Estimation of the Pore Size of the Large-Conductance Mechanosensitive Ion Channel of *Escherichia coli*

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ABSTRACT The open channel diameter of *Escherichia coli* recombinant large-conductance mechanosensitive ion channels (MscL) was estimated using the model of Hille (Hille, B. 1968. Pharmacological modifications of the sodium channels of frog nerve. *J. Gen. Physiol.* 51:199–219) that relates the pore size to conductance. Based on the MscL conductance of 3.8 nS, and assumed pore lengths, a channel diameter of 34 to 46 Å was calculated. To estimate the pore size experimentally, the effect of large organic ions on the. conductance of MscL was examined. Poly-L-lysines (PLLs) with a diameter of 37 Å or larger significantly reduced channel conductance, whereas spermine (~15 Å), PLL$_{19}$ (~25 Å) and 1,1'-bis-(3-(1'-methyl-(4,4'-bipyridinium)-1-yl)-propyl)-4,4'-bipyridinium (~30 Å) had no effect. The smaller organic ions putrescine, cadaverine, spermine, and succinate all permeated the channel. We conclude that the open pore diameter of the MscL is ~40 Å, indicating that the MscL has one of the largest channel pores yet described. This channel diameter is consistent with the proposed homohexameric model of the MscL.

INTRODUCTION

Mechanosensitive channels are a class of ion channels gated by mechanical force. These channels have been found in a variety of cells and tissues (Martinac, 1993; Sackin, 1995), including aortic baroreceptor neurons (Cunningham et al., 1995), vascular epithelial cells (Lansman et al., 1987), renal proximal tubule tissue (Sackin, 1989), and supraoptic neurons (Oliet and Bourque, 1993), where they have been suggested to mediate several physiological processes including osmosensitivity. Recently, the first large-conductance mechanosensitive channel (MscL), which is present in the *Escherichia coli* inner membrane (Blount et al., 1996a; Häse et al., 1997b), was cloned and sequenced (Sukharev et al., 1994a, b).

The MscL is one of at least three types of mechanosensitive channels found in *E. coli* (Berrier et al., 1996). However, their physiological significance is as yet unclear. The conductance of each channel type is related to its mechanosensitivity where the channel with the smallest conductance is activated at the lowest membrane tension while the MscL, having the largest conductance, requires the highest membrane tension (Berrier et al., 1996; Sukharev et al., 1997). Therefore, these channels have been proposed to play a role in osmoregulation under extreme conditions, where efflux of organic osmoprotectants such as glycine-betaine may lead to a reduction of intracellular osmotic pressure (Sukharev et al., 1993; Berrier et al., 1992; Cui et al., 1995).

Hydropathy plot analysis of the MscL has suggested that the protein monomer consists of two α-helical membrane-spanning regions (Sukharev et al., 1994a) and the functional

MscL channel has been proposed to have a homohexameric quaternary structure (Blount et al., 1996a). The results of molecular biological and electrophysiological studies revealed several regions that are important for channel gating and/or mechanosensitivity (Blount et al., 1996a, b; Häse et al., 1997a). However, to date there have been no estimates of the open pore diameter of the MscL. A knowledge of this pore size may assist in determining the types of molecules that can pass through this channel and therefore contribute to an understanding of its physiological function(s).

In the present study, the diameter of the open channel pore was estimated experimentally by measuring blockade of the channels by large organic polymers. In addition, several organic ions important in bacterial metabolism were demonstrated to permeate the channel consistent both with the estimated pore size and with the proposed physiological role for these channels. The experimentally determined diameter of MscL was in close agreement with that calculated from a homohexameric model of the channel in bacterial membranes.

MATERIALS AND METHODS

Chemicals

Putrescine, cadaverine, and spermine were used as the hydrochloride salts and succinate was obtained as the sodium salt (Sigma Aldrich, Castle Hill, NSW, Australia). The poly-L-lysines (PLLs) used were PLL$_{19}$, PLL$_{46}$, PLL$_{120}$, and PLL$_{268}$, (Sigma Aldrich) with the numbers referring to the average number of lysine monomers per molecule. 1,1'-Bis-(3-(1'-methyl-(4,4'-bipyridinium)-1-yl)-propyl)-4,4'-bipyridinium hexaperchlorate (BMBB) was synthesized by McGeachie and Summers (1986). All other materials were of analytical grade.

