16 Molecular Dynamics Simulation Approaches to K Channels

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16.1 Introduction: Potassium Channels

Ion channels are proteins that form pores of nanoscopic dimensions in cell membranes. As a consequence of advance in protein crystallography we now know the three-dimensional structures of a number of ion channels. However, X-ray diffraction techniques yield an essentially static (time- and space-averaged) structure of an ion channel, in an environment often somewhat distantly related to that which the protein experiences when in a cell membrane. Thus, additional techniques are required to fully understand the relationship between channel structure and function. Potassium (K) channels (Yellen, 2002) provide an opportunity to explore the relationship between membrane protein structure, *dynamics*, and function. Furthermore, K channels are of considerable physiological and biomedical interest. They regulate K^+ ion flux across cell membranes. K channel regulation is accomplished by a conformational change that allows the protein to switch between two alternative (closed vs. open) conformations, a process known as *gating.* Gating is an inherently dynamic process that cannot be fully characterized by static structures alone.

The elucidation of the structures of several K^+ channels (Mackinnon, 2003; Gulbis and Doyle, 2004) has shed light on the structural basis of the mechanisms of ion selectivity and permeation (Doyle et al., 1998; Morais-Cabral et al., 2001; Jiang et al., 2002a,b, 2003; Kuo et al., 2003; Zhou and MacKinnon, 2003; Lee et al., 2005; Long et al., 2005). All K channels share a common fold in their pore-forming domain, while exhibiting differences in their structures corresponding to their various gating mechanisms: KcsA is gated by low pH; MthK is gated by Ca^{2+} ions; KvAP and Kv1.2 are gated by transmembrane voltage; and KirBac is presumed to be gated by binding of ligands to its intracellular domain. The core pore-forming domain of K channels is tetrameric, with the monomers surrounding a central pore. The domain is formed of four M1-P-M2 motifs, where M1 and M2 are transmembrane (TM) helices (corresponding to S5 and S6 in Kv channels), with the short P-helix and extended filter (F) region forming a re-entrant loop between the two TM helices (see Fig. 16.1). In voltage-gated potassium (Kv) channels each subunit also contains a voltage sensor domain composed of four TM helices (S1 to S4). In inward rectifier channels, exemplified by the KirBac1.1 structure, there is an additional helix in the pore domain, called "the slide helix," that runs parallel to the cytoplasmic face of

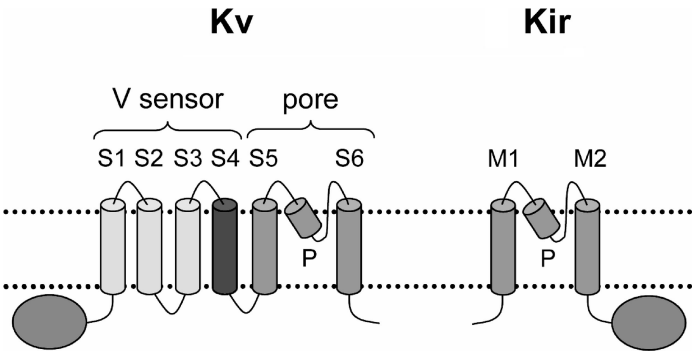

Fig. 16.1 K channel topologies: schematic transmembrane topologies are shown for Kv and Kir channels. The voltage-sensor (S1 to S4 in Kv channels) and pore-forming domains (i.e., S5-P-S6 in Kv channels, M1-P- M2 in Kir channels) are indicated. The intact channel is formed by four subunits.

the membrane. Along with an extensive C-terminal intracellular domain, the slide helix is thought to play a role in the gating mechanism of the channel (Kuo et al., 2003).

16.2 Homology Modeling

The X-ray structures of K channels are all, except for that of Kv1.2 (Long et al., 2005), of bacterial homologues of mammalian channels. It is therefore of some interest to what extent homology modeling (Marti-Renom et al., 2000) and related techniques may be used to extrapolate from bacterial K channel structures to the structures of their mammalian and human homologues (Capener et al., 2002). There have been a number of studies that have employed homology modeling of K channels to help to explain structure/function relationships (Capener et al., 2000; Ranatunga et al., 2001; Eriksson and Roux, 2002; Durell et al., 2004; Laine et al., 2004; Antcliffe et al., 2005). Here we will focus on one particular application of this technique, to the pore-forming domain of the much-studied *Shaker* Kv channel from *Drosophlia* (Tempel et al., 1987). Models of two states of the *Shaker* Kv pore domain have been generated: one, based on the X-ray structure of KcsA, represents a closed state of the channel; the other, based on KvAP, represents an open state pore domain (Fig. 16.2).

The main steps followed in building a homology model of channel are as follows:

1. Sequence alignment of target sequence against the sequence of a suitable template structure.
2. Identification of TM helices within the target sequence using a range of prediction methods (Chen and Rost, 2002).

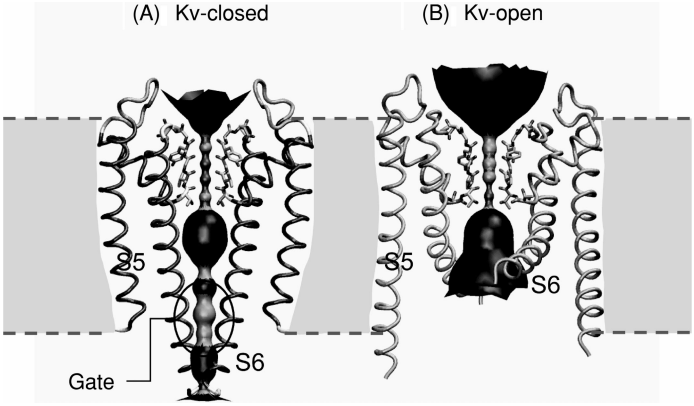

Fig. 16.2 Homology models of the *Shaker* Kv pore domain, based on KcsA [for the Kv-closed model in (A)] and on KvAP [for the Kv-open model in (B)]. In both cases the model is restricted to the core pore-forming domain (i.e., S5-P-S6) and only two of the four subunits are shown for clarity. The approximate location of the lipid bilayer is shown by the horizontal band. The surface of the pore [calculated using HOLE (Smart et al., 1996)] is shown, and the region of the hydrophobic gate is indicated for the Kv-closed model.

