Mechanosensitive Ion Channels

Forewords by Max J. Lab and Jürgen Hescheler
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Chapter 4
Computational Studies of the Bacterial Mechanosensitive Channels

Ben Corry and Boris Martinac

Abstract  Bacterial mechanosensitive (MS) channels were first documented in giant spheroplasts of *Escherichia coli* during a survey of the bacterial cell membrane by the patch clamp some twenty years ago. Two major events that greatly advanced and kept the research on bacterial MS channels at the forefront of the MS channel research field include: (i) cloning of MscL and MscS, the MS channels of Large and Small conductance, and (ii) solving their 3D crystal structure. In addition to advancing further experimental studies of the bacterial MS channels by enabling the use of new techniques, such as EPR and FRET spectroscopy, these events also enabled theoretical approaches to be employed. In this chapter we will review recent computational approaches used to elucidate the molecular dynamics of MscL and MscS, which has significantly contributed to our understanding of basic physical principles of the mechanosensory transduction in living organisms.

Key words: MS channels  Patch clamp  Bilayer model  Mechanosensory transduction  EPR spectroscopy  FRET  Molecular dynamics  Brownian dynamics

4.1 Introduction

Studies of mechanosensitive (MS) channels in bacteria were facilitated by the advent of the patch-clamp technique (Hamill et al., 1981), which has removed the constraints of being able to study electrophysiologically only cells that are large enough to be impaled with glass microelectrodes. Development of a "giant spheroplast" preparation, which was essential for the examination of a bacterial cell membrane by the patch clamp technique (Ruthe and Adler, 1985; Martinac et al., 1987), led to the discovery of MscS and MscL, two of the several types of MS channels existing in bacteria (Berrier et al., 1996; Martinac, 2001; Blount et al., 2007). Both channels were cloned several years after their discovery (Sukharev et al., 1994; Levina et al., 1999). Given that bacteria can be grown in large quantities delivering milligram amounts of channel proteins, 3D structures of both MscL (Chang et al., 1998) and MscS (Bass et al., 2002) became available just a few years after their cloning (Fig. 4.1). This opened a window of opportunities to conduct studies relating
the structure and function of these MS channels by employing a whole array of structural methods and techniques including 2D electron crystallography (Saint et al., 1998), electronparamagnetic resonance (EPR) spectroscopy (Perozo et al., 2001; Perozo et al., 2002a, 2002b; Tsai et al., 2005), fluorescence resonance energy transfer (FRET) spectroscopy (Corry et al., 2005) as well as computational modelling based on molecular dynamics (Gullingsrud et al., 2001; Gullingsrud and Schulten, 2003; Sotomayor and Schulten, 2004; Sotomayor et al., 2006; Meyer et al., 2006) and Brownian dynamics (Vora et al., 2006). Together, all these approaches have provided a wealth of information on the structural determinants of gating and conduction of the two MS channels. In this review we have summarized the key theoretical findings on the structure and function of MscL and MscS. See the Chapter by Paul Blount and colleagues in this volume for general information on bacterial MS channels not covered here.

4.2 Molecular Dynamics Studies of MscL

The determination of the 3D structure of the MscL homologue from *Mycobacterium tuberculosis* (Tb-MscL) by X-ray crystallography showed that the MscL protein forms a homopentameric channel (Chang et al., 1998) (Fig. 4.1A). Each subunit of the pentamer consists of two transmembrane α-helices TM1 and TM2 and a third cytoplasmic α-helix. The TM1 transmembrane domain (residues 15–43) is connected to TM2 domain (residues 69–89) by a periplasmic loop which extends
into the pore region and lines the external face of the channel. The TM2 helix is continued by a second loop (residues 90–101) leading to the cytoplasmic helix (residues 102–115) at the C-terminal end of the protein facing the cytoplasm. Each TM1 helix is in contact with two TM1 helices from adjacent subunits and two TM2 helices of the channel pentamer, one from the same subunit and the other from the neighboring subunit. The helices are slanted and the five TM1 helices join together at the cytoplasmic side of the pentamer (Fig. 4.1A). The ion conduction pathway of the MscL channel is formed by the TM1 transmembrane helices with their hydrophilic residues lining the channel pore. The pore is approximately 18 Å in diameter at the periplasmic side but narrows to an occluded apex at the cytoplasmic side (approximately 2 Å in diameter). The tight constriction, which is believed to act as the channel gate, is formed by hydrophobic residues (5 Val and 5 Ile) that are highly conserved among bacterial members of the MscL family (Oakley et al., 1999). The open channel conductance of the pore, on the other hand, suggests a much wider channel in the order of 30 Å in diameter which implies a very large conformational change upon channel gating (Crucickshank et al., 1997).

