The large mechanosensitive ion channel (MscL) of *Escherichia coli* was expressed on a plasmid encoding MscL as a fusion protein with glutathione S-transferase in an *Escherichia coli* strain containing a disruption in the chromosomal mscL gene. After purification of the fusion protein using glutathione-coated beads, thrombin cleavage allowed recovery of the MscL protein. The purified protein was reconstituted into artificial liposomes and found to be fully functional when examined with the patch-clamp technique. The reconstituted recombinant MscL protein formed ion channels that exhibited characteristic conductance and pressure sensitivity and were blocked by the mechanosensitive ion channel inhibitor gadolinium. The recombinant MscL protein was also used to raise specific anti-MscL polyclonal antibodies which abolished channel activity when preincubated with the MscL protein.

Mechanosensitive ion channels have been found in organisms of different phylogenetic origin including animals, plants, fungi, and bacteria (1-4). Although exclusively documented in patch-clamp experiments, the ubiquity of mechanosensitive channels suggests that they have important physiological functions in various types of biological cells. Increasing evidence indicates that the physiological role of these channels is to modulate cell responses to mechanical stimuli such as stretch, contraction, or osmotic stress (5-7). Microorganisms, such as the enterobacterium *Escherichia coli*, are constantly exposed to changes in environmental osmolarity. A recent study by Berrier et al. (7) demonstrated that the loss of metabolites following osmotic down-shock was blocked by gadolinium, the well-documented inhibitor of mechanosensitive channels (4).

Patch-clamp studies of giant spheroplasts of *E. coli* have revealed the presence of two distinct types of mechanosensitive ion channels in the bacterial cell envelope: a small weakly anion-selective mechanosensitive channel (MscS) with a conductance of approximately 1 nS (8, 9) and a large nonselective channel (MscL) with a conductance of 2.5-3.0 nS (9). Both ion channels could be reconstituted into artificial liposomes either by fusing bacterial membrane vesicles (9, 10) or by reassembly of detergent-solubilized membrane extracts (9, 11), without loss of their mechanosensitive properties. Furthermore, functional reconstitution was used as an assay for the biochemical isolation of mechanosensitive channel proteins (11) leading to the recent molecular identification and cloning of the mscL gene encoding the large mechanosensitive channel of *E. coli* (12, 13).

In the present study, we have used a common method for expressing recombinant proteins in *E. coli* to produce significant amounts of purified MscL protein (14, 15). The recombinant protein was found to be fully functional when reconstituted into artificial liposomes and was used to raise polyclonal anti-MscL antibodies. This work was presented in preliminary form (16).

**EXPERIMENTAL PROCEDURES**

**Materials—** *E. coli* strain DH5α was from Life Technologies, Inc.; plasmids pGEM12zf(+) and pGEX-2T were from Promega and Pharmacia LKB Biotechnology (Uppsala, Sweden), respectively. Bacto-tryptone and yeast extract were from Oxoid Ltd. (Hampshire, United Kingdom). The following reagents were purchased from Sigma: octyl glucoside, Tris, EDTA, lysozyme (L 6876), dianisidine, thrombin (T 7009), HEPEs, SDS, MOPS, bovine serum albumin (A2153), phosphatidylcholine (P 3644), and cholesterol. Isopropyl-1-thio-β-D-galactopyranoside (IPTG) and Tween 20 were obtained from Aldrich. Ampicillin and chloramphenicol were from Boehringer Mannheim (Mannheim, Germany). Other chemicals were analytical reagent grade. Glutathione-Sepharose 4B beads and cyanogen bromide-activated Sepharose beads were purchased from Pharmacia, and Calbiorobin beads were obtained from Calbiochem-Novabiochem Corp.

**Bacterial Strains, Plasmids, and Culture Conditions—** *E. coli* strain AW737-KO, carrying a chromosomal insertion in the mscL gene (12), was used for the protein expression experiments. Plasmid pS-2-2 carrying the entire open reading frame (ORF) of mscL on a XhoI DNA fragment, generated by the polymerase chain reaction method, was described previously (12). Plasmids pGEM12zf(+) and pGEX-2T were used for generating pGEX1.1. Bacterial cells were grown at 37 °C in Luria-Bertani broth (10 g/liter Bacto-tryptone, 5 g/liter yeast extract, 5 g/liter NaCl) with 50 μg/ml chloramphenicol for the mscL knock-out *E. coli* mutant, added when required.

**DNA Preparation, Manipulation, and Analysis—** Plasmid DNA was extracted from *E. coli* cells using the alkaline lysis method (17). Standard techniques (18) were used for the generation of recombinant plasmid constructs described under "Results." Restriction enzymes and DNA ligase were purchased from Promega and used as specified by the manufacturer. DNA restriction fragments were electrophoresed in horizontal 0.8% agarose gels in 40 ml Tris, 1 mM EDTA, pH 8.0, and stained with ethidium bromide (0.5 μg/ml). DNA fragments were excised from 1% low melting agarose gels (Promega), melted at 55 °C, and used directly in ligations reactions.