3. Generation of a model via one of a number of homology modeling programs [e.g., Modeler (Sali and Blundell, 1993; Fiser et al., 2000)], using the sequence alignment, template structure, and predicted TM helices as inputs.
4. Validation of the resultant model by, e.g., molecular dynamics (MD—see below) simulations in a bilayer-like environment (Capener et al., 2000; Capener and Sansom, 2002).

The first step is crucial: a poor alignment will inevitably yield a poor model. It has been shown that there is a strong correlation between the percentage sequence identity between two proteins and the similarity of their three-dimensional structures (Chothia, 1984). In general, it can be difficult to obtain a good homology model when the percentage sequence identity is $<30\%$. However, the presence of conserved sequence motifs provides a good indicator of structural and functional similarity among apparently distant-related proteins. In the case of K channels, the sequence motif TVGYG in the selectivity filter region of the pore domain is highly conserved, and thus aids alignment. Along with prediction of TM helices, this means it is in general possible to obtain a reasonable alignment of the pore domain (i.e., M1-P-M2 or S5-P-S6) of a target K channel sequence to a template, even though the overall percentage residue identity may be quite low (e.g., $<20\%$). As several structures of bacterial K channels are available, it is also possible to model both closed state (e.g., using KcsA as a template) and open state (e.g., using MthK or KvAP as a template) conformations of a K channel pore domain (Holyoake et al., 2003). This is illustrated in Fig. 16.2, where models of a closed and open state of the pore domain of the *Shaker* Kv are shown. It is evident that in the Kv-closed model (based on a KcsA template) the intracellular mouth of the channel, which is

believed to be the location of the channel gate, is much narrower than in the Kv-open model. The functional consequence of this will be discussed in more detail below.

16.3 MD Simulations of Ion Channels

Molecular dynamics simulations have been used to over 25 years to simulate the conformational dynamics of biomolecules (Karplus and McCammon, 2002). In MD simulations a trajectory (i.e., a series of conformations evolving in time) of a molecular system is generated by simultaneous integration of Newton's equations of motion for all the atoms in the system. To achieve this, MD simulations require a potential energy function, which describes the interactions of all the atoms in the system. This energy function includes terms representing interactions between atoms that are covalently bonded to one another, and also terms for van der Waals and electrostatics interactions between nonbonded atoms. The potential energy is evaluated via an empirical force field, in which atoms are treated as van der Waals particles carrying a point charge. Bonds between atoms are modeled by simple harmonic functions for bond lengths and bond angles, and simple sinusoidal functions for torsion angles (Leach, 2001). Integration of the equations of motion is performed in small time steps, typically 1 or 2 fs. Equilibrium quantities can be calculated by averaging over a trajectory, which therefore should be of sufficient length to sample a representative ensemble of the state of the system.

A simulation starts from initial atomic coordinates and velocities. The initial coordinates are generally obtained by embedding in membrane protein structure in a pre-equilibrated model of a lipid bilayer (Tieleman et al., 1997), followed by addition of water molecules (>30 per lipid molecule) and ions. The initial velocities are taken from a Maxwellian distribution, corresponding to the desired temperature of the simulation (usually 300 K).

Several issues determine the "quality" of a simulation. One of these is the nature of the empirical force field used to describe interactions between atoms of the simulation system. There are a number of force fields that are routinely used in macromolecular simulations, which continue to evolve and improve. Without addressing the details of comparison between different force fields, it is important to be aware of their relative strengths and limitations, especially in the context of membrane simulations. A further issues, especially if simulations are being employed to explore possible conformational changes of a membrane protein, is the extent to which the duration of the simulation allows available conformations of the protein to be sampled (Faraldo-Gómez et al., 2004).

The treatment of long-range electrostatic interactions, and the method used to control the system temperature are also of importance in governing the accuracy of a simulation. There are two principal approaches to approximating long-range electrostatic interactions in membrane simulations. The simplest approach is to neglect the small interactions between any pair of atoms further apart than a given cut-off

distance (e.g., 1.0 nm). This method is efficient from a computational point of view, but introduces inaccuracies that may detract from the quality of a membrane simulation (Tobias, 2001). These inaccuracies may be avoided by using Ewald summation-based methods to treat long-range electrostatic interactions (Darden et al., 1993; Essmann et al., 1995; Sagui and Darden, 1999). However, Ewald methods may introduce artifacts in some simulations (Hunenberger and J.A., 1999; Hunenberger and McCammon, 1999; Weber et al., 2000; Bostick and Berkowitz, 2003), and so should not be employed uncritically. With respect to control of simulation temperature, in most protein simulations the system is coupled to an external thermostat with which it can exchange energy. This may be achieved simply via use of, e.g., a Berendsen thermotstat (Berendsen et al., 1984), or via the Nosé-Hoover method (Nose and Klein, 1983).

