Molecular simulations of the large conductance mechanosensitive (MscL) channel under mechanical loading

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Abstract The MscL channel is a mechanosensitive channel which is gated by membrane stress or tension. Here, we describe a series of simulations which apply simulated mechanical stress to a molecular model of the MscL channel using two methods – direct force application to the transmembrane segments, and anisotropic pressure coupling. In the latter simulations, pressures less than that equivalent to a bilayer tension of 12 dyn/cm did not cause the channel to open, while pressures in excess of this value resulted in the channel opening. These results are in approximate agreement with experimental findings. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Molecular dynamics; Mechanotransduction; Ion channel; Membrane tension

1. Introduction

The MscL family of large conductance mechanosensitive ion channels are thought to be involved in the regulation of osmotic pressure within cells [1]. They are well known for their ability to open and close in response to mechanical stresses in the lipid bilayer [2]. Chang et al. [3] determined the crystal structure of the bacterial Tb-MscL channel, and their structure (1msl.pdb) forms the starting point for the work described in this paper. They determined the bacterial MscL channel to have a pentameric structure, with each pentamer containing two transmembrane helices. The inner of these helices (TM1) lines the pore, which is gated by a hydrophobic apex on the cytoplasmic side[3], while the outer helix (TM2) would be in contact with the lipid bilayer.

Experimental workers have characterised five subconductance states for the MscL channel during the closed to open transition, of which the first is the limiting step [1]. They estimate the conductance to be approximately 3.5–3.7 nS in the open state and predict a pore diameter of 30–35Å to achieve this. In the closed state, however, the diameter of the gate region is approximately 2 Å [3]. Various authors have proposed theories which could explain this large change in pore diameter. Batiza et al. [4] suggest that the pore-lining helices could rotate and straighten vertically, moving out of the way to create a large pore, while Chang et al. suggest that the outer helices could move radially to create the larger pore [3]. Recent modelling results by Gullingsrud et al. [5] showed that surface tension on the extracellular side may greatly increase the angle of the transmembrane helices, giving a wider, shorter pore.

The intention of this study was to simulate the effect of membrane tension on the MscL channel. In reality, the membrane tension is transmitted to the protein by interactions between the lipids and the transmembrane segments embedded therein. In our simulations, two simple methods have been investigated. The first modelled the localised effects on the transmembrane segments of a tensile stress, by directly applying a radial force to the outermost transmembrane segment. The second applied an equibiaxial negative pressure to the whole channel, rather than the localised force. Ideally, molecular simulations would transmit force to the channel through a lipid bilayer model, and such simulations are currently being conducted by the authors. The work described here represents an initial attempt to examine the role of mechanical tension on the MscL channel. Recent work by Gullingsrud et al. [5] have used surface tension for a similar purpose. It is not clear whether representing the membrane stress as a surface tension is a realistic model of how the stress is transferred to the channel in real cells, or indeed if either of the methods here are more or less suited to the task. In all three cases, however, the computational workload is significantly reduced by modelling only the bare protein and solvent instead of explicitly including the lipid bilayer.

2. Materials and methods

The coordinates for the bacterial MscL channel were obtained from the PDB file 1msl.pdb [3]. Residues 1–9, which were disordered in the crystal structure, were omitted from our analysis. Missing atoms were fixed using the program MOLMOL [6] and the channel's main axis aligned with the z-axis of the coordinate system. The bare channel was placed in a simulation box and solvated with SPC water molecules. The complete system consisted of 5180 protein atoms and 8067 water molecules, giving a total of 29 381 atoms. No explicit lipids were included, and the simulation used periodic boundary conditions. The simulation box size is the minimum size of the protein plus twice the cutoff length. This gives a minimum of three layers of hydration around MscL at the closest point to the periodic boundaries, which also ensures that the molecule cannot interact directly with itself, as the periodic images are significantly further apart than the cutoff distances. A larger box is used in the channel axis direction to minimise artifacts from the use of periodic boundary conditions in that

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Abbreviations: MscL, large conductance mechanosensitive

