2 Ion selectivity and conductance

Dorothy M. Kim, Jason G. McCoy, and Crina M. Nimigean

Contents
2.1 Introduction 13
2.2 Structural basis for selectivity in ion channels 15
  2.2.1 K channel selectivity 15
    2.2.1.1 Close-fit model of selectivity 16
    2.2.1.2 Field-strength model of selectivity 16
    2.2.1.3 Coordination model of selectivity 16
    2.2.1.4 Kinetic model of selectivity 17
    2.2.1.5 Site number model for selectivity 17
    2.2.1.6 Other K channel selectivity determinants 17
  2.2.2 Na channel selectivity 18
2.3 Conductance 19
References 21

2.1 INTRODUCTION

Ion channels function to orchestrate an exquisite array of physiological processes, including nerve impulses, muscle contraction, regulation of cell volume, and cell signaling in all organisms. The electric current in a signaling event is generated by ion flux across the cell membrane that is controlled by the opening and closing of ion channels, including those permeable to potassium, sodium, calcium, and chloride ions. The direction of the ion fluxes is determined by the membrane potential and preset transmembrane ionic gradients, which are established and maintained by specific ion channels and the Na⁺/K⁺ ATPase.

For certain signaling modes, such as the generation of the action potential, it is important that the ion channels involved are only permeable to specific ions and impermeable to others. Particularly important to signaling in nerve and muscle are potassium and sodium channels, which display a very high level of selectivity. Therefore, some ion channels have evolved to exhibit high ionic selectivity, which is fundamental to electrical signaling.

As early as 1902, Julius Bernstein predicted that excitation was the result of a change in membrane permeability of excitable cells (Bernstein, 1902), hypothesizing that cells at rest were only permeable to K⁺ and that permeability to other ions occurred during excitation; this provided the first suggestion of ion-selective components in the membrane. Breakthrough studies of the squid giant axon in the 1940s and 1950s by Hodgkin, Huxley, Keynes, Goldman, and Katz (Goldman, 1943; Hodgkin and Huxley, 1945, 1946, 1947, 1952a,b,c,d; Hodgkin and Katz, 1949; Hodgkin et al., 1952; Hodgkin and Keynes, 1955) identified the action potential of the axon to be the result of a composite of currents carried by different ions.

Using voltage-clamp on the membrane of a squid giant axon bathed in a solution with controlled ion concentrations, they concluded that at rest the membrane was predominantly selective to K⁺ resulting in a negative transmembrane potential (according to the electrophysiological convention, the transmembrane potential is measured inside the cell relative to the outside). On the other hand, Na⁺ was responsible for the inward current causing the cell membrane to become more positive than at rest, referred to as depolarization. Hodgkin and Huxley identified these two major ionic components responsible for the generation of the action potential in their seminal 1952 papers (Hodgkin and Huxley, 1952a,b,c,d; Hodgkin et al., 1952), and developed a model directly correlating Na⁺ and K⁺ fluxes with excitation and electric conduction in the squid giant axon, earning them the Nobel Prize in 1963. These studies informed the conclusion that nerve impulse propagation is an electrical process involving a delicate balance of ion fluxes across the cell membrane controlled by the opening and closing (gating) of highly selective ion channels. Ion selectivity and regulated gating are crucial to generating the action potential.

The key to understanding the mechanism of selectivity in channels lies in the aqueous pore, a narrow canal comprising the permeation path for ions. The simplest illustration of the pore is that of a molecular sieve that can only pass ions not exceeding a certain radius. Although pore diameter was initially suggested to be a major determinant in ion permeability, it was insufficient to explain the permeability sequences exhibited by some channels. The region within the channel protein that directly interacts with the conducting ions is called the selectivity filter and both the dimensions and chemical properties of this region influence ion selectivity and conductance. In addition to containing binding
Ion selectivity and conductance

sites that can accommodate only certain ions of specific sizes and valences, the pore provides an electrostatic landscape that favors permeation of certain ions over others, thus requiring ions to overcome various energy barriers to permeate the pore. The crystal structure of the bacterial potassium channel KcsA (Doyle et al., 1998; Zhou et al., 2001), for example, reveals that the chemistry within the pore provides binding sites for potassium ions, and may not accommodate sodium ions.

Prior to the elucidation of the atomic structures of these channels, pioneers in the ion channel field predicted some of these pore characteristics for channels of varying ion selectivity. One early hypothesis claimed that in order for channels to discriminate between ions they would need to dehydrate upon entry into the pore (Mullins, 1959; Bezanilla and Armstrong, 1972). The energy required for this step would then need to be balanced by stabilizing interactions between the ions and the walls of the pore (Mullins, 1959; Eisenman, 1962; Hille, 1975). The energetic landscape of a pore consists of specific binding sites for ions representing energetic minima separated by energetic barriers that can also be encountered upon entering and exiting the pore (Hille, 1975; Begenisich and Cahalan, 1980a,b). Ions with lower permeability thus may face a higher energy barrier at external binding sites or alternatively may encounter very deep energy wells within the selectivity filter. This concept can also explain why channels often are permeable to several ions but favor some over others. The determination of permeability ratios led to the conclusion that voltage-gated sodium channels have a large pore that can accommodate many different cations (Hille, 2001). Potassium channels, on the other hand, were imagined to contain a narrower pore than most Na channels, resulting in higher selectivity due to increased interaction and contact between the ion and the pore walls (Hille, 2001). This prediction was confirmed by the determination of crystal structures of voltage-gated Na channels from bacteria, which have a selectivity filter that is ~4.6 Å wide (Payandeh et al., 2011, 2012; Zhang et al., 2012), compared with the ~3 Å wide selectivity filter of the KcsA channel (Doyle et al., 1998; Zhou et al., 2001). Accordingly, potassium channels can conduct other cations such as Tl⁺, Rb⁺, and NH₄⁺ but remain mostly impermeant to other large cations as well as Na⁺ and Li⁺.