Purification and reconstitution of the MscL

MscL protein was purified and reconstituted into azolectin liposomes following the procedure of Häse et al. (1995). Briefly, a fusion protein of glutathione-S-transferase (GST) and MscL was expressed and isolated

from E. coli cells. The GST portion was used to bind the fusion protein to glutathione-coated Sepharose beads, allowing purification in a single step. After incubation with thrombin to cleave the fusion protein, the MscL protein was extracted with n-octyl-β-D-glucopyranoside. After dialysis to remove the detergent, the purified MscL protein was reconstituted into artificial liposomes according to the method of Delcour et al. (1989) and examined using the patch-clamp technique (Hamill et al., 1981).

Single-channel recording

Currents were recorded using borosilicate microcapillary pipettes (Sigma Aldrich) made using a micropipette puller (Flaming/Brown model P-87, Sutter Instrument Co., Novato, CA). Pipettes were of bubble number 3.8–4.2 in absolute ethanol (Corey and Stevens, 1983) corresponding to a resistance of ~5 MΩ in recording solution (200 mM KCl, 40 mM MgCl$_2$, 5 mM HEPES, pH 7.2, adjusted with KOH). Bath and pipettes were filled with recording solution or a low-potassium buffer, K_L (10 mM KCl, 40 mM MgCl$_2$, 5 mM HEPES, pH 7.2, adjusted with KOH). A Leitz micromanipulator (Ernst Leitz Wetzlar GmbH, Wetzlar, Germany) was used to move pipettes to suitable liposomes that were viewed with a phase-contrast microscope (IMT-2, Olympus Optical Co., Tokyo, Japan). Seals between the liposome membrane and patch-clamp pipettes formed spontaneously or following the brief application of negative pressure (<30 mm Hg). Liposome membrane patches were excised and channels were activated by applying suction to the interior of the pipette via the same port used for seal formation. A Ag/AgCl$_2$ electrode and agar salt bridge (2% agarose in 1M KCl) were used as the reference electrode. Currents were filtered at 1 kHz using a patch-clamp amplifier (AxoPatch 1D, Axon Instruments, Foster City, CA) and digitized at 5 kHz by a computer running the pCLAMP 6 package (Axon Instruments, Foster City, CA). Applied pressure was converted into a voltage signal using a piezoelectric pressure transducer (Omega, Stamford, CT) and digitized together with the current signal.

Conductance measurements

Channel conductance was estimated either as the slope of the least-squares regression line fitted to the plot of single-channel current amplitude against applied pipette potential or from a series of continuous voltage ramps (−60 to +60 mV, duration 3.3 s) that were applied at 3.5-s intervals. For voltage step experiments, currents were measured as the separation in peaks of the current amplitude histograms, or by the difference between cursors aligned at peak and baseline currents within the pCLAMP program. Measurements were obtained from at least three patches at each holding potential. For voltage ramp experiments, conductance was estimated as the slope of single-channel openings following correction for the leak current.

Effect of organic ions on conductance

Pipette tips were filled with recording solution and backfilled with 100 μM of a test compound in recording solution (pH 7.2). Compounds examined were spermine, BMBB, PLL$_{19}$, PLL$_{46}$, PLL$_{120}$, and PLL$_{268}$. After seal formation, channels were activated by negative pressure above an activation threshold and control data were collected. The pressure was then released and after 30–40 min incubation to allow the test compound to diffuse to the patch, negative pressure was again applied to the patch and the currents recorded.

To determine whether various ions permeated the MscL, pipettes were filled with K_L while the bath contained 100 mM of the test compound in K_L. Compounds examined were the positively charged polyamines putrescine, cadaverine, and spermine, and the negatively charged succinate. Single channel currents were recorded at pipette potentials between −60 and +60 mV in 10-mV steps, and the conductance and reversal potentials were estimated following correction for junction potentials (Axoscope, Axon Instruments, Foster City, CA; Barry and Lynch, 1991). Ion mobilities used for junction potential calculations were estimated from their radii according to the Einstein–Stokes equation (Robinson and Stokes, 1959)

and the relationship between diffusion coefficient and mobility (Hille, 1992).