In order to conduct molecular simulations of an ion channel, it is crucial to have atomistic resolution structures of the pore. Thus, a large number of simulation studies aimed primarily at unravelling the gating pathway of the channel and how the protein detects changes in membrane tension or composition have emerged since the publication of the Tb-MscL crystal structure. Bacterial MS channels directly sense tension developed in the bilayer alone (Markin & Martinac 1991), and this mechanosensitivity is maintained when the channel has been reconstituted into artificial liposomes in which no other proteins or cytoskeleton are present (Perozo et al., 2002; Delcour et al., 1989; Berrier et al. 1989; Häse et al., 1995). In particular, evidence suggests that MS channels respond only to tension in the plane of the membrane and not that perpendicular to it (Gustin et al., 1998; Sokabe & Sachs 1999). The two main questions that computational studies have aimed to elucidate, therefore, is how the lateral tension in the membrane is sensed and transformed into structural changes, and what are the conformational changes involved in moving the closed to open states of the channel.

MS channel proteins could in principle sense bilayer tension by interacting with the membrane in a number of ways. These interactions could simply be Van der Waals contacts between the non-polar regions of the protein and lipid, or could rely on specific hydrogen bonds forming between parts of the protein and the lipid head groups. EPR studies of MscL demonstrated that hydrophobic mismatch, although not the driving force that triggers MscL opening, could stabilize intermediate conformational states of the channel along the kinetic path towards the open state (Perozo et al., 2002a, 2002b). The same studies suggested further that addition of polar lipids, such as lysophosphatidylcholine (LPC), to one monolayer of liposomes reconstituted with MscL channels created local stresses in the lipid bilayer leading to Redistribution of the transbilayer pressure profile sufficient to open the channel. MscL could sense these stresses and open in the absence of externally applied membrane tension.

A number of computational studies have been undertaken to help determine if coupling between the membrane and the MscL protein is simply a result of
van der Waals interactions and hydrophobic matching, or whether hydrogen bonds with the lipid headgroups are important. In an analytic study of the energetics of channel opening, Wiggins and Phillips (2004) proposed the competition between the hydrophobic mismatch of the protein and the applied membrane tension result in a bistable system representing the open and closed states of the channel. This model suggests that hydrophobic matching may be all that is required to induce gating within the protein. Furthermore it is able to make some predictions about how altering the composition of the lipids or protein can influence the hydrophobic contacts and thus alter the energetics of channel gating. Similarly the influence of membrane curvature can be examined within this framework.

Atomistic simulations have been used to help understand why changing the nature of the lipid headgroups or the length of the lipid chains alters the pressures required to initiate channel gating. Elmore and Dougherty (2001) examined the Tm-MscL in a POPE bilayer using molecular dynamics (MD) simulations. A very large number of hydrogen bonds were formed between the lipid headgroups and the protein, primarily with either the C-terminal domain or a localised region of the periplasmic loop suggesting that these portions of the protein may be important for sensing changes in membrane tension. Exchanging the POPE bilayer for POPC resulted in many less hydrogen bonds forming, which provides a possible mechanism for explaining the different gating tensions observed in membranes of different lipid composition (Elmore and Dougherty, 2003). Although positng hydrogen bonds with the lipid headgroups as being important for linking the protein to the membrane appears contrary to the notion that hydrophobic mismatch is the cause of channel gating it is possible that these two effects could reinforce each other. Both these links between the protein and membrane would be affected in complimentary ways by membrane thinning. The more specific nature of the hydrogen bonds, however, may impose more conformational restrictions upon the protein during gating than would generic hydrophobic contacts.