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direction. Gromacs v2.0 was used for the molecular simulations [7]. The gromacs forcefield was used, with cutoffs for electrostatic interactions [8]. The complete system then underwent an energy minimisation of the protein/water system to remove close water contacts, using the conjugate gradient algorithm, with the temperature kept at a constant 310 K, using a Berendsen thermostat. The energy minimised system was then further equilibrated with position restraints on the protein molecules to allow water molecules to equilibrate freely through the protein [9,10]. The system after equilibration, prior to simulation of the membrane stress is shown in Fig. 1. Full scale MD simulations were then performed using two methods to apply a simulated membrane tension:

1. Direct load application to the transmembrane segments. A radial acceleration was directly applied to the outer (TM2) transmembrane segments of the channel, at various magnitudes to simulate the lipid bilayer stress being transmitted to the outermost transmembrane segment.
2. Anisotropic pressure coupling. The whole simulation box was coupled to an anisotropic pressure bath. The z-direction pressure was held constant (along channel axis direction), while the x- and y-pressures (in the plane of the cell membrane) were allowed to relax to a predetermined value [9].

Details of the implementation of these methods can be found in the Gromacs manual [8]. Pore geometry was analysed using the HOLE program [11]. The intracellular helices were deleted prior to calculation of the minimum pore radius with HOLE, similar to the methods adopted by Gullingsrud et al. [5]. Conductances were calculated by the HOLE program, as outlined in Smart et al. [12].

3. Results and discussion

The gross structural changes and analysis of the pore geometry demonstrated that while both methods simulated a widening of the pore, the structural rearrangement in various regions was directly related to how the loading was applied. In particular, the pressure-coupled simulations resulted in a less dramatic structural change, while the acceleration simulations resulted in large motions of the outer (TM2) segments, to which the acceleration loading was applied. Fig. 2 shows a top and side view of both types of simulations demonstrating the gross structural changes. Fig. 4 shows the structural changes in the backbone in the gate region for both types of simulations, as viewed from the top of the protein.

In both cases, there was some deviation from the symmetrical pentameric structure. In the pressure-coupled simulations, this may be due to the fact that an equibiaxial load

