channel systems in higher organisms. In particular, bacterial ion channels can be easily overexpressed in bacterial expression systems (Rees et al., 2000). Thus, a large amount—relative to that obtainable for mammalian channels—of channel protein can be produced and purified for subsequent studies. Purified channel can be used for biochemical studies, such as cross-linking (Maurer et al., 2000; Sukharev et al., 1999), and spectroscopic measurements, such as circular dichroism (Arkin et al., 1998). Other studies have successfully used electron paramagnetic resonance (EPR) spectroscopy measurements of spin-labeled bacterial channels to develop gating models (Perozo et al., 1999; Perozo et al., 2002). Bacterial channels can also be functionally characterized using electrophysiological techniques analogous to those applied to eukaryotic channels. Many channels, such as MscL, KcsA, and ClC, can be purified and reconstituted into lipid vesicles or bilayers of controlled lipid composition (Heginbotham et al., 1998; Maduke et al., 1999; Sukharev et al., 1993), allowing the effects of lipid composition on channel function to be considered. Also, bacterial cells expressing channels can be prepared as spheroplasts, or "giant round-up cells," by preventing them from separating properly after cell division (Saimi et al., 1992). This leads to unusually large "cells" that can be patch-clamped directly for electrophysiological measurements. In addition to using detailed electrophysiological measurements, some bacterial channels

and their mutants can be characterized using high-throughput *in vivo* assays of channel function (Maurer and Dougherty, 2001).

The ability to produce large amounts of channel proteins also makes direct structural determination, such as through crystal structures, feasible. In fact, a few groups have been particularly successful in obtaining crystal structures of bacterial ions channels. In 1998, the first ion channel structures were solved: KcsA, a potassium channel, by the MacKinnon group (Doyle et al., 1998) and MscL, the mechanosensitive channel of large conductance, by the Rees group (Chang et al., 1998) (Fig. 1.2). The KcsA structure allowed the first direct structural interpretation of ion selectivity in potassium channels, the understanding of which has been increased by using the structure as a basis for subsequent theoretical and experimental studies (Sansom et al., 2002). For both channels, the structures offered a starting point for studies predicting the gating transition between closed and open forms (Perozo et al., 1999; Perozo et al., 2002; Sukharev et al., 2001).

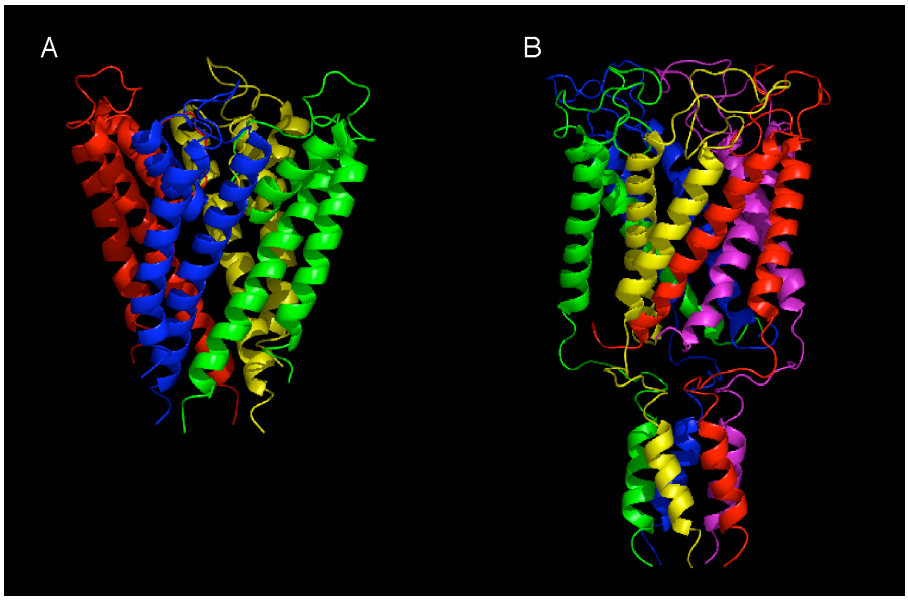

Figure 1.2: Crystal structures of the KcsA (A) and MscL (B) channels.