An alternative explanation for the influence of lipid composition on MscL gating has also been presented, in which the differing pressure profiles of the lipids could influence the forces exerted upon the MscL protein. Gullingsrud and Schulten used MD simulations to determine the lipid bilayer pressure profiles in POPE and POPC membranes (Gullingsrud and Schulten, 2004). A non uniform pressure profile exists across the membrane as the hydrophilic headgroups are squeezed together to avoid water contacting the hydrophobic tails while maintaining a nearly constant volume in the lipid. In POPC bilayers the lateral pressure is concentrated near the headgroups, but stretching the membrane concentrates the pressure closer to the start of the aliphatic chain, a phenomenon that also arises when switching to POPE. Furthermore, if the length of the lipid tails is shortened in MD simulations this results in a thinning of the protein created by pore constriction and helix kinking to avoid a hydrophobic mismatch (Elmore and Dougherty, 2003).

In parallel to the studies examining the protein-lipid interactions, a number of computational investigations have taken place to examine the conformational changes involved in gating. The closed state of the pore can be expected to be relatively stable, as the free energy difference between the closed and open states of the protein is estimated to be \( \sim 17 \) kT (Martinac, 2001). Therefore, it is not surprising
that when the protein is placed in a bilayer with no applied tension or force, the transmembrane helices all remain relatively static and the pore remains occluded in MD simulations (Colombo et al., 2003; Gullingsrud et al., 2001; Elmore and Dougherty, 2001). The extramembrane regions of the protein (C-terminal domain and periplasmic loop) do display more mobility, but it is not clear that these represent functional motions.

Large conformational changes can be observed in MD simulations, however, when the protein is influenced by an external force. In particular, it is hoped that external forces that mimic those presented to the protein by membrane tension may be able to initiate the large structural changes involved in gating within the nanosecond timescales achievable in molecular simulations. Many simulations have been carried out to investigate the nature and sequence of these conformational changes, differing most notably in the way that the external force is applied to the protein. Gullingsrud and Schulten applied surface tension to the bare Tb-MscL protein (not in a lipid bilayer) (Gullingsrud et al., 2001) as well as direct, predominantly radial forces to selected regions of the Eco-MscL protein that would contact the lipid to mimic the membrane pressure profiles (Gullingsrud and Schulten, 2003). Bilton and Mylvaganam (2002) examined direct force application to the outer TM2 helix and anisotropic pressure coupling also to a bare Tb-MscL protein. Colombo et al. (2003) applied pressure to the membrane around Tb-MscL, and finally Kong et al. (2002) used targeted molecular dynamics to force a conformational change of Eco-MscL from the crystal structure to a pre-determined end point. Not surprisingly, the different methods of force application resulted in differing conformational changes, although some common features are evident.

In all cases the application of force resulted in a flattening of the protein in the direction of the pore axis due to an increased tilt in the TM1 and TM2 helices, an increase in the in-plane area of the protein, as well as a shortening of the pore, all of which are in agreement with EPR studies (Perroz et al., 2002b) and results obtained by engineering disulfide bonds between subunits (Betanzos et al., 2002). This type of motion reproduces the models that suggest an iris like opening of the pore. In most cases some degree of opening of the pore constriction was observed during the simulations, provided that the strength of the applied force or pressure was above a critical point. In these cases water enters the entire length of the pore. The force or pressure required to observe this was always higher than that used experimentally to open the pore otherwise conformational changes would not occur during the short length of the simulations (typically <10ns).