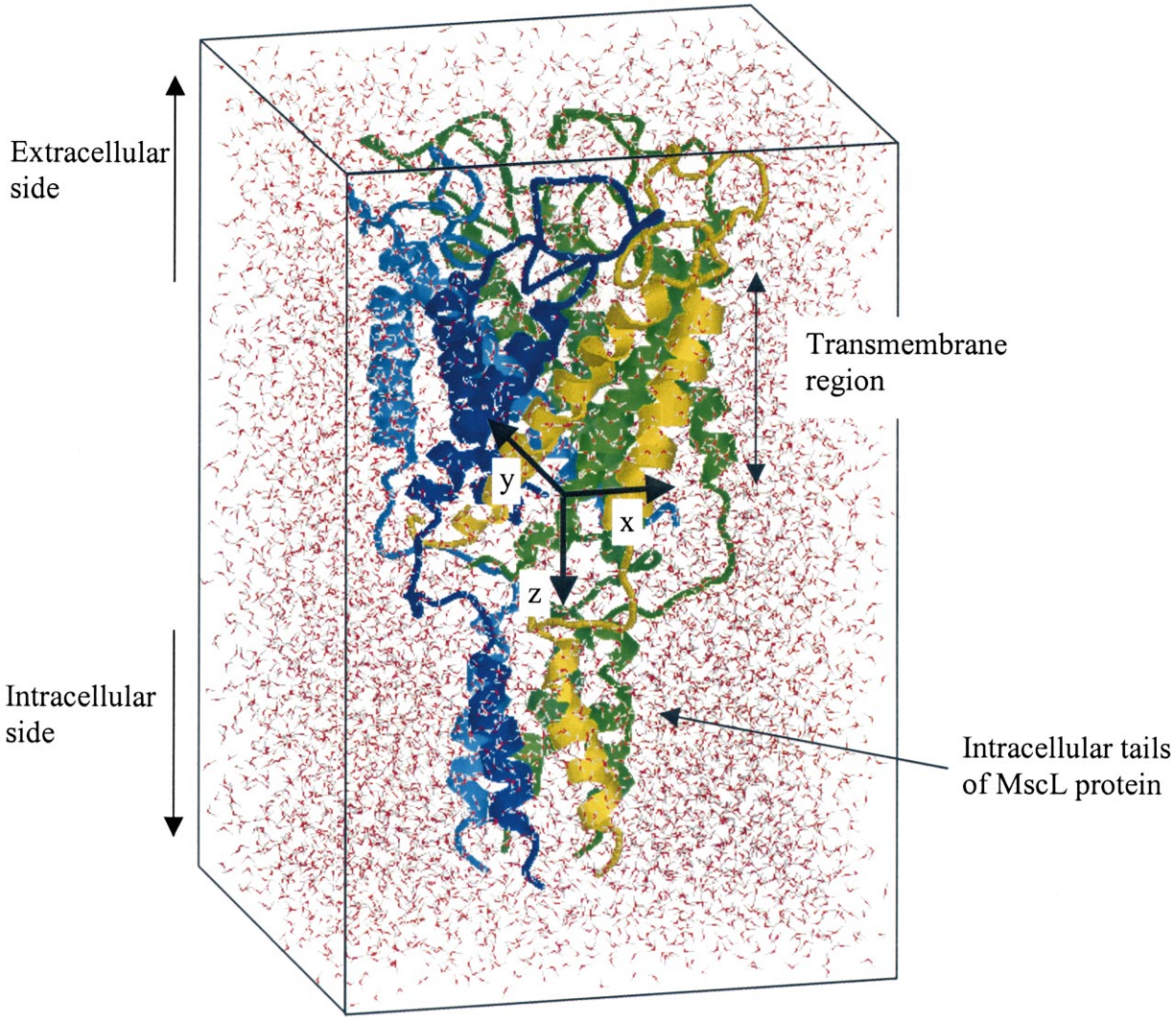

Fig. 1. Overview of the model after equilibration. The transmembrane (channel) axis of the MscL protein is aligned with the z-axis of the simulation box, with the intracellular tails shown at the bottom of the figure. The cell membrane, which is not explicitly modelled in this study, lies in the x–y plane.