Bezanilla and Armstrong (1972) and Hille (1973) proposed that K⁺ ions were stabilized inside the K channel pore by binding sites composed of a bracelet of oxygen dipoles in the selectivity filter region. The selectivity filter would therefore consist of a cylindrical pore with an inner diameter between 3 and 3.4 Å. The K channel selectivity filter was later identified on the pore loop (P-loop), a reentrant loop containing a signature sequence highly conserved among K channels that could contribute these oxygen dipoles (Heginbotham et al., 1992, 1994). These predictions, based entirely on functional data, turned out to be astonishingly accurate and were supported by the crystal structure of the KcsA potassium channel (Doyle et al., 1998; Zhou et al., 2001) with potassium binding sites comprising mostly carbonyl dipoles from amino acid residues in the signature sequence TVGYG (Heginbotham et al., 1994).

At the same time, Bezanilla and Armstrong also hypothesized that ions binding within the pore would slow conduction and thus the payoff for high selectivity should be slow permeation (Bezanilla and Armstrong, 1972). Paradoxically, K channels can have very large conduction rates while also being highly selective. This paradox could be partly addressed by the ability of the pore to accommodate multiple ions at the same time. The multi-ion theory was proposed (Hodgkin and Keynes, 1955; Heckmann, 1965a,b, 1968, 1972; Hille and Schwarz, 1978) in order to explain deviations from the independence principle (Hodgkin and Huxley, 1952b) that states that the probability that an ion crosses the membrane is independent of other ions present. Several lines of evidence support the simultaneous occupancy of more than one ion in the pore. Hodgkin and Keynes hypothesized that the observed anomalous unidirectional flux ratios in squid axons were due to ions passing single-file through long channels that can hold multiple ions at one time (Hodgkin and Keynes, 1955). This was based on the observation that the K channel exhibits flux coupling, which indicates the presence of more than one ion in the pore. The multi-ion channel theory was further supported by the so-called anomalous mole fraction effect (Neher and Sakmann, 1975; Sandblom et al., 1977; Hille and Schwarz, 1978; Hess and Tsien, 1984; Eisenman et al., 1986), which describes changes in permeability ratios in Na (Chandler and Meves, 1965; Cahalan and Begenisich, 1976; Begenisich and Cahalan, 1980a), K (Hagiwara and Takahashi, 1974), and Ca (Almers and McCleskey, 1984; Hess and Tsien, 1984) channels when more than one permeant ion type is present. These studies showed that channel conductance passes through a minimum or maximum when plotted against the mole fraction of two permeant ions and it was understood by allowing the two ions to interact inside the pore and with the pore walls (Nonner et al., 1998; Hille, 2001).

Studies providing strong evidence that supports the multi-ion model were made possible by the advent of recombinant DNA technology, which allowed for the sequencing, cloning, heterologous expression, and purification of ion channel proteins. While sodium and calcium channels had been purified from membrane preparations in the 1970s, it was not until the 1980s that the first ion channel genes, the nicotinic acetylcholine receptor channel nAChR from Torpedo californica (Noda et al., 1982, 1983) and the voltage-gated sodium channel from the electric eel Electrophorus electricus (Noda et al., 1984), were cloned. The nAChR channel was also the first to be recorded with patch clamp (Neher and Sakmann, 1976) and then eventually purified, reconstituted, and recorded in a lipid bilayer (McCarthy et al., 1986; Montal et al., 1986). Success with the discovery and cloning of potassium channels followed (Papazian et al., 1987). In addition, the discovery of homologous K channels (Milkman, 1994; Schrempf et al., 1995; Derst and Karschin, 1998) and Na channels (Ren et al., 2001; Ito et al., 2004; Koishi et al., 2004; Webster et al., 2004) in prokaryotes paved the way for numerous advancements in structural studies of ion channels. These advances, as well as developments in electrophysiological techniques, enabled the taxonomic grouping of known channels into functional superfamilies as well as the prediction of novel channels by sequence analysis. Site-directed mutagenesis studies revealed the functions of specific residues in channels, while sequence inspection also provided some insight into the structural properties of ion channels.
Ultimately, the rapid accumulation of information in the era of cloning led to a major breakthrough in the first crystal structure of an ion channel in 1998 (Doyle et al., 1998), as described below. This structural data, in conjunction with the wealth of structure-based data that followed, have revolutionized the ion channel field and inspired many new avenues of research. These structures confirmed many of the predictions of early functional data including the physical properties of the pore, the dehydration of ions within the selectivity filter, and the multi-ion nature of ion conduction. In the next section, we describe these structures in detail and explore how they have enriched our knowledge and understanding of selectivity.