Given the strength of the forces used in these molecular simulations, some caution must be taken in assuming that the conformational changes seen represent those taking place in reality. The influence of the external force on the structural changes observed can be seen from the fact that the channel opening events and their ordering differ among the various simulations. The first study of Gullingsrud et al. (2001) showed two distinct conformational changes: a widening of the extracellular end of the pore created by a retraction of the periplasmic loop, followed by a simultaneous shift in the angle of the TM1 and TM2 helices. Their second study, however, did not show much movement by the periplasmic loop (Gullingsrud and Schulten, 2003). Instead, the initial motion was a widening of the cytoplasmic end caused by a tilting
of the transmembrane helices, followed by a simultaneous expansion of both the TM1 and TM2 helices that forced the hydrophobic constriction apart. Colombo et al. (2003) saw similar helix tilting, but noted that the TM1 helix only moved after the TM1 had made space for it and also that the tilt of the TM2 helix was greater than that of the TM1 helix unlike in the simulations of Gullingsrud et al. (2001).

Due to the short length of the simulations employed, none of the studies discussed so far claim to witness the entire gating process. Kong et al. on the other hand (Kong et al., 2002) do reach a final open state due to the targeted nature of their simulations in which they force the simulation to end at a pre-determined open state. Some caution must be used, however, in interpreting the sequence of opening events seen in this study. As the driving force in this simulation is the distance of the atoms from their final location, the resulting order of events is likely to favor large changes before small ones and may not reproduce the sequence undergone in reality. Furthermore, the structural changes will be highly dependent upon the conformation chosen to represent the final open state given that there is no detailed experimental evidence to support this choice.

The radial forces applied in all these studies are used to speed up conformational changes that may be associated with gating, but it is possible that such forces bias protein motions. For example, radial movements may take place sufficiently quickly such that movements in other directions do not have time to take place. To avoid these difficulties, Meyer et al. (2006) examined the conformational changes displayed in MD simulations of Eco-MscL embedded in a curved bilayer composed of single and double tailed lipids without the application of any external force (Fig. 4.2). This is meant to reproduce the conditions seen in patch clamp experiments in which the bilayer is bent through suction, although the degree of bending is again necessarily more in the simulation than in reality. Although the conformational changes seen in this 9.5ns simulation were not as large as in some of those using external forces and channel opening was not seen, some interesting motions possibly associated with channel gating were observed. In particular, the

![Fig. 4.2 Conformational changes of MscL in a curved bilayer studied by molecular dynamics simulation. (A) Side view and (B) top view of MscL at the beginning (left) and end (right) of the 9.5ns simulation. The C-terminus has been cut-off in the simulation and is not shown. (Reproduced from Meyer et al., 2006).](image-url)
periplasmic loop was seen to be particularly mobile, something that may have been overlooked in previous simulations. There is evidence that this loop is important in channel gating as cutting it with proteases (Ajouz et al., 2000) or reconstituting protein without this connection between the C- and N-terminal halves of the protein (Park et al., 2004) resulted in functional channels with increased pressure sensitivity. This loop may possibly have to move first to allow the transmembrane helices to adjust to pressure.

Two alternatives to direct simulations of the protein have also been utilised to witness channel openings: normal mode analysis in which local fluctuations of the protein about a minimum energy conformation are extrapolated to show large scale conformational changes, and continuum models in which a non-atomistic description of the system is used. Some common motions were extracted from a normal mode analysis starting from a number of different closed and open structures (Valadie et al., 2003). Most notable was a twist and tilt of the helices, in particular TM1, as suggested previously. Notably the top half of the TM2 helix was seen to be less mobile than the bottom which resulted in a kinking of this helix, a property that had also been suggested in some of the MD studies (Meyer et al., 2006; Kong et al., 2002). Another result in common with previous studies included the lack of interaction between the C-terminus and the remainder of the protein and the lack of motion seen in the central gating residues. In order to witness large scale motions in a short time span, Tang et al (2006) developed a continuum modelling approach in which the lipid bilayer and protein are represented as elastic sheets and rods rather than as atomistic structures. Using this simplified model a widening of the pore is observed upon membrane stretching created by helix tilting and radial movement caused by a thinning of the membrane, but no significant opening was seen with membrane bending.