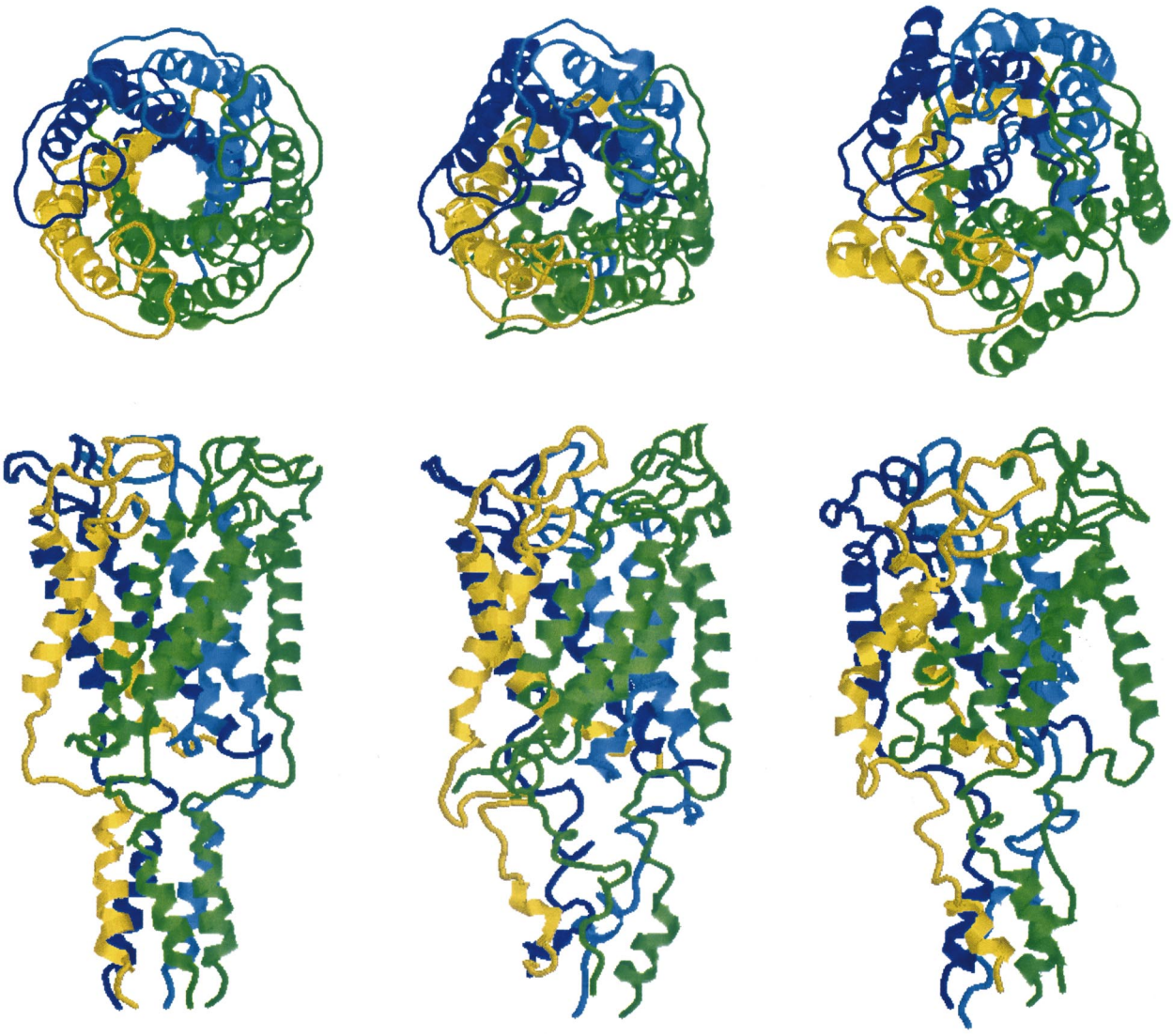

Fig. 2. Structural changes in MscL due to pressure coupling and acceleration loadings. The leftmost panels are the original structure. The middle panels are the pressure-coupled results for a pressure of 50 bar, and the right panels are the acceleration results for an acceleration of 0.2. Note that the structures visible in the centre of the pore in top panels the are the intracellular tails, not portions of the transmembrane helices.

was applied to the channel in the x- and y-directions, which encouraged the protein to move preferentially in these directions rather than moving in a purely radial direction. However, asymmetry also developed in the acceleration simulations, in which the loading was radial, relative to the initial weighted average position of the membrane helices. However, during the simulations, the loading direction was kept constant, and this may have meant that the loading was not completely radially symmetrical relative to the current position of the helices at all times. It is also possible that the loading exaggerates any small deviations from ideal symmetry in the initial crystal structure in both types of simulations. The acceleration simulations create a more localised loading, which leads to large motions of the outer TM2 helices, as a result of which other structural changes occur. The pressure-coupled simulations, however, load the entire structure rather than applying load only to the transmembrane segments which would be in contact with the lipid bilayer in vivo.

In both pressure-coupled and acceleration simulations, the channel widened and shortened during the simulations and the outer transmembrane helices moved more horizontally (tangentially) as the pore widened. This can be seen from the pore profile plots in Fig. 3a, and from the channel schematics in Fig. 2. This is consistent with the results from Gullingsrud et al. [5], and from some other models of pore opening in the literature [4]. Recently, Sukharev et al. [13] suggested that the outer membrane helices may expand first in response to membrane tension, acting as tension sensors, followed by motion of the inner helices. This type of motion was seen in the acceleration simulations (see rightmost panel of Fig. 2), but not in the pressure-coupled simulations, where the helices tended to move together. It is not clear which of these motions is more realistic. It appeared that the extracellular end of the helices did not move outward quite as much as the intracellular end. In the acceleration simulations, the helices also bowed outward due to the acceleration being directly applied to them. Significant disorder was seen in the intracellular tails in both types of simulations. In both cases, this disorder created a constriction which was of similar size to the narrowest part of the pore in the transmembrane sec-