2.2 Structural basis for selectivity in ion channels

2.2.1 K channel selectivity

Analysis of Drosophila mutants resulted in the discovery of a region of genomic DNA encoding a protein involved in the conductance of potassium. The observation of a fly that exhibited uncontrolled shaking led to the identification of the Shaker locus. This gene encodes a member of the voltage-gated potassium channel (Kv) family and was the first K channel to be cloned (Kamb et al., 1987; Papazian et al., 1987). This milestone allowed for the heterologous expression of a K channel in Xenopus oocytes and functional characterization (Iverson et al., 1988; Timpe et al., 1988). Electrophysiological studies in oocytes revealed that Shaker demonstrates a clear selectivity preference for K⁺, Rb⁺, and NH₄⁺ over Na⁺ (Heginbotham and MacKinnon, 1993). In addition to these discoveries, the cloning of additional potassium channels provided a rich trove of amino acid sequence information.

Analysis of these sequences identified a stretch of highly conserved residues, and this region is the site of various mutations that lead to changes in ionic selectivity (Heginbotham et al., 1994). These conserved residues (T₁X₂X₃T₄X₅G₆Y₇G₈) were coined the signature sequence. Within this sequence, the GYG motif is by far the most conserved region, although some channels such as the EAG-like K channels and the inwardly rectifying Kir6 family of K channels contain a phenylalanine in place of tyrosine.

The significance of these residues was unveiled upon the publication of the first crystal structure of a potassium channel (Doyle et al., 1998; Zhou et al., 2001). The structure of KcsA, from the soil bacterium Streptomyces lividans, reveals a tetramer with fourfold symmetry and a clear pore along the fourfold symmetry axis (Figure 2.1a). Each subunit contains two transmembrane domains (T1 and T2). Between the T1 and T2 helices a short helix (the pore helix) is positioned such that its C-terminus points into the center of the channel pore. Immediately following this helix, the selectivity filter loop containing the signature sequence residues, T₇₂A₇₃T₇₄T₇₅V₇₆G₇₇Y₇₈G₇₉, extends into the center of the channel. The backbone carbonyl oxygens of Y₇₈, G₇₇, V₇₆, and T₇₅ as well as the side-chain hydroxyl of T₇₅ point directly into the center of the protein, forming the narrowest region of the channel pathway. These oxygens coordinate dehydrated K⁺ ions as they permeate the pore, surrounding each K⁺ by eight oxygen atoms in a cage-like structure (Figure 2.1b and c). The four potassium binding sites are numbered S₁–S₄, starting from the most extracellular cage formed by the backbone carbonyls of Y₇₈ and G₇₇ (the S₁ site), and ending with the most intracellular cage formed by the side-chain hydroxyl and backbone carbonyl oxygens of T₇₅ (the S₄ site). In addition to the four K⁺ ions fully coordinated by the protein, a fifth K⁺ binding site is observed at the extracellular surface of the channel, coordinated by the backbone carbonyl oxygens of Y₇₈ and four water molecules (the S₀ site). The KcsA crystal structure confirmed many of the early predictions about the potassium channel selectivity filter, including its multi-ion nature and the rings of oxygen atoms involved in coordinating K⁺ (Bezanilla and Armstrong, 1972; Hille and Schwarz, 1978; Neyton and Miller, 1988a,b).

The structure of the KcsA potassium channel and the three-dimensional architecture of the selectivity filter reveal a detailed picture of the molecular mechanisms by which potassium channels distinguish between different ions. The structures provide not only the identities of functional groups that interact

![Figure 2.1](image-url)
Ion selectivity and conductance

with the K⁺ ions and the bond distances, but they also serve as good starting models for molecular dynamic simulations that can also provide a detailed understanding of selectivity. In the following subsections, we describe several models that have been proposed as the basis of selectivity. Many of these models are not necessarily mutually exclusive.

2.2.1.1 Close-fit model of selectivity

The underlying assumption of the close-fit model is that the selectivity filter distinguishes between different ions based on ionic radius. In this model, the distances between the conducting ion and the selectivity filter backbone carbonyl oxygens are tuned specifically to optimize the coordination of K⁺ relative to that of smaller or larger ions (Figure 2.2a). This partially alleviates the energetic penalty for dehydrating the K⁺, and this is necessary for entry into the selectivity filter. Despite this high affinity, the ions are still rapidly processed through the selectivity filter presumably due to electrostatic repulsions between multiple K⁺ ions in close proximity in the filter. In the published 2 Å resolution crystal structure of KcsA, the average coordination distance between the K⁺ ions and the selectivity filter oxygen atoms is 2.85 Å, nearly identical to that observed in the potassium-selective antibiotic nonactin (Zhou et al., 2001). Na⁺, with an ionic radius approximately 0.4 Å smaller than that of K⁺, would require a positional shift of the selectivity filter for optimal binding. It should also be noted that crystal structures depict a positional average over time. Therefore, while the structures show K⁺ density in each of the selectivity filter binding sites, the K⁺ ions probably occupy only alternate binding sites as they pass through the channel (e.g., S4 and S2 or S3 and S1) (Morais-Cabral et al., 2001).