### 4.3 Computational Studies of MscS

The mechanosensitive channel of small conductance, MscS, is an archetypal mechanosensor. Unlike MscL proteins, which are almost exclusively found in bacterial cells, MscS-like proteins are widely spread among Gram-negative and Gram-positive bacteria, archaea, fungi and plants where they function in cellular processes underlying osmoregulation and growth (Martinac, 2006). They constitute a large sub-family of MS channels (Martinac and Kloda, 2003; Pivetti et al., 2003).

Shortly after MscS was cloned (Levina et al., 1999) its structure was solved by X-ray crystallography at a resolution of 3.9 Å (Bass et al., 2002). MscS forms a homoheptameric channel with a diameter of 80 Å and a length of 120 Å (Fig. 4.1B). Each subunit contains three transmembrane domains, with N-termini facing the periplasm and large C-termini extending into the cytoplasm. The transmembrane helices TM1 and TM2 are considered to constitute the sensors for membrane tension and voltage (Bass et al., 2002; Bezanilla & Perozo, 2002), whereas TM3 helices line the channel pore and facilitate the channel opening by slight iris-like rotations and tilting (Edwards et al., 2005) and possibly unkinking (Sotomayor et al., 2007).
A Gly-Ala pattern in this helix faces the pore and appears to be an important structural motif supported by the fact that it is highly conserved in the MscS sub-family of MS channel proteins (Martinae and Kloda, 2003; Pivetti et al., 2003).

Computational studies have hoped to help answer a number of unresolved questions relating to this channel. The first of these is surprisingly simple: what is the conduction state of the crystallized protein? It is obvious from the published structure that there is a large non-occluded pore passing across the membrane (Fig. 4.3) indicative of an open ion conductive pore. Noting this, Bass et al (2002) suggested that the protein has most likely been imaged in an open state. This is somewhat surprising, as the closed state of MscS is usually favoured by ~7 kcal/mol in membranes in the absence of lateral tension, but as the protein was crystallized from detergent micelles rather than in lipid this may have altered the resting state of the channel. Closer inspection of the structure, however, indicates that the narrowest region of the pore has a diameter of only ~3.5 Å, and is surrounded by two rings of non-polar leucine residues (L105 and L109). It has been suggested that such narrow hydrophobic pores can prevent the passage of ions without presenting a physical occlusion.

One way to try and ascertain the conduction state of the imaged structure is to try to determine the conductance of the protein directly from simulations and compare these to known experimental values. Although this approach is appealing, it suffers one major problem: it is computationally very demanding to conduct atomistic simulations for long enough to witness many ions passing through the pore as is required to accurately determine the channel conductance. To overcome these problems, mesoscopic simulation techniques have often been employed in which some of the atoms in the system are not treated explicitly in order to reduce the

**Fig. 4.3** MscS channel model used for Brownian dynamics simulations of the conduction properties of the channel. (A) Schematic of the MscS crystal structure with the top half removed to reveal the pore. (B) Schematic of the transmembrane region of an expanded pore structure. The highlighted residues, starting from the intracellular side, are residues L109 and L105 that line the expanded part of the channel, and R88, the only charged amino-acid residue lining the pore. (Reproduced from Vora et al., 2006)
computational demand and allow the current to be determined explicitly. In two such studies of the MscS channel (Sotomayor et al., 2006; Vora et al., 2006), the protein, lipid and water are treated as continuous dielectric media (Fig. 4.3). Ions are treated explicitly and move under the electrostatic force induced by the membrane potential, partial charges of the protein atoms and other ions, as well as experiencing scattering and frictional forces to replicate the collisions with water molecules or the protein boundary. Both studies showed that when the protein is held in its crystal structure the current (carried almost exclusively by anions) is much below recorded single open channel currents, supporting the notion that this does not represent the open state of the pore. Simulations using widened structures demonstrate that only minimal structural changes are required to create a highly conductive pore (Sotomayor et al., 2006; Vora et al., 2006).