After the initial mechanosensitive and potassium channel structures, high-resolution structures have been determined for other bacterial ion channels. These have included a chloride selective channel, ClC (Dutzler et al., 2002); a mechanosensitive and voltage modulated channel, MscS (Bass et al., 2002); and a voltage-gated potassium channel, KvAP (Jiang et al., 2003). Thus, it appears that bacterial channels can provide useful structural information for many types of ion channels. This is particularly notable as decades of concerted effort towards determining the high-resolution structure of ion channels from higher organisms has led to limited success. For example, the dedicated work of Unwin and co-workers towards obtaining cryo-EM structures of nAChR from *Torpedo* electroplaques has only led to structures at about 4 Å resolution, too low to resolve atomic-level details (Miyazawa et al., 2003).

Applying Computational Modeling and Experimental Biochemistry to Ion Channel Structures

While the determination of several high-resolution structures of bacterial ion channels has provided the first atomic-level interpretations of many phenomena, the structures also raise even more questions, including figuring out the most effective way to utilize structural data to learn about channel function. One such approach that seems particularly promising is using the structures as a starting point for computational modeling and experimental biochemical studies performed directly in tandem with one another. The solving of the first crystal structures of KcsA and MscL in 1998 coincided with the increasing feasibility of performing multi-nanosecond molecular dynamics (MD) simulations on membrane proteins embedded in explicitly represented hydrated lipid

membranes. For example, one landmark study was the simulation of a porin, OmpF, in a phosphatidylethanolamine membrane (Tieleman and Berendsen, 1998), and this was followed in rapid succession by similar ion channel simulations in several other groups (Forrest and Sansom, 2000; Roux, 2002). These types of simulations, which developed from initial MD studies on explicit hydrated lipid membranes in the early 1990s (Egberts et al., 1994; Heller et al., 1993), allow people to consider channel dynamics and atomic-level interactions that might not be apparent from the static picture provided by a crystal structure. As well, many other types of computations, such as Brownian Dynamics simulations that use structures to predict channel conductances (Chung et al., 2002; Im et al., 2000) and electrostatic calculations (Roux and MacKinnon, 1999), have been used along with crystal structure information. Alone, information from these computations is intriguing, but it is most compelling if it can be tied to experimental results. This can be done relatively readily for bacterial channels, since they are amenable to a wide range of biochemical, spectroscopic, and electrophysiological studies. Thus, a useful synergy can be developed where computation drives experiments, and in turn, experiments drive additional computation.

The following chapters describe my attempts to utilize this in tandem computational-experimental approach to study mechanosensitive, voltage-sensitive, and ligand gated ion channel systems. Chapters 2 through 5 describe different studies of MscL, which is a bacterial channel thought to be gated only by tension in the cell membrane. Chapter 2 describes some initial studies on MscL, including cross-linking studies designed to verify its crystal structure conformation, circular dichroism studies comparing the secondary structure of a number of MscL homologues, and additional

homologue comparisons using a bioinformatics approach. Chapter 3 discusses the use of MD simulations and circular dichroism studies of multiple channel mutants to probe the curious helical bundle conformation of the MscL C-terminal region seen in the crystal structure. Many different molecular dynamics simulations of the full MscL channel crystal structure embedded in a lipid membrane are presented in Chapters 4 and 5. The initial setup of these MD simulations and the ability of the simulations to consider channel mutations are discussed in Chapter 4. These first simulations are extended in Chapter 5 to consider how the membrane lipid composition may affect MscL structure and function. Simulations of MscL in gradually thinner membranes predicted that kinking of transmembrane helices might be an important element of channel gating. This prediction was then tested by experiments and additional computations described in Chapter 5 that characterized MscL mutants with a designed transmembrane kink.

In other studies described in Chapter 6, I have probed the voltage-sensitivity of the mechanosensitive channel of small conductance, MscS. These studies utilized MD simulations of MscS similar to those performed on MscL to structurally verify the supposed voltage sensitivity of the channel and to identify specific amino acid residues likely to be important for voltage sensitivity. These residues were then experimentally mutated and characterized electrophysiologically to verify the computational predictions.

The final chapter, Chapter 7, discusses the use of small molecule *ab initio* calculations and modern solvation models to predict the conformation of the nicotine molecule in aqueous solution. Nicotine is an important agonist of the nicotinic acetylcholine receptor (nAChR), a ligand-gated ion channel. Experimental studies have found that nicotine appears to bind to the channel differently than other agonists, such as

acetylcholine (Beene et al., 2002). Thus, these computations aimed to better characterize the conformational subtleties of nicotine with the goal of gaining insight into its apparently unusual binding behavior.

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