Permeability ratios for the test ions were calculated using the corrected reversal potentials (V) according to the following equation (Lewis, 1979):

$$\sum_X \left(\frac{F^2}{RT} z_X^2 P_X V \frac{[X]_o - [X]_i e^{z_X FV/RT}}{1 - e^{z_X FV/RT}} \right) = 0 \tag{1}$$

where P_X is the permeability, z_X is the valence, and $[X]_o$ and $[X]_i$ are the activities of the ion in the bath and pipette, respectively. R, T, and F have their usual thermodynamic meanings. Activity coefficients used to derive the permeability ratios for divalent organic ions were assumed to be equal to that of succinate (0.76; Robinson and Stokes, 1959). For the tetravalent spermine, the activity coefficient of Th^{4+} was used (0.29; Robinson and Stokes, 1959). All other activity coefficients were obtained from Lide (1995).

Sizing of poly-L-lysine molecules

The PLL compounds used in this study were synthesized without protection of side-chain amines of the lysine monomers (Sigma Aldrich). As a consequence, polymerization was possible at both the side-chain and peptide amines. Therefore, the molecules were assumed to be approximately spherical for the purpose of calculating their molecular sizes, estimated using the Einstein–Stokes equation (Robinson and Stokes, 1959). This equation relates the diffusion coefficient, D, of a solution of a compound to the radius, r, of that molecule:

$$D = \frac{kT}{6\pi\eta r} \tag{2}$$

where k is the Boltzmann constant (1.38 × 10^{-23} J K^{-1}), T is the absolute temperature (298 K), and η is the viscosity coefficient (0.00101 N s^{-1} m^{-2}, Chang, 1981) of the solution. The coefficients of diffusion and molecular weights, M, were obtained for thirteen compounds and plotted on a logarithmic scale (Fig. 1). The equation relating D (10^{-11} m^2 s^{-1}) and M was:

$$\log D = 2.75 - 0.422 \times \log M \tag{3}$$

By using Eqs. 2 and 3, the sizes of poly-L-lysines used in the present study were calculated and presented in Table 1.

RESULTS

Calculation of the MscL pore size using the channel conductance

The model proposed by Hille (1968) relating pore size and resistance to ion flow in the channel was used to estimate the pore diameter of the MscL. The model assumes that the channel is a uniform cylinder of radius r, length l, in a solution of resistivity ρ. Then the resistance through the channel, R_C, is given by:

$$R_C = \left(l + \frac{\pi r}{2} \right) \frac{\rho}{\pi r^2} \tag{4}$$

and since the conductance, g, is the inverse of R_C, Eq. 4 can be rearranged to give the channel diameter, d, from:

$$d = \frac{\rho g}{\pi} \left(\frac{\pi}{2} + \sqrt{\frac{\pi^2}{4} + \frac{4\pi l}{\rho g}} \right) \tag{5}$$
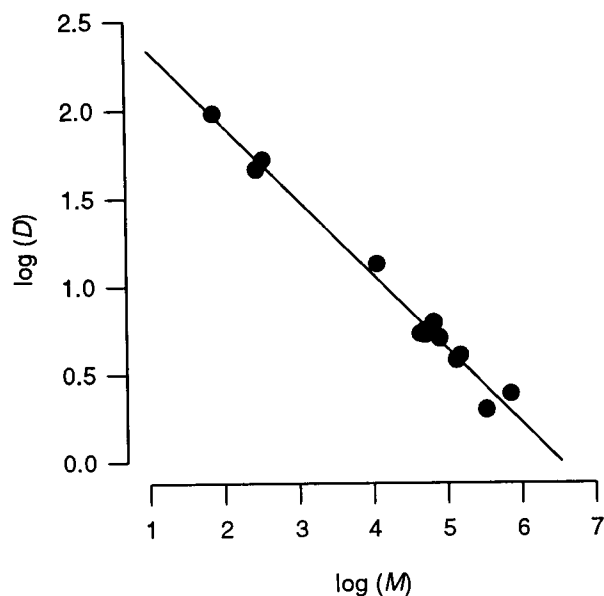
FIGURE 1 Plot of diffusion coefficient and molecular weight. Coefficients of diffusion and molecular weights were obtained for thirteen compounds (Lentner, 1984; Atkins, 1992) and plotted. A linear relationship between the logarithm of both the diffusion coefficient and molecular size was determined to be $\log D = 2.75 - 0.422 \times \log M$. The correlation coefficient for the fitted line was 0.985.