Recently, all atom molecular dynamics simulations have also been used to estimate the conductance of the pore. Spronk et al (2006) witnessed ionic currents in their MD simulations of the MscS pore under large applied potentials and Sotomayor et al. (2007) also measured currents in a range of situations. Some caution should be applied when directly interpreting the conductance measured in these high voltage simulations as most channel I-V curves become non-linear under large applied potentials, but the picture emerging is consistent with other results. When the protein is held constrained near the imaged structure, the conductance of the pore is much smaller (~200ps) than that measured experimentally for the open state (~1000ps) (Sotomayor et al. 2007). Furthermore, the current is carried almost exclusively by anions, rather than being only slightly anion selective as is found in patch clamp recordings (Martina et al., 1987; Sukharev et al., 1993). Together these results support the idea that the pore has been crystallized in neither the fully open nor fully closed states, and more likely represents some intermediate or inactive configuration.

The likely conductance state of the crystallized structure can also be probed by a less direct method that examines the behaviour of water in the pore. A number of molecular dynamics investigations of model pores (Beckstein et al., 2001; Beckstein and Sansom, 2003; Beckstein and Sansom, 2004), carbon nanotubes (Hirmer et al., 2001; Wan et al., 2005), and the nicotinic acetylcholine receptor (Corry, 2006; Beckstein and Sansom, 2006) have shown that water tends to evacuate the hydrophobic regions of such pores under certain conditions. In particular, if the radius of the pore is under a certain critical value that depends on its particular geometry and surface character, then the pore is often empty. But, if the radius is increased or a more polar surface is introduced then water will fully hydrate the channel. This critical radius is around 4–4.5 Å for the model pores (Beckstein and Sansom, 2004) and nicotinic receptor (Corry, 2006), roughly the radius required to allow three shells of water to enter at which stage the average number of hydrogen bonds per water molecule can approximate that found in the bulk (Anishkin and Sukharev, 2004). If water evacuates a region of a pore it is unlikely that an ion will traverse through it. In these conditions the ion will have to be stripped of most of its hydration shell (some waters may be pulled through the channel with the ion) without being able to compensate this energy with coordination to any polar
groups in the protein as is the case, for example, in the selectivity filter of potassium channels.

As the radius of the hydrophobic constriction in the MscS pore lies below the critical radius for hydration seen for other pores, it is possible that a similar hydrophobic gating mechanism is at play in MscS. Initial molecular dynamics studies of this pore indicated that water does indeed evacuate the constricted region of the MscS pore when it is held constrained near the crystal structure (Sotomayor and Schulten, 2004; Anishkin and Sukharev, 2004). Adding a polar gain of function mutation L109S at this constriction leads to stable hydration of the pore and significantly reduces the barrier to ion permeation (Anishkin and Sukharev, 2004), demonstrating that a change in the surface character of the pore can significantly alter its characteristics.

If an evacuated pore can be equated with a non-conducting state of the channel, then these results support the notion that the crystal structure of the protein represents a non- or low-conducting state. A further complication has emerged from a more recent molecular dynamics study that showed the evacuation of the narrow region of the pore seen in earlier simulations is voltage dependent (Spronk et al., 2006), a result similar to that seen in model hydrophobic pores (Dzubiella et al. 2004). This result has significant implications, suggesting that application of a large enough membrane potential may result in leakage currents in hydrophobically gated pores. It should be noted, however, that a large electric field was required to observe these results. When a more modest fields of 20–50 mV/nm (transmembrane potential ～220–550 mV) were applied the pore did not remain fully hydrated suggesting that the MscS pore could still use a hydrophobic gating mechanism to prevent ion conduction under physiological conditions.