For simulations of ion channels (Roux, 2005) and related membrane proteins (Ash et al., 2004), there are two main choices of environment for the protein. The first is to embed the protein within a model of a lipid bilayer. This provides an accurate representation of the environment of the protein, at least in vitro, and is of particular use if, e.g., protein–lipid interactions are to be analyzed (Domene et al., 2003). However, the effective viscosity of a lipid bilayer is quite high, and this may restrict the ability to observe conformational changes in channel proteins on the time scales ($\sim$20 ns) currently available to MD simulations. A lower viscosity approximation to a lipid bilayer is provided by a "slab" of octane molecules. Combined with water molecules on either side, this provides a membrane-mimetic environment that has been successfully employed in a number of K channel simulations (Capener et al., 2000; Capener and Sansom, 2002; Arinaminpathy et al., 2003). An example of such a system, showing an octane slab within which the TM domain of KirBac1.1 is embedded, is provided in Fig. 16.3.

16.4 Essential Dynamics of Ion Channels

One of the challenges of analysis of MD simulations is to extract functionally relevant motions of the protein from the simulation "noise." Recent studies have suggested that such motions occur along directions of a few collective coordinates, which may dominate simulated atomic fluctuations (Garcia, 1992; Amadei et al., 1993; Kitao and Go, 1999). It can be difficult to extract functionally relevant motions from simulation results, mainly because simulation times are generally too short to yield proper sampling of the corresponding conformational changes. A possible solution of this problem is to make use of collective coordinates. A collective coordinate can be calculated by determining a set of eigenvectors via diagonalization of a second moment matrix. This approach forms the basis of principal component analysis (PCA). In the case of a simulation of a protein, a covariance matrix is derived from a set of Cartesian coordinates as a function of time:

$$a_{ij} \equiv \left\langle \left(x_i - \langle x_i \rangle\right) \left(x_j - \left\langle x_j \right\rangle\right) \right\rangle ,$$

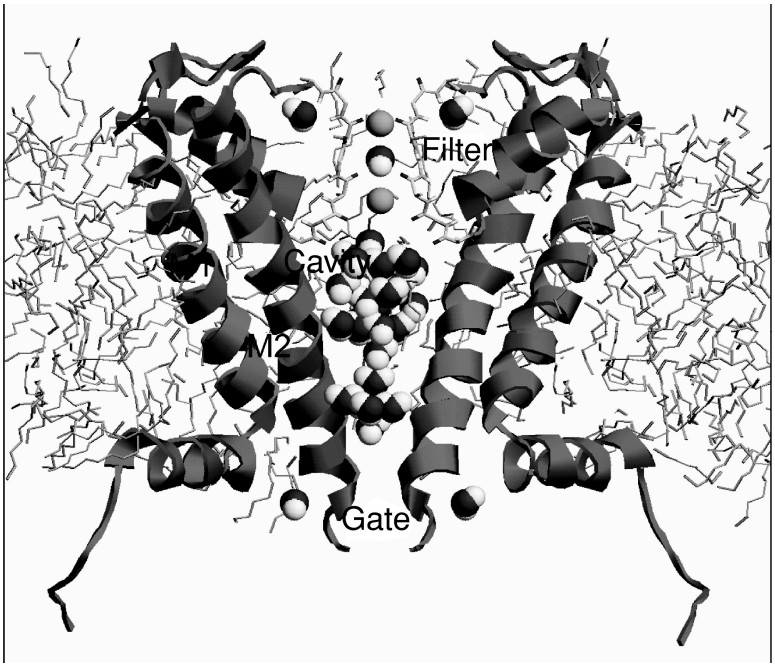

Fig. 16.3 MD simulations of the KirBac TM domain. The TM domain (two subunits only are shown, for clarity) is shown embedded with a membrane-mimetic octane slab. Water molecules and ions within the filter, and water molecules within the cavity of the channel are shown.

where $<>$ denotes a time average over the instantaneous structures sampled during the simulation, and x_i is a mass-weighted atomic coordinate. A set of eigenvalues and eigenvectors is calculated by solving the standard eigenvalue equation:

$$\mathbf{A\Gamma} = \mathbf{\Gamma}\Lambda,$$

where $\Lambda = \mathrm{diag}\,(\lambda_m)$ is a diagonal matrix whose mth diagonal element is the eigenvalue λ_m, Γ is a matrix whose mth column vector is the eigenvector of λ_m. It can be shown that the eigenvalues correspond to mean square fluctuation of the collective coordinates along selected components (Amadei et al., 1993). Collective coordinates are a powerful tool to investigate conformational changes in proteins. They are calculated by projecting the MD trajectories onto the mth eigenvector.

This technique has been useful in analyzing the dynamics of proteins, especially in probing, e.g., large conformational changes related to protein folding/unfolding (de Groot et al., 1996; Roccatano et al., 2001, 2003; Daidone et al., 2003, 2004). As shown by Amadei et al., the overall conformational space sampled by a protein can be divided into a low-dimensional essential subspace of $\sim$10 eigenvectors, within which the principal collective motions of proteins are usually confined, and