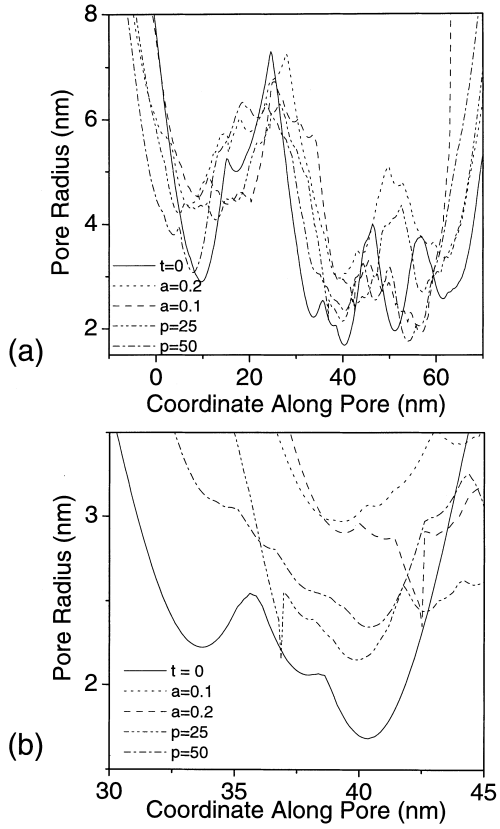

Fig. 3. (a) Profile of the pore as a function of position along the pore, as calculated by the program HOLE for pressure-coupled and acceleration simulations. (b) Enlarged view of gate region. The gate region has widened in all simulations.

tion of the channel. These tails can be seen in Fig. 2. Mutagenesis experiments have shown that these regions are not required for mechanosensitivity and do not affect the conductance, although they may be involved in adjusting the sensitivity of the channel [14]. Therefore, these segments were not considered in the minimum pore diameter and conductance calculations presented in Table 1. More recently, the same group has suggested that the N-terminus may be involved in gating, on the basis of structural models [13] and some experimental evidence [15]. Our results are not inconsistent with this data, however the methods used for simulating mechanical tension in these models are not accurate enough to seriously address this issue.

The structural change in the channel during pressure-coupled simulations was related to the magnitude of the pressure applied. A pressure of 12 bar did not cause any appreciable opening of the pore (as shown in Fig. 5, Table 1) at 150 ps, although there was some fluctuation during the early part of the simulation, as shown in Fig. 5. At pressures of 25 bar and above, the pore widened significantly, and the pore radius fluctuated more throughout the simulation, as shown in Fig. 5a. The literature quotes a figure of 12 dyn/cm as the membrane tension required for gating of this channel, which is similar to the rupture tension for the lipid bilayer membrane [1]. This is approximately equivalent to a pressure of 24 bar, for a 50 Å thick bilayer. The approximate similarity in magnitude of pressure required to open the channel is encouraging, however this should not be over-interpreted, given the simplified model of pressure application used in this study.

The nominal channel conductances were also affected by the loading, as shown in Table 1. All simulations increased the conductance of the channel, as predicted by the HOLE program, with the exception that a simulation run with isotropic pressure of unity (i.e. no loading) showed a decreased conductance compared to the starting structure, although the gate diameter was not significantly affected. This may be a more realistic reference state for the conductance and diameter comparisons than the starting structure, which represents the protein in a vacuum, since all the simulations with load