If the imaged protein structure is neither in the open or closed states an obvious question that simulations can help to answer is exactly what the open and closed states look like and how do they differ from the crystallized structure. When no harmonic restraints are applied to the protein in MD simulations to keep it near the imaged structure, the pore has been seen to constrict and become physically occluded (Sotomayor and Schulten 2004; Spronk et al. 2006; Sotomayor et al. 2007). These results support the idea that the imaged state of the channel does not represent the closed state of the pore. In addition, they cast some doubt on whether the pore uses a hydrophobic gating mechanism in which ions are prevented from passing through a non-occluded pore as described previously, as this would not be necessary if the closed state of the pore contains a physical occlusion.

As noted previously, mesoscopic simulations indicate that only minimal widening of the pore is required to greatly increase the channel conductance (Sotomayor et al 2006; Vora et al 2006). Similarly, if the hydrophobic model of channel gating is to be believed, then evidence from other pores would suggest only minor conformational changes are required to open the pore. Such conformational changes have been observed in a number of MD simulations. When a large tension (20 dyn/cm, i.e. 20 mN/m) was applied to the membrane, the pore was seen to widen and remain fully hydrated (Sotomayor and Schulten, 2004). More surprisingly, when a large electric field (～1V) was applied across the membrane in the absence of any constraints, the pore was seen to widen significantly (Spronk et al. 2006;
Sotomayor et al. (2007), presumably related to the ability of water and ions to enter the hydrophobic region of the pore. The widening seen in the most recent study (Sotomayor and Schulten, 2004) has a direct impact on the channel conductance determined directly from MD simulations, yielding a value of 900–1100 ps in agreement with measured open channel values (Martina et al., 1987; Sukharev et al., 1993). Thus, the widened pore witnessed in this study may be representative of the open state of the channel.

The conformation changes involved in gating MscS appear to be much smaller than those required to open MscL, which is not entirely surprising given the difference in currents carried by the two pores. Simulations in which the pore becomes physically constricted suggest a possible location of an occlusion gate. The pore lining TM3 helix in one of the seven subunits buckles slightly, moving Leu105 toward the center of the pore (Sotomayor and Schulten, 2004; Spronk et al., 2006). Salt bridges have also been noted between Asp62 in the TM1-TM2 linker and Arg128 at the cytoplasmic end of the TM3 helix on the adjacent subunit (Sotomayor and Schulten, 2004). The widening of the pore obtained under an applied field proceeded with a straightening of the TM3 helix to remove a kink midway through the membrane and establish contact between this helix and TM2 (Sotomayor et al., 2007). Interactions between the lipid headgroups with a number of charged and polar residues in the TM1 and TM2 helices have been noted (Sotomayor and Schulten, 2004) that may provide a mechanism of linking membrane tension to channel opening. It has been suggested that this link between the lipid contacting TM1-TM2, cytoplasmic domain and pore lining TM3 may be important for gating or stability although no clear conclusions have been drawn.

The crystallized structure of MscS contains a very large cytoplasmic domain (Fig. 4.1B) whose function remains unclear. It extends a long way from the membrane and contains a large central chamber accessible through seven side openings and one distal pore. This domain has been suggested to play a role in channel gating (Koprowski and Kubalski, 2003), desensitization and stability (Miller et al., 2003; Schuman et al., 2004) as well as in ion transport and selectivity (Edwards et al., 2004). Although the seven side entries to the chamber are surrounded by a number of basic amino acid residues that could play a role in valence selectivity, both K⁺ ions and Cl⁻ ions have been seen to pass through them and enter the cytoplasmic chamber in MD (Sotomayor and Schulten, 2004; Sotomayor et al., 2007) and mesoscopic simulations (Sotomayor et al., 2006; Vora et al., 2006), but no ions pass through the distal opening. The distribution of ions within the chamber, however, is such that more Cl⁻ than K⁺ ions reside near the transmembrane pore mouth, thus it is possible that this domain does play an important role in ionic selectivity. However, anion selectivity is also seen in simulations of only the transmembrane portion of the protein, and is largely controlled by the charged residue Lys169 (Vora et al., 2006; Sotomayor et al., 2007). It is possible that this residue is not normally charged, which may explain the extra anion selectivity seen in simulations compared with experiments. The conformation of the cytoplasmic domain has also been shown to influence the conductivity of the pore (Sotomayor et al., 2007). It has also been suggested that this domain may act as a molecular sieve (Koprowski and Kubalski, 2003), preventing large solutes from passing through the channel where they may either block it, or be
Sotomayor et al. (2007), presumably related to the ability of water and ions to enter the hydrophobic region of the pore. The widening seen in the most recent study (Sotomayor et al. 2007) has a direct impact on the channel conductance determined directly from MD simulations, yielding a value of 900–1100 pS in agreement with measured open channel values (Martinac et al., 1987; Sukharev et al., 1993). Thus, the widened pore witnessed in this study may be representative of the open state of the channel.