The resistivity of the recording solution was measured as 49.7 Ω cm. Although the true length of the MscL is unknown, an estimate of the length of the shorter membrane-spanning region (Sukharev et al., 1994a, Blount et al., 1996a) was used as an approximation. This region is likely to be α-helical formed from 28 amino acid residues (Blount et al., 1996a). Given that a single turn of an α-helix spans 5.4 Å and corresponds to 3.6 amino acids (Zubay, 1993), the minimum length of the membrane spanning region is ~42 Å. The single channel conductance of the MscL measured in symmetric recording solution was 3.8 ± 0.1 nS (Fig. 2) and using the values of ρ = 49.7 Ω cm and l = 42 Å, the diameter of the pore of MscL was calculated to be 42 Å.

Conductance measurement in the presence of large organic ions

Since there are no known specific channel blockers of the MscL (Hamill and McBride, 1996), large branched amino

TABLE 1 Estimated size of the poly-L-lysine polymers

Compound	Average Molecular Weight	Calculated Average Diameter (Å)	Range* (Å)
PLL$_{19}$	3970	25	17–29
PLL$_{46}$	9600	37	27–42
PLL$_{120}$	25000	55	55–63
PLL$_{268}$	56000	78	64–79

*Range of polymer diameter was calculated from the range of molecular weights obtained from the supplier.
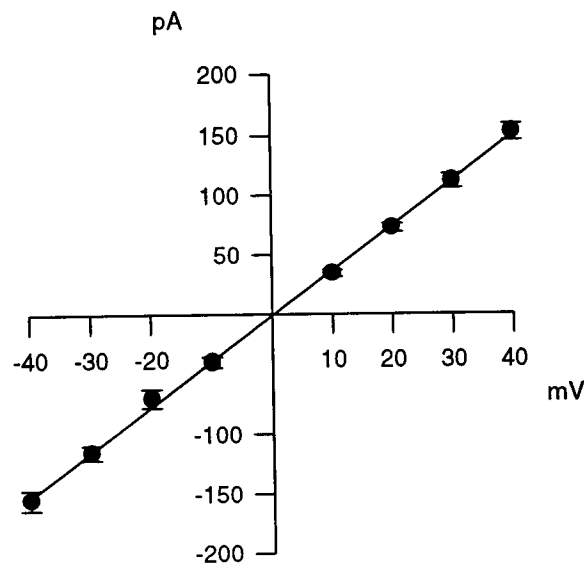

FIGURE 2 Current voltage relationship of the MscL. Single-channel currents were measured in symmetric recording solution at pipette voltages between −40 mV and +40 mV. Linear regression analysis gave a conductance of 3.8 ± 0.1 nS (mean ± SE, df = 7).

acid polymers were used in an attempt to physically occlude the channel pore. Fig. 3 shows the activated channel currents in response to voltage ramps. Blockade of the channel at positive pipette potentials was observed only in the presence of large poly-L-lysines (PLL$_{46}$, PLL$_{120}$, and PLL$_{268}$), and this blockade was reversed at negative potentials (Fig. 3). The magnitude of the reduction in conductance by the blocking compounds was not significantly different between the agents (Fig. 4). Incomplete blockade was observed for all compounds, indicating residual current through the unblocked portion of the channel.

Permeation of polyamines through the MscL

Fig. 5 shows the conductance of the MscL in the presence of organic ions. The bath and pipette solutions contained low potassium solution (K$_L$) and for test compounds, 100 mM was added to the bath solution. An increase in the conductance above the control values at negative pipette potentials indicated permeation of the MscL by the positively charged test compounds. For negatively charged succinate, an increased slope at positive potentials was observed (Fig. 5).