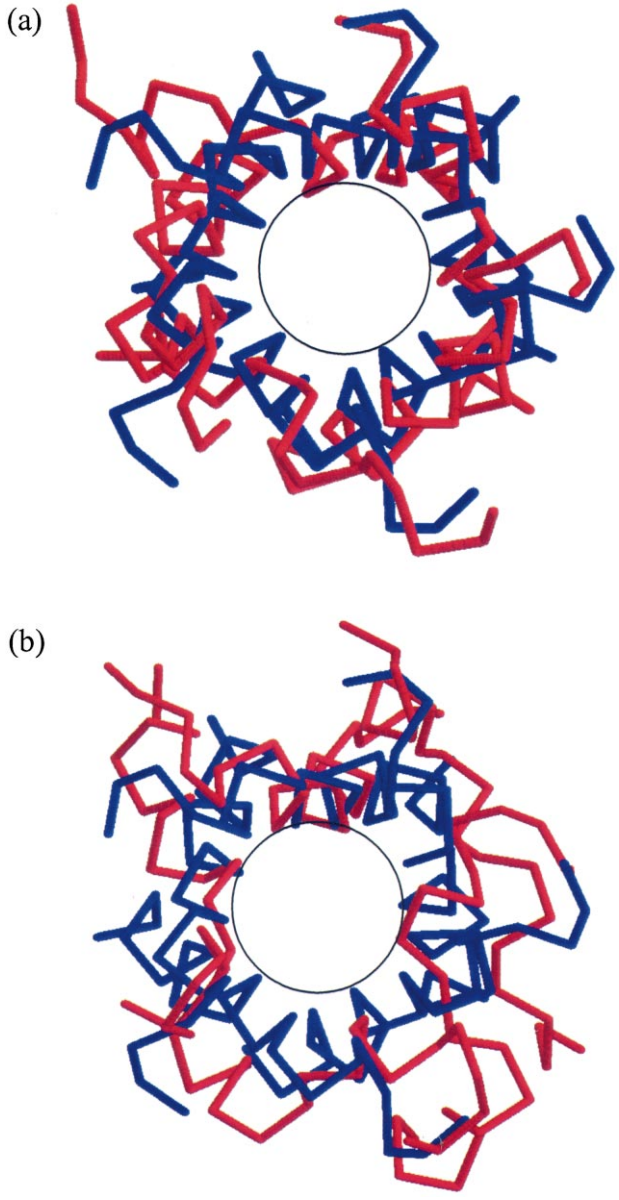

Fig. 4. This shows a detailed view of the structural changes in the protein backbone near the gate region for the two different simulation methods. The circle denotes the original minimum gate radius. The blue structure is the starting structure. The red structure is the structure after 150 ps of simulation. (a) Anisotropic pressure-coupled simulations at a pressure of 50 bar. (b) Acceleration simulations at an acceleration of 0.2 nm/ps^2. In both cases, there is a widening of the gate region and the expansion is not perfectly symmetrical. Numerical values for minimum pore diameter are shown in Table 1, and a plot of the pore profile is shown in Fig. 3.

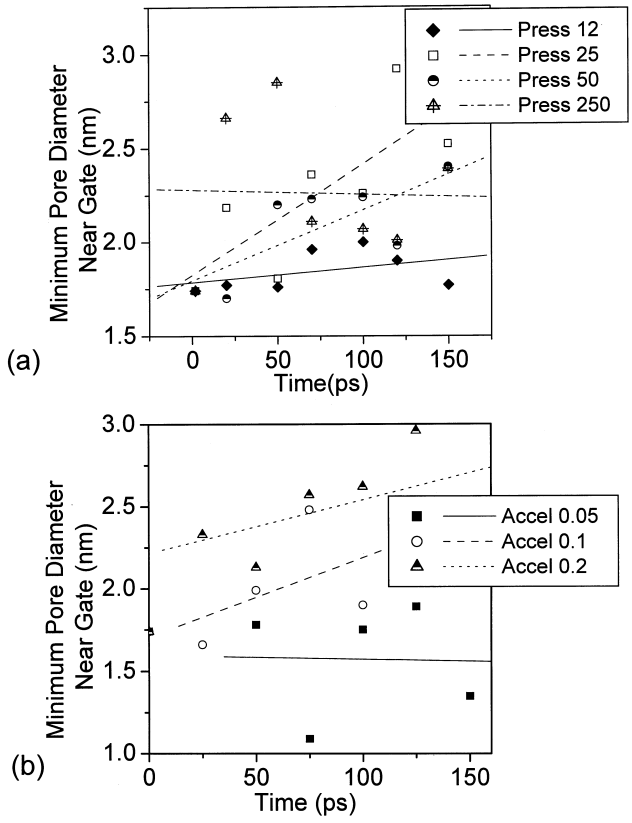

Fig. 5. Minimum radius in the gate region for the first 150 ps of (a) pressure and (b) acceleration simulations.

applied were conducted with the pressure-coupled to unity in the z-direction (all directions in the case of the acceleration simulations). In simulations where conductances were increased while the diameters were not significantly changed, the conductance change occurred a result of changes in the shape of the pore rather than the diameter at the gate.