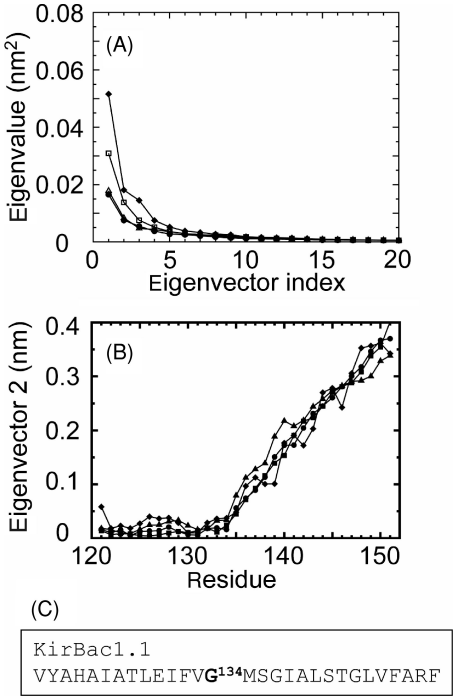

Fig. 16.4 (A) Eigenvalues vs. eigenvector index from principal components analysis (PCA) of the motions of the M2 helices in a simulation of KirBac1.1 in a phospholipid bilayer. Each line represents the M2 helix of a different subunit, according to the following symbols: circles = subunit 1; squares = subunit 2; diamonds = subunit 3; and triangles = subunit 4. (B) PCA analysis of M2 helix motions: mean square displacements of the Cα atoms according to eigenvector 2 are shown as a function of residue number. The fitting of the helices uses only the Cα atoms of the N-terminal half (residues 121–134) of M2. Thus, the mean square displacements increase after the molecular hinge at residue 134. (C) Sequence of the M2 helix from KirBac1.1, highlighting the hinge residue at Gly134.

a near-constraint space within which simple small fluctuations occur. Indeed, the first 10 eigenvectors usually account for 70–80% of the total fluctuations spanned by the principal components. This implies that it may be possible to capture the nature of large amplitude biological relevant motions (as might occur in potassium channels).

As an example of PCA, in Fig. 16.4, we illustrate its application to analysis of the motion of the pore-lining M2 helices in a simulation of KirBac1.1 in a lipid bilayer. A comparable analysis has been performed for the related channel KirBac3.1 (Grottesi et al., 2005). As discussed in more detail below, a major element of the motion of the M2 helices in this and related simulations appears to be a bending motion about a central glycine residue (Gly134) which forms a molecular hinge. In Fig. 16.4A, the first 10 eigenvalues are shown, as derived from PCA of the

motions of the M2 helices in the simulation. From this it can be estimated that the first 10 eigenvectors account for ∼80% of the motion of the M2 helices. For each eigenvector, the resultant motions may be analyzed by plotting the mean square displacement along the eigenvector as a function of residue number. This is shown in Fig. 16.4B for the second eigenvector, after fitting the Cα atoms of M2 residues N-terminal the location of the molecular hinge. The results of this analysis clearly support the existence of a molecular hinge at residue Gly134 (Fig. 16.4C), midway down the M2 helix. The functional consequences of this are discussed in more detail below.

The application of the PCA technique to protein dynamics analysis is limited by the time scale accessible to current MD techniques and computers power. However, by means of ED sampling techniques it is possible to try overcoming this limitation and to investigate conformations that are normally not accessible by classic MD (de Groot et al., 1996).

16.5 What Can Simulations Tell Us?

In considering MD simulations of ion channels, and of related membrane proteins, it is useful to consider the time scales involved. For a simulation of an ion channel embedded in a lipid bilayer plus waters, which yields a system size of ∼50,000 atoms, simulation times of ∼20 ns are readily achievable. This time is of the same order of magnitude as the mean time for the passage of a single ion through a channel. Thus, MD simulations may be used directly to examine the nature of ion permeation, but multiple simulations are required to lend statistical significance to the observations made. In contrast, channel gating and associated conformational changes occur on time scales of microseconds to milliseconds. Thus, MD simulations cannot be used to address such events directly, and so alternative approaches, both to analysis and to simulation, are required.

MD simulations can also provide details of the interaction of channel proteins with their membrane environment. Given the importance of interactions with lipids on the stability, function (Valiyaveetil et al., 2002; Demmers et al., 2003), and regulation (Du et al., 2004) of K channels, this aspect of analysis via MD simulations is increasingly important. However, considerations of space preclude a more detailed discussion in the current paper, and the interested reader if referred to e.g. (Domene et al., 2003; Deol et al., 2004) for further details.

16.5.1 Ion Permeation

Simulation studies of K channels from a number of laboratories have focused on the events during ion permeation through the selectivity filter, both in KcsA (Allen et al., 1999; Bernèche and Roux, 2000; Guidoni et al., 2000; Shrivastava and Sansom, 2000, 2002; Domene and Sansom, 2003) and in related K channels (Capener et al., 2000; Capener and Sansom, 2002; Domene et al., 2004). The results of these simulations

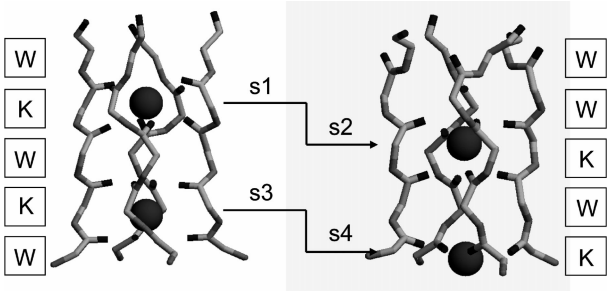

Fig. 16.5 Two snapshots from a simulation of KirBac1.1 in a POPC bilayer (Domene et al., 2004). The snapshots show just the selectivity filter backbone, plus two K^+ ions, and correspond to structures 0.1 ns apart, just before and after the concerted translocation of the two ions from sites S1 and S3, to sites S2 and S4. Note the small changes in backbone conformation coupled to translocation of the K^+ ions. Schematics of the two patterns of occupancy of the five sites (S0 at the top to S4 at the bottom) are given on either side; W = water; K = potassium ion.

have confirmed that ion permeation occurs via concerted single file motion of K^+ ions and water molecules through the filter. The oxygen atoms that line the filter region form five distinct binding sites for K^+ ions, from S0 at the extracellular mouth of the filter to S4 at the opposite end of the filter next to the central cavity. Ion translocation through the filter occurs via a switch between two patterns of occupancy of the filter: between ions at sites S0, S2, and S4, and ions at sites S1 and S3. This result from simulations is in good agreement with structural data (Morais-Cabral et al., 2001; Zhou et al., 2001).