For each of the organic compounds, conductance was greater than under control conditions (Fig. 5, Table 2). Conductance in the presence of spermine was greater than that for the other compounds (putrescine, cadaverine, succinate, and potassium), presumably because spermine has four charges. The calculated permeability ratios show that the larger organic ions permeated the MscL less readily than the smaller potassium ion (Table 2).
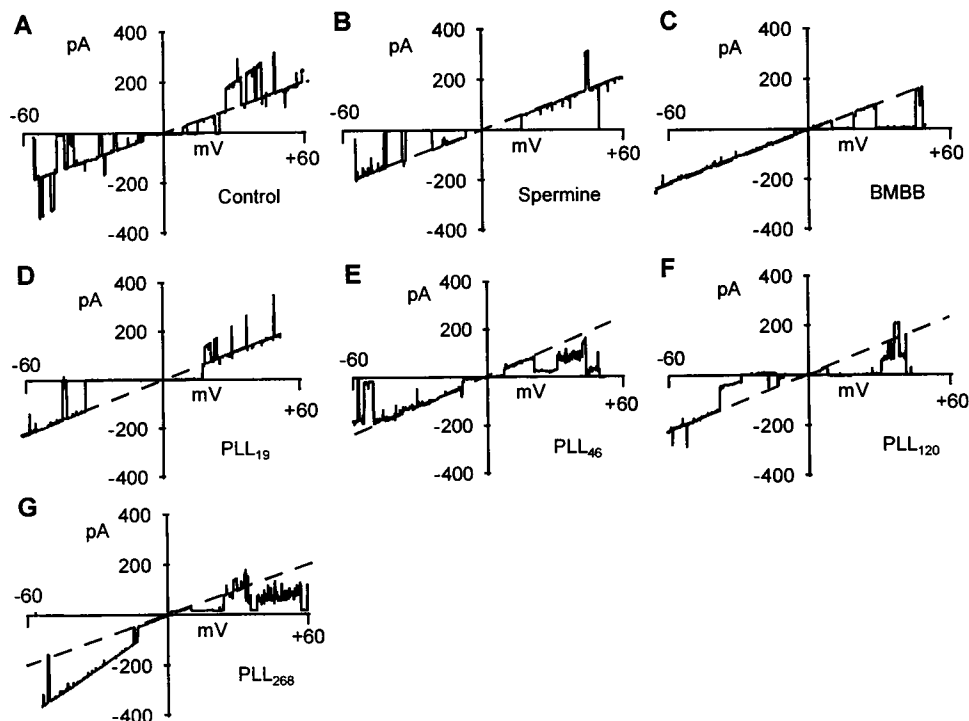
FIGURE 3 Effect of polycationic compounds on channel conductance. Voltage ramps from −60 to +60 mV were applied across patches of liposome membrane containing activated MscL. Pipette tips were filled with recording solution and backfilled with recording solution only (A), or 100 μM of a polycation (B–G). Polycations used were spermine hydrochloride (B), BMBB (C), and bromide salts of PLL$_{19}$ (D), PLL$_{46}$ (E), PLL$_{120}$ (F), and PLL$_{268}$ (G). Sample traces shown were recorded 40 min after patches were obtained.

DISCUSSION

Ion channel diameters can be estimated by investigating the permeability of molecules of various sizes through the pore. Using this approach, channel diameters have been explored with metal and organic ions (Hille, 1992). In general, chan-

nels that exhibit ion selectivity have pore diameters <10 Å (Bezanilla and Armstrong, 1972; Cecchi et al., 1982; Yang, 1990; Villarroel et al., 1995; Sabovcik et al., 1995). Among
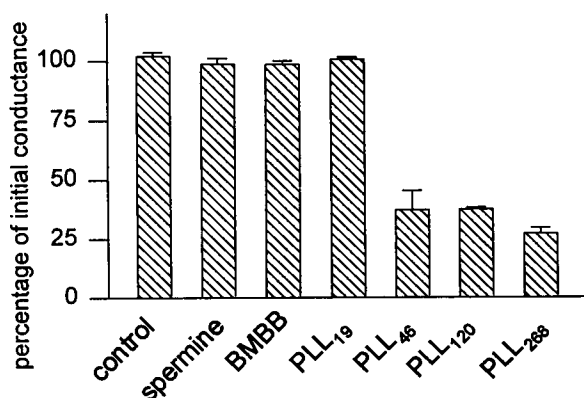