2.2.1.2 Field-strength model of selectivity

The field-strength model foregoes the idea of rigid cages, and instead treats the selectivity filter as a flexible, liquid-like environment. The dipole moments of the selectivity filter backbone carbonyls generate electrostatic forces that repel each other and attract the cation leading to selectivity for K⁺ (Noskov et al., 2004; Noskov and Roux, 2006) (Figure 2.2a). In this model, it is the physical properties of the ligand groups (i.e., the selectivity filter backbone carbonyls) that lead to selection for K⁺ over other ions. In simplified simulations, a ligand dipole between 2.5 and 4.5 debye (similar to a carbonyl) selects for K⁺, whereas decreasing or increasing the ligand dipole leads to optimal selection for larger or smaller cations, respectively (Noskov et al., 2004).

2.2.1.3 Coordination model of selectivity

The coordination model differs from the close-fit and field strength models in that the number of groups that interact with the ligand also contributes to selectivity. In other words, a protein framework with eight liganding moieties, whether they belong to waters or carbonyls, as in the KcsA channel, will inherently select for K⁺ over Na⁺ (Bostick and Brooks, 2007) (Figure 2.2a). In another version of the coordination model, the electrical properties of the local environment of the binding sites create strong selectivity by over-coordinating K⁺ with eight carbonyl

![Figure 2.2 Principles of selectivity. (a) Ion coordination in the K channel selectivity filter demonstrates principles of the close-fit, field-strength, coordination, and kinetic models. K⁺ binds with octahedral coordination in the center of a cage formed by eight carbonyl oxygens (left). In KcsA this bond distance is 2.9 Å (PDB ID 1K4C). In contrast Na⁺ binds with square planar coordination in the plane of the carbonyls (right). In KcsA this bond distance is 2.5 Å (PDB ID 3OGC). (b) The number of contiguous ion binding sites demonstrates principles of the site number model of selectivity. K⁺ ions are shown as spheres in single file in the filter. Water molecules are shown only for NaK as spheres coordinating the top two ions. The nonselective NaK channel lacks the S1 and S2 binding sites (left panel). Restoration of the S2 site through mutagenesis of the selectivity filter to TVGDTPP (called NaK-CNG), a sequence very similar to that of nonselective cyclic nucleotide-gated (CNG) channels, fails to increase the K⁺ selectivity of the channel (center). Restoration of site S1 through mutagenesis of the NaK selectivity filter to TVGYGDF (called NaK2K), a sequence very similar to that of KcsA, results in a K⁺ selective channel (right).](https://example.com/figure2.2.png)
ligands instead of the usual six ligands coordinating K⁺ in water (Varma and Rempe, 2006; Varma et al., 2008).

### 2.2.1.4 Kinetic model of selectivity

In the selectivity mechanisms described above, the origins of selectivity are ultimately derived from a difference in the free energy of binding for different ions within the selectivity filter. An alternative kinetic-based model suggests that an additional layer of K⁺ selectivity over other ions may be the result of a high-energy barrier preventing other ions from advancing through the selectivity filter. Molecular dynamics simulations of KcsA predict that the S4 site is slightly selective for both Na⁺ and Li⁺ over K⁺; however, the energy minima for the Na⁺ and Li⁺ lies in the plane formed by the carbonyl oxygens dividing the S3 and S4 sites (Thompson et al., 2009) (Figure 2.2a). Crystal structures of KcsA in the presence of Na⁺ have also shown spherical densities between the carbonyl oxygens instead of centered in the carbonyl cage as observed for K⁺ (Lockless et al., 2007; Cheng et al., 2011). The presence of K⁺ may then obfuscate the preferred binding sites of smaller ions such as Na⁺, generating a large Coulombic repulsion that prevents Na⁺ from entering the selectivity filter (Thompson et al., 2009; Egwolf and Roux, 2010; Kim and Allen, 2011).

### 2.2.1.5 Site number model for selectivity

Determination of the crystal structure of the NaK channel from *Bacillus cereus* (Shi et al., 2006) led to the hypothesis of the site number model. Unlike KcsA, the NaK channel does not prefer K⁺ to Na⁺. This difference in selectivity appears to be largely due to a change in the sequence of the selectivity filter. The selectivity filter sequence in NaK is TVGDGNF, in contrast to the KcsA sequence of TVGYGDL. The result of this change is that sites S1 and S2 are disrupted, creating a large cavity in place of the two cage-like binding sites (Figure 2.2b). Modifying the NaK selectivity filter sequence to TVGDTPP, similar to what is observed in nonselective cyclic nucleotide-gated channels, reestablishes the S2 site but this channel still fails to demonstrate selectivity (Derebe et al., 2011). Modifying the NaK selectivity filter to TVGYGDF, similar to that of KcsA, reestablishes both the S1 and S2 sites and results in heightened selectivity for K⁺ (Derebe et al., 2011). This suggests that the presence of four contiguous binding sites is a critical component for establishing K⁺ selectivity.