As an example of the results from such simulations, Fig. 16.5 shows two snapshots (separated in time by 0.1 ns) of the filter from a simulation of the bacterial inward rectifier homologue Kirbac1.1 embedded in a lipid bilayer (Domene et al., 2004). It can be seen that there is a spontaneous, concerted switch between K^+ ions at sites S1 and S3, to ions at sites S2 and S4. Close examination of the structures reveals small changes in the conformation of the filter, with the oxygen atoms lining the pore moving by <0.1 nm as the ion switches from site to site. Such limited flexibility of the pore is needed to facilitate rapid translocation of the ions between adjacent binding sites.

It is also possible to use MD simulations to examine the energetics of ion translocation along the filter. Early studies by Åqvist and colleagues (Åqvist and Luzhkov, 2000) established that configurations with ions at S1, S3 and at S2, S4 were of similar energy. More extensive exploration of the free energy surface of ion translocation along the filter has been performed by Roux et al. (Bernèche and Roux, 2001), who have also examined the role of filter flexibility in ion translocation and ion selectivity (Allen et al., 2004; Noskov et al., 2004). These results indicated that inclusion vs. omission of thermal atomic fluctuations resulted in significant differences in permeation energy profiles.

In addition to considerations of the effects of flexibility, it must be recalled that such energetic analyses are an approximation, as the underlying force fields do not

allow for any electronic repolarization as the cation moves relative to the oxygen atoms lining the filter. Treatment of the latter effect by density functional theory calculations (Guidoni and Carloni, 2002) suggests it may play an important role in the mechanism of permeation and selectivity.

16.5.2 Filter Flexibility

The conformational dynamics of the selectivity filter of K channels are also of interest in the context of possible gating mechanisms. For example, MD simulations of KcsA and of KirBac1.1 provide evidence both for limited (<0.1 nm) filter flexibility during the concerted motion of ions and water molecules within the filter (see above), but also for more substantial distortions (Fig. 16.6). In particular, in simulations of both KcsA and KirBac, occasional peptide bond "flips" are seen, especially for the valine carbonyl of the TVGYG motif, such that one of the peptide backbone oxygen atoms point away from the filter instead of toward it. Clearly, such events will result in a significant change in the permeation energy landscape for the duration of the "flip." If simulations are performed in the *absence* of K^+ ions, then a more profound distortion of the filter occurs, resulting in, e.g., three of the four valine carbonyls

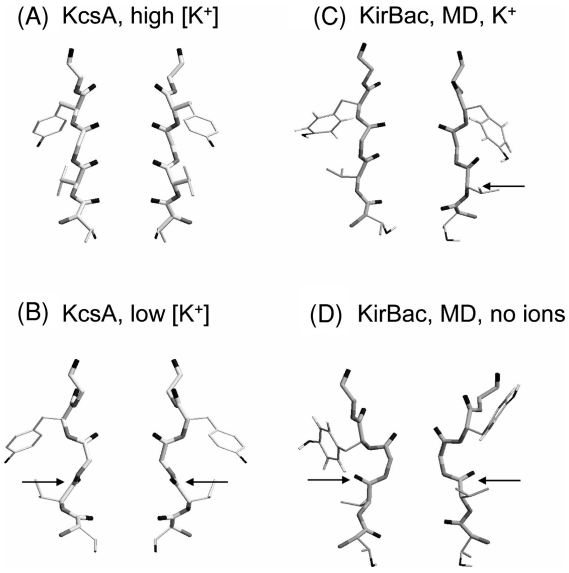

Fig. 16.6 Comparison of the structures of K channel selectivity filter in X-ray crystallographic structures and MD simulations. In each case the backbone (thick bonds) and side chain (thin bonds) atoms of just two subunits of the filter are shown. (A) KcsA, crystallized in the presence of a high concentration of K^+ ions (PDB code 1K4C); (B) KcsA, crystallized in the presence of a low concentration of K^+ ions (PDB code 1K4D); (C) KirBac, midway through a 10 ns MD simulation in the *presence* of K^+ ions (at sites S2 and S4 of the filter—see Fig. 16.4); and (D) KirBac, at the end of a 10 ns MD simulation in the *absence* of K^+ ions. The flipped carbonyls of the valine residue of T**V**GYG are indicated by arrows.

pointing away from the pore. Such changes in filter conformation are expected to lead to functional closure of the channel. These simulations suggest that filter distortions may provide a mechanism of K channel gating, in addition to larger scale changes in the conformation of the hydrophobic gate formed at the intracellular crossing point of the M2 helices. Indeed, certain mutations of Kir6.2 channels in the filter region have been shown to result in changes in "fast gating" of the channel (Proks et al., 2001), and simulations based on models of such mutants suggest they may promote a change in the conformational dynamics of the filter region (Capener et al., 2003).

It is of particular interest that the simulation results on filter flexibility correlate well with structural studies. Thus, comparison of the filter conformations generated during simulations in the absence of K^+ ions (Fig. 16.6D) with the structural change in KcsA crystals resulting from a low concentration of K^+ ions (Fig. 16.6B) shows that in both cases the change in conformation relative to the X-ray structure in the presence of a high concentration of K^+ involves movement of the carbonyl oxygen of the valine away from the pore. The conformational change is less pronounced in the X-ray structure/reflecting the presence of K^+ ions, albeit at reduced occupancy. The relationship of such local changes in conformation, which will result in changes in the energy landscape of permeation, to changes in channel conductance and fast gating continues to be an active area of research. For example, simulation-based estimates of ion permeation free energies based on the low K^+ conformation of KcsA suggest it is nonconducting (Bernèche and Roux, 2005). It is clear from all of these studies that relatively minor changes in channel conformation can have a profound effect on channel properties.