FIGURE 4 Summary of the effects of various organic ions on conductance. Initial conductance was measured immediately after seal formation, while the final conductance was measured after 30 or 40 min. Conductance was estimated from the slope of single-channel currents in response to voltage ramps between ±60 mV. Data are presented as final conductance as a percentage of the initial conductance. Conductances were measured at positive pipette potentials only, and data are presented as means ± SE (n = 3 except for PLL$_{268}$; n = 4).
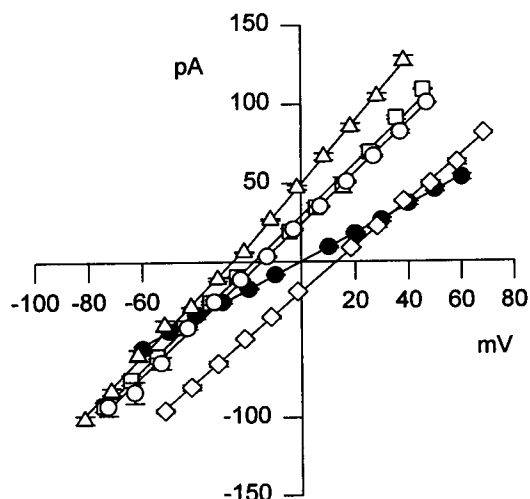

FIGURE 5 Current voltage plot of the permeation of organic ions through the MscL. Current-voltage relationships of single-channel current amplitude against pipette potential were generated in symmetric low potassium solution (K$_L$, ●) and also in configurations in which 100 mM test compound was added to the bath solution. Compounds examined were putrescine (○), cadaverine (□), spermine (△) and succinate (◇). All data are presented as means ± SE (df = 11).

TABLE 2　Summary of the organic ion permeation studies

Test Ion	Charge	Conductance (nS)	Reversal Potential (mV)	P_X/P_K
	—	0.900 ± 0.07	-0.5 ± 0.2	
Potassium	1+	1.40 ± 0.01	-2.2 ± 0.2	1.0
Putrescine	2+	1.62 ± 0.02	-15.1 ± 0.4	0.53
Cadaverine	2+	1.64 ± 0.02	-18.1 ± 0.2	0.58
Succinate	2−	1.45 ± 0.01	$+12.9 \pm 0.3$	0.48
Spermine*	4+	$(+)1.99 \pm 0.02$	-27.6 ± 0.5	0.49
		$(-)1.77 \pm 0.06$		

Recordings were made in low potassium buffer, K_L (10 mM KCl, 40 mM $MgCl_2$, 5 mM HEPES, pH 7.2), with 100 mM test ion in the bath solution. Data are presented as mean $\pm$ SE ($df = 11$, except for spermine $df = 5$). Permeability ratios, P_X/P_K, where X represents the test ion, were calculated according to Lewis (1979).
*Since a significant difference was found between conductance at negative and positive pipette voltages for spermine, they are shown separately.

the largest channels are cell-to-cell junctional membrane channels in insect salivary glands with pore diameters of 20–30 Å (Schwarzmann et al., 1981) and staphylococcal α-hemolysin with a pore of $\sim$40 Å (Song et al., 1996). In this study, the size of the MscL pore was investigated using large organic ions such as PLLs.

To obtain sizes for the PLL compounds the molecules were assumed to be spherical and the radii were calculated using the Einstein–Stokes equation. A relationship was found between the molecular weights and coefficients of diffusion for several other compounds, including globular proteins, which provided the basis for the estimate of the size of each test compound.

We have found experimentally that PLLs with an average diameter larger than 37 Å (PLL_{46}) reduced the channel conductance, whereas PLL_{19} ($\sim$25 Å), spermine ($\sim$15 Å), and BMBB ($\sim$30 Å) did not. Although there was some overlap in the size distribution of PLL_{19} and PLL_{46}, the lack of any blockade by PLL_{19} suggests that the MscL pore is greater than or equal to the average diameter of PLL_{46} (37 Å, Table 1).