In the acceleration simulations, there was also a threshold above which the structural changes were significantly different. In simulations with accelerations in excess of 0.2 nm/ps^2, the outer transmembrane segments were pulled away from the rest of the protein, becoming significantly distorted at larger acceleration values. In contrast, at lower accelerations, the outer transmembrane segments moved radially outward, but the inner segments tended to move with them rather than separating. At accelerations below the rupture threshold, the speed of pore opening was related to the magnitude of the acceleration, as shown in Fig. 5b. The separation of the outer membrane helices and associated helix distortion may not be a realistic motion for a channel embedded in a lipid bilayer. At the lowest accelerations (e.g. 0.05 nm/ps^2) used in this study, the pore shaped fluctuated during the simulations, as shown in Fig. 5. This may indicate that this level of acceleration is not sufficient to completely overcome the interactions between the protein chains. It is not straightforward to make a direct comparison of the acceleration applied using this method with the known tension required to open the MscL channel.

In examining the results from the two different methods of modelling applied mechanical load, it is clear that neither is an ideal method. In the case of the acceleration load, prescribing the acceleration forces directly to the outer transmembrane helices results in very localised changes in the structure, with quite dramatic bending of the helices in some simulations. This is unlikely to be the case where the channel is embedded in the bilayer and the forces are transmitted through the surrounding lipid molecules. On the other hand, while the pressure-coupled simulations apply a less localised loading, they apply the load over the whole molecule rather than only to the transmembrane segments which would be in contact with the lipid bilayer. The alternative approach taken by Gullingsrud et al. [5] to apply loading as a surface tension is also not an ideal model of the real situation, in that it predisposes the protein to widen at the outer surface where the surface tension is applied. Simulations where mechanical tension is applied through an explicit lipid bilayer model may be able to address some of these concerns. The inclusion of explicit lipids in this type of simulation, while very computationally intensive, will allow a more realistic modelling of the application of load to the MscL channel through the bilayer. This is expected to result in less dramatic structural changes to those seen in the acceleration simulations, where the transmembrane helices were deformed quite significantly due to the somewhat artificial loading by direct acceleration. A different pore shape due to interactions between the transmembrane helices and the lipids is also quite possible, which would be accompanied by a change in the pore conductivity, although the details of the changes are difficult to predict a priori.

We have ongoing simulations with MscL embedded in an explicit POPC lipid bilayer, which are not yet complete. Comparison of some preliminary results from an equilibrated, unloaded MscL/bilayer with the bare protein system indicate

Table 1
Minimum diameter in the gate region after 150 ps of simulation

Type of simulation	Magnitude of loading	Minimum pore diameter (Å)	Nominal conductance (pS/m)
Initial configuration	–	1.74	560
Pressure-coupled	1	1.73	330
Pressure-coupled	12	1.77	490
Pressure-coupled	25	2.52	688
Pressure-coupled	50	2.47	740
Pressure-coupled	250	2.42	930
Acceleration	0.05	1.35	520
Acceleration	0.1	2.97	983
Acceleration	0.2	2.47	740

The last column is the nominal conductance, as calculated by HOLE [12]. Note that these are significantly less than those reported by Sukharev at al [1], however, the simulation durations may not be long enough for the full effect of the loading to be seen in the conductance changes. Note that the pore diameter for the 0.05 nm/ps^{2}acceleration simulation fluctuated significantly during the simulation, and this may not be a 'typical' value.

that the presence of the explicit lipids slightly alters the conductivity of the pore due to mild structural rearrangement as a result of interaction between the membrane helices and the lipids. There is also, not unexpectedly, an altered distribution of water molecules around the protein, due to the exclusion of water molecules from the hydrophobic central region of the lipid bilayer.

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