16.5.3 Channel Gating

As noted above, the time scale of channel gating is too long to enable this process to be studied directly by MD simulation. However, a combination of simulations of simple models, homology modeling of various channel states, and PCA analysis of K channel simulations has provided information on the nature of the conformational transitions that may underlie K channel gating.

Examination of the structures of K channels suggests that the main gate is at the intracellular mouth of the channel, where the pore-lining M2 (or S6) helices come together in the closed state of the channel to form a narrow hydrophobic region. Comparison of the structures of KcsA (closed state) and of MthK (crystallized in an open state) indicated a change in conformation and orientation of the M2 helices resulted in opening of the gate, seen as a loss of the hydrophobic constriction at the intracellular mouth.

The fundamental properties of hydrophobic gating in ion channels have been studied using simulations of simple nanopore models (Beckstein et al., 2001, 2003, 2004; Beckstein and Sansom, 2003, 2004), in particular to establish the relationship between pore radius and hydrophobicity of residues lining the ion pathway. The results of these studies suggest that a purely hydrophobic pore of radius $<\sim$0.8 nm may be functionally closed, even though it is not sterically occluded. This is

because even at a radius of ∼0.6 nm there is a considerable energetic barrier to water entering a hydrophobic pore, which therefore prevents a complete solvation shell entering alongside an ion (Beckstein et al., 2004).

As mentioned above, comparison of X-ray structures suggests a role for the conformational dynamics of the pore-lining M2 (or S6) helices in the gating mechanism of K channels. This is supported by spectroscopic data (Perozo et al., 1998, 1999; Liu et al., 2001), by early simulations (Biggin et al., 2001), and by mutation studies on a number of K channels. In particular, it is suggested (Jiang et al., 2002b) that a glycine residue in the M2 helix of, e.g., KcsA and MthK channels forms a molecular hinge that enables hinge-bending of the M2 helices so as to switch the channels from a closed to an open conformation. The proposed molecular hinge corresponds to a conserved glycine residue (Jiang et al., 2002b) present in the sequence of most K channels. In Kv channels the situation may be somewhat more complex, with a possible second hinge lower down the S6 helix associated with a conserved PVP sequence motif (Camino and Yellen, 2001; Bright et al., 2002; Beckstein et al., 2003; Bright and Sansom, 2004; Webster et al., 2004). In the case of Kv channel mutations in the vicinity of both the conserved glycine hinge (Magidovich and Yifrach, 2004) and the second (PVP) hinge result in perturbations of channel gating (Hackos et al., 2002; Labro et al., 2003; Sukhareva et al., 2003). For Kir channels, proline scanning mutations combined with molecular modeling (Jin et al., 2002) support the proposed M2 helix glycine molecular hinge. Mutational studies of the more distantly related bacterial sodium channel NaChBac suggest a glycine hinge may also be present in the pore-lining helices of sodium channels (Zhao et al., 2004).

A number of modeling and simulation studies, ranging from early in vacuo simulations (Kerr et al., 1996) to more recent simulations in either a membrane mimetic octane slab and/or in a lipid bilayer (Shrivastava et al., 2000; Tieleman et al., 2001; Bright et al., 2002), have been performed on models of the isolated S6 helix from *Shaker* Kv channels. All of these indicated the presence of a dynamic hinge (Sansom and Weinstein, 2000) in the S6 helix induced by the PVP motif, demonstrating that this is a region of intrinsic flexibility in the pore-lining helix. More recent studies have focused on simulations of a model of the pore domain [i.e. $(S5\text{-}P\text{-}S6)_4$] of *Shaker* Kv channels, again in a membrane mimetic environment (Bright and Sansom, 2004). These also yield S6 structures kinked in the vicinity of the PVP motif. Comparison with the corresponding S6 helix from the X-ray structure of Kv1.2 (see Fig. 16.7) reveals that the conformations of S6 from the simulated model are very similar to those captured in the experimental crystal structure. It is also informative to compare the structure of the S6 tetrameric bundle from the modeling and simulation study with that present in the X-ray structure (Fig. 16.8). From this it is evident that not only are the S6 helices kinked in both structures, but the way in which the helices are packed around the central pore is very similar. This suggests that a combined modeling/simulation approach can be genuinely predictive in terms of understanding how pore-lining helices behave during channel gating.

Based on these studies of modeling the S6 helix of Kv channels, more extended simulation studies have been used to explore the S6 (or M2) hinge-bending

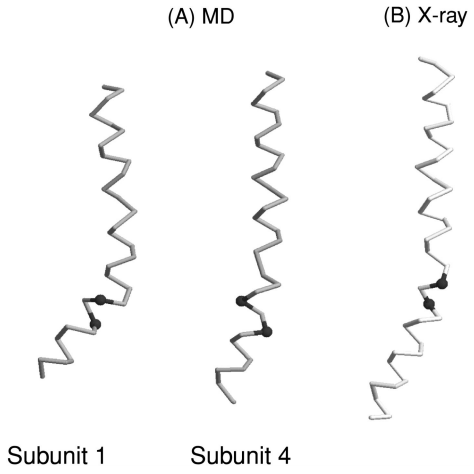

Fig. 16.7 Kv S6 helices from simulations and X-ray structures compared. In (A) two S6 helices from a simulation of the *Shaker* Kv pore domain [i.e. $(S5\text{-}P\text{-}S6)_4$] are shown (Bright and Sansom, 2004); in (B) the corresponding S6 helix from the X-ray structure of Kv1.2 (Long et al., 2005) is shown. The Cα atoms of the prolines of the PVP motif are shown as dark grey spheres.