The conformation changes involved in gating MscS appear to be much smaller than those required to open MscL, which is not entirely surprising given the difference in currents carried by the two pores. Simulations in which the pore becomes physically constricted suggest a possible location of an occlusion gate. The pore lining TM3 helix in one of the seven subunits buckles slightly, moving Leu105 toward the center of the pore (Sotomayor and Schulten, 2004; Spronk et al., 2006). Salt bridges have also been noted between Asp62 in the TM1-TM2 linker and Arg128 at the cytoplasmic end of the TM3 helix on the adjacent subunit (Sotomayor and Schulten, 2004). The widening of the pore obtained under an applied field proceeds with a straightening of the TM3 helix to remove a kink midway through the membrane and establish contact between this helix and TM2 (Sotomayor et al. 2007). Interactions between the lipid headgroups with a number of charged and polar residues in the TM1 and TM2 helices have been noted (Sotomayor and Schulten, 2004) that may provide a mechanism of linking membrane tension to channel opening. It has been suggested that this link between the lipid contacting TM1-TM2, cytoplasmic domain and pore lining TM3 may be important for gating or stability although no clear conclusions have been drawn.

The crystalized structure of MscS contains a very large cytoplasmic domain (Fig. 4.1B) whose function remains unclear. It extends a long way from the membrane and contains a large central chamber accessible through seven side openings and one distal pore. This domain has been suggested to play a role in channel gating (Koprowski and Kubalski, 2003), desensitization and stability (Miller et al., 2003; Schuman et al., 2004) as well as in ion transport and selectivity (Edwards et al., 2004). Although the seven side entries to the chamber are surrounded by a number of basic amino acid residues that could play a role in valence selectivity, both K+ and Cl− ions have been seen to pass through them and enter the cytoplasmic chamber in MD (Sotomayor and Schulten, 2004; Sotomayor et al., 2007) and mesoscopic simulations (Sotomayor et al., 2006; Vora et al., 2006), but no ions pass through the distal opening. The distribution of ions within the chamber, however, is such that more Cl− than K+ ions reside near the transmembrane pore mouth, thus it is possible that this domain plays an important role in ionic selectivity. However, anion selectivity is also seen in simulations of only the transmembrane portion of the protein, and is largely controlled by the charged residue Lys169 (Vora et al., 2006; Sotomayor et al., 2007). It is possible that this residue is not normally charged, which may explain the extra anion selectivity seen in simulations compared with experiments.

The conformation of the cytoplasmic domain has also been shown to influence the conductivity of the pore (Sotomayor et al., 2007). It has also been suggested that this domain may act as a molecular sieve (Koprowski and Kubalski, 2003), preventing large solutes from passing through the channel where they may either block it, or be
expelled undesirably from the cell. Further molecular simulations including solutes may shed further light on this issue.

4.4 Conclusions

This review provides a brief summary on theoretical approaches used to model molecular dynamics and conduction properties of MscL and MscS, the two most extensively studied MS channels. Computational modeling of the two channels outlined here has significantly contributed to our understanding of basic principles of mechanosensory transduction in living cells. In particular it has helped to elucidate the nature of the imaged structures of the protein, the interactions between the protein and the lipid, the conformational changes involved in channel gating, and the specific roles played by various protein domains. We may expect further exciting developments of this research area in the future.

References


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