### 2.2.1.6 Other K channel selectivity determinants

While it is clear that the selectivity filter is crucial to mediating channel selectivity, many aspects of selectivity cannot be explained entirely by the filter. For example, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels contain a variant of the signature sequence CIGYG. Replacement of the threonine in K⁺-selective channels with cysteine would presumably remove the S4 site and reduce K⁺ selectivity. In accordance with this hypothesis, Na⁺ permeability in these channels can be up to one-third of that of K⁺. However, mutation of cysteine to threonine actually further reduces the K⁺ selectivity (D’Avanzo et al., 2009). In addition, channels with identical selectivity filters can exhibit differences in selectivity. Although several channels share the selectivity filter sequence TTVGYG, the voltage-gated channels Kv1.5 and Kv2.1 will conduct Na⁺ in the absence of K⁺ while KcsA and *Shaker* do not (Heginbotham and MacKinnon, 1993; Korn and Ikeda, 1995; LeMasurier et al., 2001). Addition of negative charges into the cavity of the inwardly rectifying Kir3.2 channel restores K⁺ selectivity in a nonselective mutant of the channel, demonstrating that the pore cavity and not just the selectivity filter can influence selectivity (Bichet et al., 2006). C-type inactivation, a process by which the selectivity filter changes conformation to halt K⁺ conductance in response to prolonged opening of the channel, has also been shown to influence selectivity (Starkus et al., 1997; Kiss et al., 1999; Cheng et al., 2011). Thus, other channel features in the pore outside of the selectivity filter can clearly modulate selectivity.

### 2.2.2 Na CHANNEL SELECTIVITY

Channels selective for Na⁺ over K⁺ were originally observed by Hodgkin and Huxley in studies of the squid giant axon (Hodgkin and Huxley, 1952b). Early work suggested that the Na⁺ channel contains a rectangular opening of approximately 3 × 5 Å surrounded by oxygen atoms as well as a negatively charged carboxylate ion (Hille, 1971). The size of this opening suggests that the Na⁺ ions conduct in a partially hydrated state. Furthermore, since the selectivity sequence of Na⁺ channels (Na⁺ > Li⁺ > Tl⁺ > K⁺ >> Ca++) closely follows the electrostatic model of Eisenman (1962), it was argued that selectivity was dependent on the field strength of the binding site (Hille, 1972). A high field-strength anion, such as a glutamate side chain, would be required to increase the Na⁺ selectivity relative to that of K⁺.

Cloning and sequencing of mammalian voltage-gated Na channels (Noda and Numa, 1987) and subsequent blocking studies (Terlau et al., 1991) led to the identification of four conserved residues instrumental for maintaining Na⁺ selectivity. Unlike K channels, eukaryotic Na channels are monomeric proteins containing four similar repeated domains, thus lacking the fourfold symmetry of K channels. The four residues each reside in a distinct domain but occupy the same location in the primary structure based on sequence alignments of each individual domain. These residues consist of an aspartate in the N-terminal-most repeat followed by a glutamate, a lysine, and an alanine in the following repeats. This is referred to as the DEKA motif. Mutation of these residues has profound effects on selectivity. As Hille predicted, the filter sequence contains two residues with carboxyl-containing side chains (Hille, 1972). Mutation of glutamate to alanine leads to an increase in K⁺ permeability (Favre et al., 1996), while mutation of the aspartate to alanine has little effect. Therefore, glutamate appears to play a larger role in selectivity in the Na channel filter. Removal of the lysine side chain leads to an even greater loss in selectivity against K⁺ as well as Ca⁺⁺ (Favre et al., 1996). Voltage-gated Ca channels have an EEEE motif in a similar location to that of voltage-gated Na channels. Mutation of the DEKA motif in Na channels to DEEEE confers Ca⁺⁺ selection properties on the channel (Favre et al., 1996).

The crystal structures of several bacterial voltage-gated Na channels (Payandeh et al., 2011; McCusker et al., 2012; Zhang et al., 2012) show that in contrast to their eukaryotic counterparts, these channels are homotetramers. The NavAb channel, the first of these to be published, contains a glutamate in the signature position, similar to eukaryotic Ca channels (Payandeh et al., 2011) (Figure 2.3a and b). The four glutamates...
are positioned along the pore in the center of the channel near the extracellular opening. The cavity between the four glutamate side chains is the most constricted region within the channel allowing just enough room for the conducting Na⁺ to remain partially hydrated while directly interacting with one of the glutamates. Below this glutamate, the backbone carbonyl oxygens of the two following amino acids (Leu and Thr) point into the pore (Figure 2.3b). Solvent molecules coordinated to these carbonyl oxygens presumably help rehydrate Na⁺ as it passes through the filter. Molecular dynamics simulations have shown that Na⁺ can occupy two sites simultaneously; one site in which it interacts with the side chains of the glutamate, a nearby serine, and multiple solvent molecules, and one site in which the ion is fully hydrated and coordinated by the backbone carbonyl oxygens that point toward the pore of the channel (Corry and Thomas, 2012; Furini and Domene, 2012) (Figure 2.3b). The Na⁺ ions in these sites are weakly coupled but do not require the sort of simultaneous ion movement observed in K channels (Corry and Thomas, 2012; Furini and Domene, 2012). The largest energy barrier for Na⁺ occurs in between these two sites (Corry and Thomas, 2012; Furini and Domene, 2012). However, as the EEEE sequence of NavAb is more similar to eukaryotic voltage-gated Ca channels than voltage-gated Na channels, it seems likely that there are additional features of the channel that tune its selectivity to Na⁺. A similar arrangement is observed in other bacterial Na channels. The NavRh channel contains a serine in the signature position (Figure 2.3c). However, the structure has shown that a glutamate exterior to the selectivity filter points into the pore such that the carboxylate occupies a similar location to that of NavAb, presumably forming a similar Na⁺ binding site (Zhang et al., 2012). The interior carbonyl oxygens from a leucine and a threonine form a secondary site that coordinates a hydrated Ca²⁺ in the crystal structure.