**Protein Purification and Analysis—** Recombinant fusion protein was purified essentially as described previously (14, 15). Bacterial cells harboring the plasmid pGEX1.1 were subgrown for 1 h at 37 °C (1 ml of an overnight culture in 20 ml of broth), and fusion protein gene expression was induced for 3 h with 0.1 mM IPTG. The cells were harvested, resuspended in 5 ml of 150 mM NaCl, 1 mM EDTA, 50 mM Tris, pH 8.0, and lysed by addition of lysozyme (0.1 mg/ml) and detergent (1.5% octyl glucoside). After batch sonication for approximately 60 s (Unisonics Pty. Ltd., Sydney, Australia), cell debris was pelleted (16,000 rpm, 20 min;
Functional Purification of the Recombinant MscL of E. coli

J2-ML (Beckman), and 0.5 ml of glutathione-Sepharose 4B beads were added to the supernatant for 1 h at room temperature (20–22 °C). The beads were then washed at least three times (by centrifugation using a desk top centrifuge at 4,000 rpm for 5 min) in phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.2, adjusted with NaOH) and resuspended in PBS containing an additional 150 mM NaCl, 2.5 mM CaCl₂, and 50 mM Tris. Thrombin was added to a final concentration of ~1 unit/μg of protein and incubated for 1 h at room temperature. Oxytetracyclins in PBS was added to a final concentration of 1% (w/v) for 10 min, the beads were pelleted (4,000 rpm, 5 min), and the supernatant was found to contain the MscL proteins. J2-ML, purified from plasmid p5-2-2 (12) was first subcloned into plasmid vector pGEM11Zf(+) in the desired orientation as determined by restriction enzyme analysis. The gene was then excised and cloned into the expression vector pGEX-2T as a BamHI-EcoRI DNA fragment (now named pGEX1.1), thus generating a continuous ORF with the GST gene (Fig. 1). This genetic construct should lead to the production of a 41-kDa hybrid protein consisting of a N-terminal GST portion (26 kDa) and a C-terminal MscL portion (15 kDa) separated by a thrombin cleavage site. Upon thrombin cleavage of the fusion protein, nine amino acids as well as the initial methionine residue, which are not present in purified wild-type MscL, are expected to be present on the N terminus of the hybrid protein. Two female New Zealand albino rabbits were prebled and injected with approximately 100 μg of purified MscL protein in TiterMax adjuvant (Vaxcel, Inc., Norcross, GA). Antibody titers were checked after 3 and 6 weeks by Western blot analyses essentially as described (18). Briefly, proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose in transfer buffer (20 mM Tris, 150 mM glycine, 20% methanol). The filters were reacted with rabbit antibodies (diluted 1:1100 for whole sera, 1:50 for affinity-purified antibodies), incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Sigma) (diluted 1:1,000), and developed with substrate solution (0.2 mM o-dianisidine, 0.01% H₂O₂ in 10 mM Tris, pH 7.4). MscL-specific antibodies were purified by coupling purified MscL protein to cyanogen bromide-activated Sepharose beads with subsequent washes and elution conditions as described previously (19). Affinity-purified antibodies were dialyzed against 100-fold diluted PBS and then concentrated 100-fold by lyophilization (Dynavac, Pty. Ltd., Sydney, Australia). For enzyme-linked immunosorbent assay (ELISA), the wells of microtiter plates were coated with various concentrations of purified MscL protein in PBS overnight at 4 °C. The coated plates were then washed in PBS with 0.02% Tween, blocked with 2% bovine serum albumin in PBS for 2 h at room temperature, washed again, and reacted with antisera (diluted 1:1,000 for whole sera, 1:100 for affinity-purified antibodies) at 4 °C overnight. The plates were washed again and incubated with peroxidase-labeled secondary antibody (diluted 1:5,000) for 2 h at room temperature. Substrate was added, and absorbance at 410 nm was measured on a microplate reader (Spectra, Cambridge Technologies, Inc., Watertown, MA).

Electrophysiological Recordings—The improved patch-clamp techniques of Hamill et al. (20) were used to record single-channel currents from isolated membrane patches. Pipettes were made from borosilicate glass (Drummond Scientific Co., Broomall, PA) using a Flaming/Brown Micropipette puller (P-87, Sutter Instrument Co., Novato, CA) and pulled to a diameter which gave bubble numbers of 3.2–3.5 in 100% ethanol, corresponding to pipette resistances in the range 6.4–8.5 MΩ, respectively, when in recording solution. Pipettes were coated with clear nail enamel (Super Shine Top Coat, Sally Hansen, North Ryde, Australia) and filled with recording solution (200 mM KCl, 40 mM MgCl₂, 5 mM HEPES, pH 7.2 adjusted with KOH). A small aliquot (1–2 μl) of rehydrated liposomes was placed in the 0.5-ml patch-clamp chamber containing recording solution, and the chamber was situated on an inverted phase contrast microscope (IMT-2, Olympus Optical Co., Tokyo, Japan). The reference electrode was a Ag/AgCl pellet separated from the bath by an agar bridge (2% agarose in 1 mM KCl). Pipettes were positioned with a Leitz micromanipulator (Ernst Leitz Wetzlar GmbH, Wetzlar, Germany) and were touched against unilamellar blisters arising spontaneously from the liposomes as described (9, 10). Seals (>20 GΩ) either formed immediately or following application of a brief (1–2 s) pulse of negative pressure (<50 mmHg), applied to the interior of the patch pipette.