The channel diameter was calculated from its conductance according to a model proposed by Hille (1968) and was found to be 42 Å. This model assumed that the channel has a uniform bore and a length equal to the shorter of the two putative α-helices spanning the membrane in each MscL monomer (Blount et al., 1996a). Although the precise length of the pore is unknown, this parameter has less influence on the pore diameter compared to conductance (Eq. 5). For a range of pore lengths 20–50 Å, the pore diameter was 34–46 Å.

A model can be constructed that uses the pore diameter to test for the number of α-helices that could line the pore. The MscL is proposed to be a homohexamer (Blount et al., 1996a) where each MscL monomer consists of two α-helical membrane-spanning regions (Sukharev et al., 1994; Blount et al., 1996a) giving a total of 12 α-helices. Of these, the number of α-helices that form the pore, n, may be inferred from the diameter d, the distance between centers

of adjacent α-helices x, and the diameter of an α-helix y (Fig. 6) using the following expression:

$$n = \frac{\pi(d + y)}{x} \quad (6)$$

The distance between the centers of adjacent α-helices is $\sim$10.2 Å (Harris et al., 1994) and the diameter of an individual α-helix is $\sim$6.8 Å (Sybesma, 1977). These values are similar to those obtained for the membrane-bound protein bacteriorhodopsin from its crystal structure in the Brookhaven Database (Upton, NY). Using the range of pore diameters 34–46 Å, the number of α-helices ranged from 12 to 16. If the MscL is a homohexamer (Blount et al., 1996a), then this suggests that all 12 α-helices need to line the pore to provide such a large channel size.

It is interesting to consider possible implications of the large pore size of the MscL in terms of its mechanosensitivity and the conformational change corresponding to channel opening. According to the model proposed by Howard et al. (1988) that mechanosensitive channels are displacement-sensitive molecules, the change in the free energy term ΔG is a linear function of membrane tension γ:

$$\Delta G = \Delta G_0 + \gamma \Delta a \quad (7)$$

where ΔG_0 is the difference in free energy between closed and open conformations of the channel in the absence of membrane tension, and Δa is the difference in membrane area occupied by these two conformations at a given membrane tension. Taking into account that the bacterial mech-
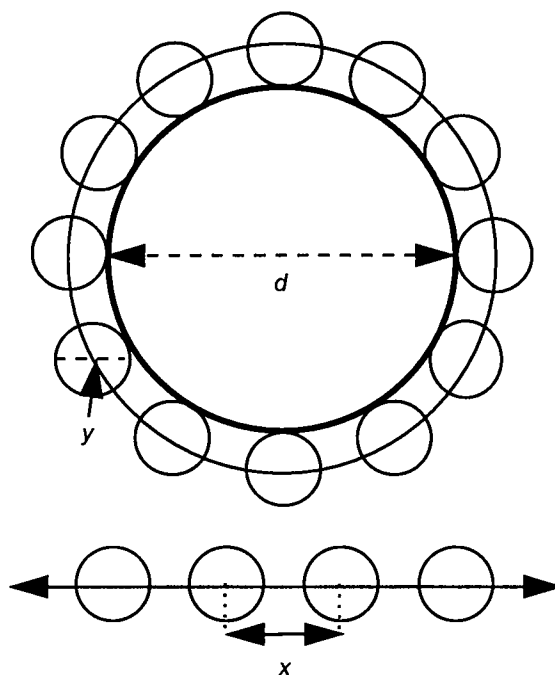
FIGURE 6　Model of the pore size of the homohexameric MscL. Twelve α-helices (*small circles*) from six subunits form the MscL pore (*bold circle*). The distance between adjacent α-helices is x, the diameter of each α-helix is y, and the diameter of the MscL pore is d.

anosensitive channels including MscL are gated by mechanical force transmitted through the lipid bilayer alone (Martinac et al., 1990; Sukharev et al., 1993; Häse et al., 1995), it is plausible to apply the above model to these channels. This should allow us to derive a possible meaning of Δa in the case of the MscL.