The structural data described above provide compelling insight into selectivity and conduction mechanisms, and it is evident that the key characteristics of the selectivity filter are observed in both prokaryotic and eukaryotic K channels. Thus, the selectivity filter appears to be highly conserved across the kingdoms for K channels. It remains to be seen whether this universality applies to Na and Ca channels, and future studies of their eukaryotic counterparts are critical for identifying important deviations from the structures of bacterial homologues. These discoveries are paramount for understanding the evolution of selectivity and conduction in ion channels.

### 2.3 CONDUCTANCE

Ion conductance is a measure of the ease with which ions flux across the membrane. Ion conductance (in picoSiemens) can be calculated from measurements of electrical current generated by the movement of ions through the membrane, normalized to the voltage applied across it (via voltage-clamp), by simply using Ohm’s law (g = I/V). In electrical terms, conductance is the inverse of resistance (g = 1/R). There are two ways to determine the ionic flux rate through a single ion channel (unitary conductance). One approach is to directly measure single-channel currents with patch-clamp and lipid bilayer recordings (Bean et al., 1969; Neher and Sakmann, 1976). Another approach is to indirectly calculate the unitary conductance from a macroscopic current composed of a large number of identical channels by using noise-analysis methods (Neher and Stevens, 1977; Sigworth, 1980a,b; Silberberg and Magleby, 1993). Comparisons of ion conductance among different channels are a useful first metric for assessing the ionic flow within their pores, providing a basis for understanding their respective permeation paths.

Because conductance is given by the ionic flux, it generally increases with increasing permeant ion concentration. However, a linear relationship between conductance and ion concentration is not obeyed at very high ion concentrations due to intrinsic structural properties of the channel or the presence of blockers. Instead, the conductance curves obey simple saturation kinetics that follow a Michaelis–Menten relationship (Michaelis and Menten, 1913; Hille, 2001; Michaelis et al., 2011). Saturation occurs when the steps of ion binding and unbinding are rate limiting: for instance, at high ion concentrations, the rate of ion entry increases and it will approach the maximum rates of unbinding (Hille, 2001). Therefore, as ion concentrations increase further, the rates of unbinding determine the overall rate of conduction and a plateau at maximum conductance is reached. Intrinsic properties of the channels alter this maximum conductance by changing the rate of ion exit from the pore.
In contrast, attractive forces at ion binding sites within the pore, necessary for high selectivity, can decrease the rate of exit, thus decreasing conductance (Hille, 1975; French, 1976; Hille and Schwarz, 1978). These electrostatic forces contribute to the energetic landscape that the ion must traverse to cross the membrane and can greatly affect the rate of flow.

These principles have been established for many years, but were later illustrated in crystallographic studies of KcsA conducted in the presence of different ions and a range of ion concentrations (Morais-Cabral et al., 2001; Zhou et al., 2001). A model was proposed for the barrierless conduction in K channels based on the observed ionic configurations of the selectivity filter. In high permeant ion concentrations, the selectivity filter is found in a conductive state, where two ions can bind at the same time in either a water-K-water-K (S2, S4) or K-water-K-water (S1, S3) configuration (Figures 2.1 and 2.5). The permeating ions provide counter charges for the 20 electronegative oxygen atoms in the filter, which minimizes the destabilization from same charge repulsion (Figure 2.1b and c). Entry of an additional ion would destabilize this configuration, supporting the knock-on model (Hodgkin and Keynes, 1955) as a mechanism for ion conduction and coupling high conductivity with high selectivity (Figure 2.5).

Interestingly, at low permeant ion concentrations, the selectivity filter of KcsA assumes a collapsed, nonconductive conformation, with presumably only one ion bound at one time (Morais-Cabral et al., 2001; Zhou and MacKinnon, 2003). Binding of the second ion could induce a conformational change in the selectivity

---

**Figure 2.4** Conductance–activity curves. Plot showing conductance as a function of ionic activity for hypothetical channel A with a high maximum conductance ($g_{\text{max-A}}$) such as KcsA, and for hypothetical channel B with a low maximum conductance ($g_{\text{max-B}}$) such as K+ ions. The $g_{\text{max}}$ for each channel is denoted by a dotted black line.

