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Brownian dynamics investigation into the conductance state of the MscS channel crystal structure

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Abstract

We suggest that the crystal structure of the mechanosensitive channel of small conductance is in a minimally conductive state rather than being fully activated. Performing Brownian dynamics simulations on the crystal structure show that no ions pass through it. When simulations are conducted on just the transmembrane domain (excluding the cytoplasmic residues 128 to 280) ions are seen to pass through the channel, but the conductance of ~ 30 pS is well below experimentally measured values. The mutation L109S that replaces a pore lining hydrophobic residue with a polar one is found to have little effect on the conductance of the channel. Widening the hydrophobic region of the pore by 2.5 Å however, increases the channel conductance to over 200 pS suggesting that only a minimal conformational change is required to gate the pore.
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1. Introduction

Mechanosensitive ion channels provide the means by which cells respond to a range of mechanical stimuli including molecular agitation and osmotic pressure, and underlie the senses of touch and hearing. The mechanosensitive channel of small conductance (MscS) is a stretch activated channel found in *Escherichia coli* that responds to forces within the inner membrane to alleviate osmotic shock [1–4]. They are also voltage dependent [3,5] and show a slight anion selectivity (P_{Cl}/P_{Na} ~ 1.5–3.0) [4,6].

Recently, a crystal structure of the MscS channel was determined to 3.9 Å resolution, which showed the channel to be a homoheptamer with each subunit containing 3 transmembrane domains [7]. One of these domains (TM3) lines the pore while the other two (TM1 and TM2) contact the membrane where it is posited they would respond to bilayer tension. Furthermore, these outer helices were postulated to also act as the voltage sensors of the channel, as each helix contains an arginine residue near the middle of the membrane. A large cytoplasmic domain was also present, containing a central chamber connected to the bulk solution by a series of wide openings that may be responsible for ion selectivity [1].

Initially, the imaged structure of the channel was posited to be in an open, conductive state [7]. The narrowest portion of the transmembrane region of the pore was claimed to have a diameter close to 10 Å, easily wide enough to pass ions. However, since the determination of this structure there has been much discussion regarding this point. Bezanilla and Perozo [8] noted that if this structure indeed represented the channel in an open state, then envisaging how it could be closed while still maintaining 7-fold symmetry was difficult, and suggested that other regions of the protein, such as the TM2–TM3 loop could form part of the gate. Cysteine crosslinking experiments suggest that the closed state of the channel is significantly different from the imaged conformation [9].

Two molecular dynamics simulations cast further doubts on the conductance state of the imaged channel. One found that water was unlikely to pass through the narrowest region of the pore and that there was a huge barrier to ion conduction, and thus claimed that the imaged structure of the channel was not in its open state [10]. A second series of simulations also found that the density of water in the pore oscillated about a low value. However, they also discovered that removal of constraints

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holding the protein near the imaged structure led to a narrowing of the pore to a hypothesised closed state [11].

Determining the state of the imaged structure of the protein is important for understanding the mechanism of gating within this class of pore [12]. If the structure represents the channel in the open state, then gating obviously involves a closure of the pore by the TM3 or nearby regions. Alternatively if the channel is closed in this configuration, then this implies that ion conduction is not prevented by a physical blockage, but rather by other means. There are a number of ways by which the behaviour of water in the pore can block ion conduction. Green et al. [13–15] suggested that water could accumulate in high density or be immobilised by the surface geometry or large electric fields, and this could prevent ion conduction by creating a narrower effective pore through which ions could not pass with their hydration shell intact. These studies highlighted the role of charged or ionisable residues in fixing or releasing water. More recently a reverse gating process more applicable to the MscS channel has been proposed, as it has been found that narrow pores surrounded by non-polar residues can block ion conduction by providing an unfavourable home to water. If the water is at a low density in the pore, then again the ion will have to be stripped of its hydration shell to pass through the channel and thus there is a desolvation barrier rather than a physical blockage that can prevent ion conduction [16]. This 'hydrophobic gating' concept has been demonstrated in model pores [17,18] and is also believed by many to be at play in the acetylcholine receptor pore [19–23]. A small change in the radius or surface polarity of the region can be enough to open the pore. Indeed, Anishkin and Sukharev [10] conclude that such a hydrophobic gating mechanism underlies the function of the MscS channel. This suggestion gains credence from the observation that the replacement of central pore lining non-polar residues with polar ones increases the activity of the channel [24,25]. A final alternative is that the imaged structure of the channel neither represents the open nor the closed state of the channel, but an intermediate of the two, or a non-physical state induced by the process of crystallization.