hypothesis of gating of Kv (or Kir) channels. By simulating just the transmembrane pore-forming domain of Kv or KirBac channels in a membrane-mimetic octane slab, it is possible (within the 10–20 ns time scale accessible to such simulations) to explore the intrinsic flexibility of the S6 (or M2) helices. Combined with PCA of the simulation data, such studies enable us to characterize possible conformational changes underlying the channel-gating mechanism, uncoupled from the "gate-keeping" role of the of the voltage-sensor S1–S4 domain (in Kv channels) or the ligand-binding intracellular domain (in Kir channels).

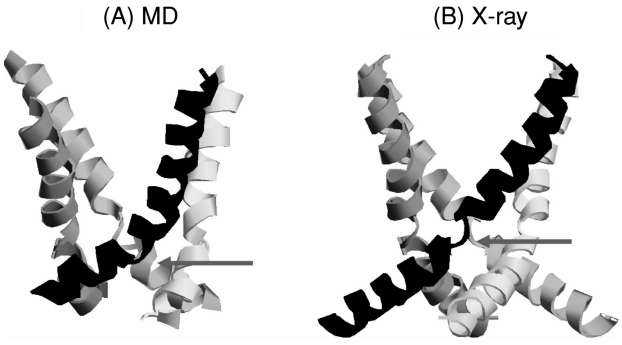

Fig. 16.8 Comparison of the S6 helix bundle from (A) a simulation of the *Shaker* Kv pore domain (Bright and Sansom, 2004); and (B) the X-ray structure of Kv1.2 (Long et al., 2005). The location of the PVP motif-induced hinge is indicated by the grey arrows.

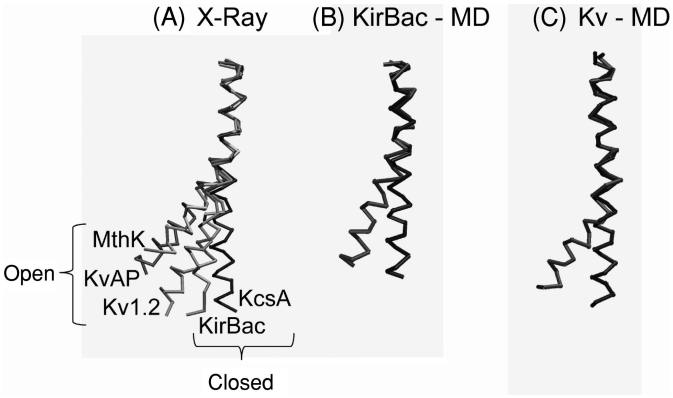

Fig. 16.9 Comparison of X-ray crystallographic and MD simulation-generated structures for the pore-lining M2 or S6 helices of K channels. In (A) the M2 helices from the crystal structures of KcsA and of KirBac1.1 (both closed pores), along with the M2 helix from MthK, and the S6 helices from KvAP and Kv1.2 (all three with open pores) are shown. In (B) selected structures are shown of M2 helices from the start (0 ns) and end (20 ns) of a simulation of KirBac1.1 in an octane slab (Grottesi et al., 2005) are shown. In (C) selected structures are shown of S6 helices from the start (0 ns) and end (9 ns) of a simulation of *Shaker* Kv channel in an octane slab. In all three diagrams, the N-terminal halves (i.e., before the molecular hinge) of the helices are used for fitting.

The results of such studies are summarized in Fig. 16.9, where snapshots from simulations are presented alongside superimposed X-ray structures of pore-lining helices from K channels. For KirBac, it can be seen that the M2 helix has a molecular hinge associated with the conserved Gly134 residue (Fig. 16.9B). PCA of the motions of M2 in KirBac simulations reveals that the first two eigenvectors correspond to helix kinking and swivelling about the glycine hinge (Grottesi et al., 2005). A comparable analysis of Kv channel simulations (Grottesi and Sansom, unpublished results) suggests that in this case the molecular hinge is associated with the PVP motif lower down the S6 helix from the conserved glycine (Fig. 16.9C). This is in agreement with the modeling and simulation studies of S6 helices in membrane and membrane-like environments discussed above.

One may attempt a simple calculation of the energetic barrier presented by the hydrophobic gate region in the Kv-closed model and how this is altered in the Kv-open model. One may employ a Born energy calculation to obtain a first approximation to the barrier height presented by a hydrophobic gate (Beckstein et al., 2004). Compared to calculations of the barrier height based on atomistic (MD) simulations and umbrella sampling, such as Born energy calculation underestimates the barrier height for hydrophobic pores of radius $>\sim$3.5 Å, and overestimates it for pores of radius $<\sim$3.5 Å. The pore radius profile of the Kv-closed model (discussed above) has an average hydrophobic gate radius of $\sim$1.4 Å, extending over a length of $\sim$20 Å. The minimum radius is $\sim$1 Å, at the cytoplasmic end of