The typical conductance–concentration saturation curve is comprised of two components: an initial quasilinear rise at low ion concentrations and a plateau at higher ion concentrations (Latorre and Miller, 1983) (Figure 2.4). The plateau portion of the curve represents the maximum conductance, or $g_{\text{max}}$, and is a measure of the exit rate of the permeating ion. As described below, this rate is dependent on the length of the pore, the number of binding sites, and the interaction between binding sites and permeating ions. In a multi-ion pore, repulsion between ions increases the rate of ions exiting from the pore, or $g_{\text{max}}$, and therefore, pores that can accommodate multiple ions can achieve a higher maximum conductance. The slope of the initial linear component of the curve measures the rate of ion entry into the pore. This rate is limited by the convergence conductance, described by Latorre and Miller as the absolute upper limit of conductance set by diffusion of ions to the mouth of the pore (Latorre and Miller, 1983). The convergence conductance depends on both the size of the pore (which also plays a role in selectivity) and the radius of the permeant ion. By increasing the capture radius of the pore, the rate of ion entry is also increased. However, widening the pore diameter will consequently decrease the selectivity. To circumvent this issue, many channels (Na and K channels, for instance) widen the pore at the base but contain a narrow constriction at the selectivity filter in order to increase entry rate and conductance but maintain high selectivity (Doyle et al., 1998; Zhou et al., 2001; Long et al., 2005; Payandeh et al., 2011, 2012) (Figures 2.1 and 2.3).

In the context of ion channels, it would be intuitive to assume that high selectivity results in low conductance, due to the fact that selectivity requires intimate contact between the ion and the pore walls, consequently slowing permeation. However, this is not the case. The energetics of ion binding dictates both selectivity and conductance. For example, inter-ion repulsion within the pore results in increased rate of exit of ions from the pore, thus increasing conductance (Hodgkin and Keynes, 1955; Eisenman, 1962; Hille and Schwarz, 1978; Morais-Cabral et al., 2001; Zhou and MacKinnon, 2003).

---

**Figure 2.5** Ion conduction pathway in multi-ion pore. (Figure adapted from Zhou, Y. and MacKinnon, R., J. Mol. Biol., 333, 965, 2003; Morais-Cabral, J.H. et al., Nature, 414, 37, 2001.) The four potassium binding sites S1–S4 in the KcsA pore are represented by boxes containing either a potassium ion (circle) or water (not shown). In the absence of an electrochemical driving force on the ions, the 1,3 (left) and 2,4 (right) configurations would have similar energies and be in equilibrium. In the presence of an outward driving force (as shown here), an incoming ion (center) enters the pore to occupy site 4, pushing the ion in site 3 to site 2 and also pushing the ion in site 1 out of the pore (top panel), resulting in a net outward K+ current.
Ion selectivity and conductance

filter, transitioning from the nonconducting to the conductive conformation. These results suggest that conduction requires the presence of two ions in the selectivity filter. Furthermore, because the nonconductive filter contains only one ion, the second ion-binding event is presumed to be the cause of the filter conformational change from collapsed to conductive. The energetic cost of this conformational change can be balanced by a decrease in the energy of ion binding in the conductive filter, thereby increasing the exit rate of ions and allowing for efficient conduction. This study illustrates yet another possibility of how high selectivity could coexist with high conduction; the presence of multiple high affinity binding sites in the pore allows for selection of certain ions over others, while high conductance only occurs in the fully occupied selectivity filter. Thus, high conduction is due to both electrostatic repulsion and, in the case of KcsA, a conformational change that further lowers ion-binding affinity and increases the exit rate of bound ions. Interestingly, other crystallized K channels do not show a collapsed selectivity filter in low concentrations of permeant ions, suggesting that multiple factors play a role in conduction and selectivity and these vary among channels (Shi et al., 2006; Ye et al., 2010; Derebe et al., 2011). Therefore, the multi-ion pore is key to understanding the seemingly paradoxical coupling of high selectivity and high conductance.

While ion selectivity does not vary widely within a channel subfamily, conductance levels can vary dramatically. Channel conductance is dependent on many factors, such as pore chemistry (Hille, 1971, 1975), pore length (Hille and Schwarz, 1978; Latorre and Miller, 1983), the size of the cavity (Jiang et al., 2002a; Breidize and Magleby, 2005; Geng et al., 2011), rates of dehydration (Mullins, 1959; Bezanilla and Armstrong, 1972), electrostatics (Breidize et al., 2003; Nimigean et al., 2003; Furini et al., 2007), the energy landscape (Bernache and Roux, 2001; Hille, 2001), rigidity of the selectivity filter (Morais-Cabral et al., 2001; Noskov et al., 2004), pore occupancy (Hille and Schwarz, 1978; Morais-Cabral et al., 2001; Zhou and MacKinnon, 2003; Jensen et al., 2010, 2013; Moscoso et al., 2012), and ionic conditions (Latorre and Miller, 1983).