A similar approach was applied previously for several other mechanically gated channels (Hamill and McBride, 1994). The model offers the possibility of calculating the change in cross-sectional area occupied by the closed and open channel from the slope of the logarithmic form of the Boltzmann function for the channel open probability. From the Boltzmann distribution:

$$\frac{P_0}{1 - P_0} = e^{-\Delta G/kT} \qquad (8)$$

the sensitivity to pressure of the MscL reconstituted in liposomes was 599 ± 266 Pa (4.5 ± 2.0 mm Hg, $n = 5$) per e-fold change in open probability (Häse et al., 1995). According to Laplace's law for an elastic thin-walled sphere, which should apply in the case of a liposome membrane patch ideally made of a single bilayer, the membrane tension is described by:

$$\gamma = \frac{pd}{4} \qquad (9)$$

where p is the applied pressure and d is the diameter of the membrane patch (Neher and Eibl, 1977; Opsahl and Webb, 1994). For a hemispherical liposome patch 2 μm in diameter corresponding to a diameter of a typical patch pipette (Howard et al., 1988; Sokabe and Sachs, 1990), the membrane tension required for the e-fold change in the MscL open probability is 0.300 ± 0.131 mN m^{-1}. From Eqs. 7–9 it follows:

$$\Delta a = \frac{kT}{\gamma} = 1512 \times 10^{-20}\,\mathrm{m}^2 \approx 1500\,\text{Å}^2 \qquad (10)$$

where the thermal noise energy $kT = 4 \times 10^{-21}$ J (at room temperature). Using the original data for each patch, calculation of the diameter of the circle corresponding to the area Δa gives a value of 43 ± 8 Å, which is very similar to the diameter of the MscL channel pore estimated in this study. However, it should be noted that the present estimate of Δa (1500 Å^2) differs from another recent independent estimate of 350 Å^2 for the MscL reconstituted in liposomes (Sigurdson et al., 1997). Our estimate of Δa is similar to the area change of 1526 Å^2 for the mechanosensitive cation channels in rat atrial muscle, but is large in comparison with an estimate of the area change of 136 Å^2 made for the small mechanosensitive ion channel in E. coli giant spheroplasts (Hamill and McBride, 1994). It is possible that we have overestimated Δa for the MscL by obtaining a lower value for γ by underestimating the radius of membrane patches in our experiments. Nevertheless, in all cases Δa is large, which is consistent with the notion that a channel undergo-

ing a substantial open-closed conformation area change should be mechanosensitive (Hamill and McBride, 1997). The area occupied by 12 tightly packed α-helices in a closed configuration of the MscL hexamer is ~ 1200 Å^2. According to the above estimates of 350 Å^2 (Sigurdson et al., 1997) and 1500 Å^2 (this study) for Δa, the open-closed conformational area change of the MscL may vary somewhere between 30% and 125% of the membrane area occupied by the closed conformation of the channel. Therefore, although probably not the sole contributor to the overall area change in the case of the MscL, the opening of the pore could be expected to present a large fraction of the total difference in area between the two conformations of the channel homohexamer.

The estimates of the pore diameter have implications to a physiological role for the MscL. Mechanosensitive channels have been shown to mediate the movement of organic molecules across the cell membrane when E. coli cells are subjected to hypo-osmotic shock (Berrier et al., 1992; Cui et al., 1995). Therefore, we examined whether the essential polycations putrescine, cadaverine, and spermine could permeate the channel. All polycations tested crossed the membrane through the open MscL to the extent given by their relative permeabilities (Table 2). Ionic charge did not appear to affect the passage of these compounds since the relative permeability of succinate, an organic anion, was similar to those for the organic cations. These results suggest that many other physiologically relevant organic compounds may cross the membrane through the open MscL.

A channel with a diameter of ~ 40 Å would be able to transfer molecules up to the size of some proteins (<10 kD) into the periplasm and this may be lethal to an E. coli cell, as is the case for pore-forming toxins (Song et al., 1996). Therefore, the present findings seem to question the proposed physiological relevance of the MscL in osmoregulation (Sukharev et al., 1997). If E. coli mechanosensitive channels are involved in osmoregulation, then it seems reasonable to assume that the MscL represents a final response in the sequence of responses of the bacterial cell to the osmotic challenge. The channel will be inactivated once osmostasis is restored. Therefore, under conditions at which the MscL is activated, the transient loss of compounds of up to 10 kD in diameter may be a necessary cost for increasing the chances of survival of the cell.

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