In this paper we aim to investigate the crystal structure of the channel using Brownian dynamics simulations in order to determine the likely conductance of the channel. This mesoscopic technique allows for simulations to be run long enough to relate the channel current to the protein structure while still retaining some atomisitic detail, a task that is difficult with other methods. We also examine channel models in which the cytoplasmic domain has been removed, or the pore widened to help understand the functioning of MscS.

2. Methods

2.1. Channel models

2.1.1. Crystal structure

The MscS channel model is created using the crystal structure coordinates (1MXM) submitted by Bass et al. [7] to the Protein Data Bank. We locate the boundary separating the protein and the water-filled pore by tracing around van der Waals radius of the pore lining atoms. A three-dimensional, cylindrically symmetric pore, connecting the inside and the outside of the cell is created by determining the minimum radius of the pore for each z value and rotating this by 360°. The resulting radius is illustrated in Fig. 1A along with the atoms forming the protein and again in Fig. 2A. Bass et al. [7] claim that the pore has a minimum diameter near the residues L105 and L109 of nearly 11 Å. Our pore, however, has a diameter nearly half of this value. To examine whether this difference was caused by asserting axial symmetry, we also calculate the pore radius using the program HOLE [26] that does not make the assumption of axial symmetry. A comparison of the two pores is given in Fig. 2A. The radius of our symmetric pore, and that found using HOLE agree well throughout the narrow section of the pore, although the minimum pore radius found using HOLE at 3.1 Å is slightly larger than in the symmetric pore at 2.5 Å.

The narrow transmembrane region opens into a wider cytoplasmic chamber with a radius of over 25 Å before narrowing again at the intracellular end. The channel has a total charge of + 28e with an important charged R88 residue found on the TM2 pointing into the pore. This charge, located at $\sim z$=48 Å, plays a crucial role for ions entering the channel and is discussed in further detail in Section 3. In our simulations, the protein atoms are treated as point charges placed in their exact positions as found in the crystal structure.

2.1.2. Modified structures

The large cytoplasmic vestibule produces an obstacle in our Brownian dynamics (BD) simulations, as our program is currently unable to cope with a protein with extra-membrane regions surrounded by water as described in more detail below. For this reason, we perform simulations both including this region, and excluding it (as noted by the box in Fig. 2A, and discussed in Section 3).

To remove the cytoplasmic vestibule, we deleted the residues numbered from 128 through to 280. Notably, the total charge of the protein was not affected by this and remained at its original value of +28e. In this truncated structure, only the transmembrane pore region was used to create a three-dimensional boundary whose radius is illustrated in Fig. 2B. Once the intracellular residues were removed, the channel was centred about the z-axis. The residue R88, which was previously at

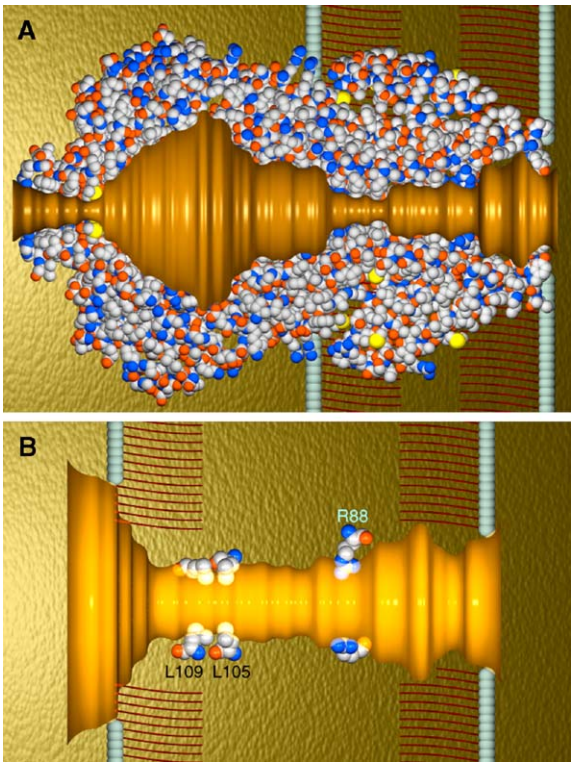

Fig. 1. MscS channel structures. (A) Schematic of the MscS crystal structure with the top half removed to reveal the pore. (B) Schematic of the transmembrane region of the *expanded structure*. The highlighted residues, starting from the intracellular side, are residues L109 and L105 that line the expanded part of the channel, and R88, the only charged amino-acid residue lining the pore.

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