Potassium channels range in conductance from 10 pS (including Kᵥ channels (Latorre and Miller, 1983), and small conductance K (SK) channels (Hille, 1971; Conti et al., 1975; Conti and Neher, 1980)) to 200–400 pS (including BK channels, (Martí, 1981; Pallotta et al., 1981; Yellen, 1984; Latorre et al., 1989; Hille, 2001)) in similar conditions (Latorre and Miller, 1983; Hille, 2001). Since the selectivity filter of K channels is highly conserved (GYG), other factors must account for these varying levels of conductance. One well-studied determinant of conductance in K channels is the size of the entrance to the inner cavity on the intracellular side of the selectivity filter. The maxi-K channels, such as the Ca²⁺-dependent BK channel and its archaeal homolog MthK, exhibit conductance rates close to diffusion limit, ranging from 250 to 300 pS (Eisenman et al., 1986), which is roughly 10–20 times higher than for other K channels (Hille, 2001). Crystal structures of MthK (Jiang et al., 2002a,b) and studies of BK using large quaternary ammonium blockers (Li and Aldrich, 2004) reveal that these channels contain a large aqueous cavity on the inner mouth of the pore that sits directly under the selectivity filter in the plane of the membrane. This cavity is larger than the equivalent in other K channels such as KcsA, Kᵥ, and

**Figure 2.6** Cavity dimensions in different K channels. Variations in cavity width are shown relative to the BK/MthK channel, which is predicted to have a cavity that is 16–20 Å wide (from Breidize, T.I. and Magleby, K.L., J. Gen. Physiol., 126, 105, 2005; Geng, Y. et al., J. Gen. Physiol., 137, 533, 2011; Jiang et al., 2002). Shaker/Kv1.2, which has a more modest cavity compared with BK (8–12 Å, (Li and Aldrich, 2004; Long et al., 2005; Webster et al., 2004)), is shown with dashed lines. Negative charges that ring the mouth of the cavity in BK (Nimigean et al., 2003; Breidize et al., 2003) are shown in circles at the internal side of the cavity.

Shaker (Doyle et al., 1998; Kuo et al., 2003, 2005; Long et al., 2005) (Figure 2.6). Potassium ions need to pass through this entrance and traverse the cavity before entering the selectivity filter. Therefore, the width of the cavity entrance is key to determining the rate of efflux. This idea was supported by studies showing that an increase in side-chain volume at the cavity entrance results in a reduction in the single-channel conductance in the outward direction (Geng et al., 2011), while having no effect on the inward current. This reduction in outward current is reversed by an increase in intracellular K⁺ concentration. This suggests that the ions do not encounter a barrier upon entrance into the cavity, and this contributes to the increase in conduction rate compared with other channels. Additional studies show that the entrance to the inner vestibule of BK is ~16–20 Å in diameter (Breidize and Magleby, 2005), in contrast to Shaker, which contains a more modestly sized vestibule of 8–12 Å (Li and Aldrich, 2004; Webster et al., 2004; Long et al., 2005) and has a correspondingly smaller conductance (30–60 pS). Molecular dynamics simulations provide further support for the importance of the width of the cavity to conductance, demonstrating the increase in conductance of K channels with a widening of the entrance to the inner vestibule (Chung et al., 2002). In addition, eight negative charges ring this entrance, attracting K ions to the cavity (Breidize et al., 2003; Nimigean et al., 2003) and reducing the diffusion limitation on conductance (Latorre and Miller, 1983), further contributing to the large outward current of BK channels in physiological ion concentrations (Figure 2.6).

In addition to cavity size, pore length has been hypothesized to contribute to the single-channel conductance level characteristic of a particular channel (Latorre and Miller, 1983). This hypothesis is supported by structural and functional studies of the bacterial inward-rectifying K channels KirBac1.1 and KirBac3.1 and the eukaryotic Kir2.2. These each contain a pore that is ~60–85 Å in length extending through the large C-terminal domains (Kuo et al., 2003, 2005; Nishida et al., 2007; Tao et al., 2009; Bavro et al., 2012), about twice the length of the pore of KcsA (Doyle et al., 1998). These channels exhibit a relatively low conductance level of ~10–35 pS at ~100 mV (Latorre and Miller, 1983; Cheng et al., 2009). Thus, the long
length of the pore of Kir channels may slow the passage of ions through the pore resulting in a decrease in the single-channel conductance compared with channels with shorter pores such as BK and KcsA. However, several other channel families such as the SK channels and Shaker/Kv channels also display a low single-channel conductance level (10–20 pS) but do not possess a similarly long pore to Kir. Therefore, while pore length may play a role in conductance levels in Kir channels, additional factors must contribute to the overall single-channel conductance in other types of channels.

The pore dimensions can thus create diversity within ion channel subfamilies by contributing to a wide range of conductance levels while retaining the evolutionarily conserved, highly specialized selectivity filter sequence. With minimal changes to the basic pore architecture, channels selective for a particular ion can exhibit an array of conductance levels that may be appropriate for different physiological situations. Perhaps of even greater significance is the ability of the channels to conduct ions at very high rates of flux without sacrificing the high degree of selectivity that is imperative for electrical signaling. Therefore, ion channels have evolved intrinsic properties critical for achieving the efficiency and specificity necessary for controlling a plethora of biological processes.

REFERENCES


Ion selectivity and conductance