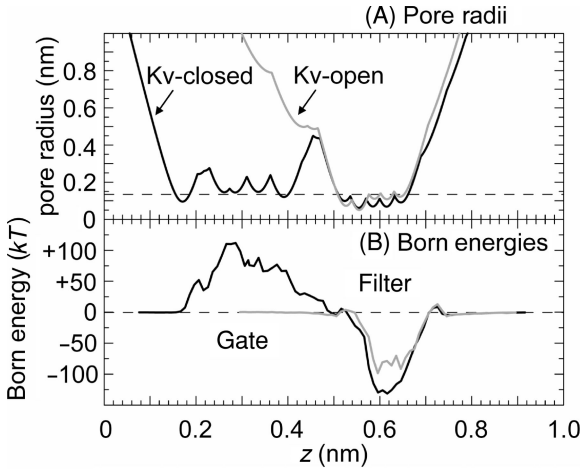

Fig. 16.10 Comparison of Kv-closed and Kv-open models. (A) Pore radius profiles and (B) Born energy profiles for the two models shown in Fig. 16.2. In each graph the black line corresponds to Kv-closed, and the grey line to K-open. [The Born energy calculation was performed essentially as described in (Beckstein et al., 2004).]

the gate (Fig. 16.10A). A Born energy calculation for the Kv-closed model yields a barrier of height $\sim 70kT$ in the center of the gate, rising to $\sim 100kT$ at the narrowest region of the gate (Fig. 16.10B). Even if, based on the comparison of Born energies and atomistic simulation-based free energies for simple nanopore models, we allow for an approximately twofold overestimation of the Kv-closed barrier height by the Born energy calculation, this still yields an estimated barrier height of $\sim 50kT$ for the Kv-closed channel. This model clearly corresponds to a fully closed conformation of the channel. For comparison, there is virtually no barrier present in the Kv-open model. Thus, the S6 helix bending motions seen in the Kv simulations are sufficient to switch fully the pore from a functionally closed to a functionally open state.

16.5.4 Regulation of Channel Gating

It is also possible to probe the motions of the "gate-keeping" domains of K channels by MD simulation. Of course, caveats regarding simulation time scales vs. gating time scales similar to those expressed for gating per se apply, but the simulations do reveal aspects of the intrinsic flexibility of domains, and hence provide valuable clues as to the overall channel-gating mechanism. For example, the X-ray structure of the intracellular domain of the Kir channel Kir3.1 has been determined (Nishida and MacKinnon, 2002), enabling simulations of the conformational dynamics of the intracellular domains of Kir3.1 and of related Kir channels. Like the TM domain, the intracellular domain forms a tetramer. Multiple 10 ns duration simulations of two

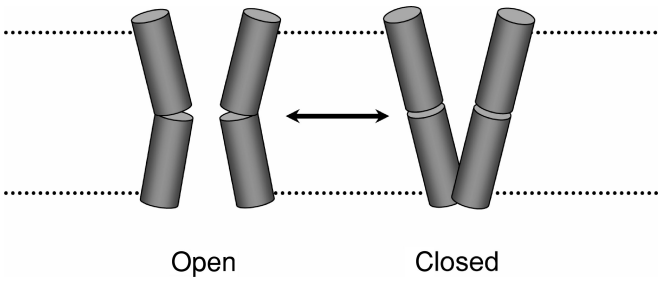

Fig. 16.11 Proposed gating mechanism of K channels. The pore-lining S6 or M2 helices of two opposite subunits are shown. In the open state these helices are kinked; in the closed state the helices are undistorted and cross at their C-termini to form a hydrophobic constriction.

different Kir intacellular domain tetramers (of Kir3.1 and Kir6.2) combined with PCA reveal loss of exact fourfold symmetry (Haider et al., 2005). This is consistent with "dimer-of-dimers" motion of the subunits in the intracellular domains. Combining this analysis with the results of simulations of the TM domain (see above), enables us to propose a Kir-gating model in which a transition between exact tetrameric symmetry (channel open) and dimer-of-dimers symmetry (channel closed) is associated with a change in M2 helix packing and kinking coupled to gating of the channel (Grottesi et al., 2005). This provides a simple example of how combining simulation and modeling studies may provide clues as to the gating mechanisms of complex ion channels. Overall, structural and simulation studies suggest a general model of K channel gating (Fig. 16.11) in which the "gate-keeper" domain (e.g., the voltage sensor in Kv channels or the intracellular domain in Kir channels) regulates the switching of the pore-lining helices between an open (kinked) conformation and a closed (distorted) conformation.

16.6 Future Perspectives

The results reviewed above indicate how MD simulations help to contribute to our current understanding of K channels. In particular, they have provided information on the nature of ion permeation through the selectivity filter, and on the flexibility of the filter in relationship to ion permeation and to "fast gating." More recently, combining MD simulations of K channel components with PCA has enabled us to isolate key motions that provide clues as to the underlying gating mechanisms of Kv and Kir channels.

Future studies will have to address at least two aspects of K channel simulations. One is that of time and length scales. There is a pressing need for simulation approaches that allow us to explore larger time and length scale motions. One possibility lies in the use of Gaussian network simulations (Erkip and Erman, 2003). However, this and other coarse-grained simulation approaches need to be explored further, and tested on a number of membrane protein systems. A second area is that of

extending models and simulations to a wider range of K channels, and to extending the realism of the biological environment present in the simulations. To date, most simulation studies have been of bacterial K channels in a simple model membrane (e.g., phosphatidyl choline). However, from a physiological perspective, one would like to extend simulations to realistic models of mammalian K channels in a complex (i.e., mixed lipid) membrane environment. This is of particular importance given the role of some lipids (e.g., phosophatidyl inositol phosphate) in regulation of channel gating. Recent simulation studies [e.g., of KcsA (Deol et al., 2005; Sansom et al., 2005)] provide some encouragement that extended simulations in more complex lipid bilayers will be able to capture at least some aspects of specific channel–lipid interactions.

Acknowledgments

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