Channel activation was normally achieved by applying pressure steps of ~20 to ~30 mmHg by mouth over a period of several seconds every 5–10 s. For rapid pressure steps (~1 s), suction was applied by mouth in a single step from 0 mmHg to a pressure exceeding the activation threshold for that particular patch. Single-channel currents were filtered at 1 kHz, recorded using a patch-clamp amplifier (List Electronics, Darmstadt, Germany), and digitized at 5 kHz by a computer running WinTida analogue to digital acquisition software (Heka Electronics, Heidelberg, Germany). Data files were analyzed off-line using commercial software or programs written in this laboratory.

RESULTS

Construction of a Plasmid Encoding a GST-MscL Fusion Protein—The XhoI DNA restriction fragment containing the entire ORF of mscL from plasmid p5-2-2 (12) was first subcloned into plasmid vector pGEM11Zf(+) in the desired orientation as determined by restriction enzyme analysis. The gene was then excised and cloned into the expression vector pGEX-2T as a BamHI-EcoRI DNA fragment (now named pGEX1.1), thus generating a continuous ORF with the GST gene (Fig. 1). This genetic construct should lead to the production of a 41-kDa hybrid protein consisting of a N-terminal GST portion (26 kDa) and a C-terminal MscL portion (15 kDa) separated by a thrombin cleavage site. Upon thrombin cleavage of the fusion protein, nine amino acids as well as the initial methionine residue, which are not present in purified wild-type MscL, are expected to be present on the N terminus of the recombinant MscL protein. The mscL ATG start codon and corresponding methionine residue are shown in boldface.

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Fig. 2. A, SDS-polyacrylamide gel electrophoresis protein patterns during purification steps of the GST-MscL fusion protein from E. coli. B, Western blot using immunoaffinity-purified anti-MscL antibodies. Lanes: 1, total E. coli cells before induction with IPTG; 2, total E. coli cells after induction with IPTG; 3, glutathione-Sepharose beads absorbed material; 4, thrombin cleavage of glutathione-Sepharose beads absorbed material; 5, purified MscL protein. Numbers on the left indicate positions of molecular weight markers. Arrows indicate positions of the fusion protein (FP), GST, and MscL.

Sepharose beads to E. coli cell lysates. Analysis of the material absorbed by the glutathione-Sepharose beads revealed a single major protein band of approximately 40 kDa (Fig. 2A, lane 3). After incubation of the Sepharose-bound fusion protein with thrombin, two additional protein bands of 26 and 17 kDa were generated, presumably representing GST and MscL, respectively (Fig. 2A, lane 4). Following thrombin digestion, the MscL channel protein was further purified by removal of the GST portion of the fusion protein with the beads fraction (Fig. 2A, lane 5). The molecular weight of these protein bands corresponds well with those predicted from the DNA sequence.

Generation and Effect of Anti-MscL Polyclonal Antisera—Polyclonal anti-MscL antisera were generated by injecting purified MscL protein into rabbits. Both animals showed significant anti-MscL titers several weeks after injection, as determined by Western blot and ELISA analyses of the collected sera. MscL-specific antibodies were purified by affinity chromatography and were used in the Western blot shown in Fig. 2B. Both fusion protein and MscL showed strong reactivity with the antibodies (Fig. 2B, lanes 3 and 5), whereas no significant reactivity was observed with any other E. coli proteins or GST (Fig. 2B, lanes 1 and 4). However, in E. coli cells strongly expressing the fusion protein, several smaller molecular weight protein bands reacted with the antibodies (Fig. 2B, lane 2), which presumably represent proteolytic degradation products of the fusion protein. Immune reactions of pre-sera, sera following MscL injection, and affinity-purified anti-MscL antibodies, against purified MscL protein were also examined in ELISA analysis. Pre-sera showed no significant reactivity with MscL protein, whereas immune blood exhibited strong reactivity; about half of the antibody titer was recovered after purification over a MscL-affinity column (data not shown).

Electrophysiological Recordings of Reconstituted MscL Protein—Purified MscL protein was reconstituted into liposomes (protein:lipid ratio of 1.6000) and observed to be functional when examined with the patch-clamp technique (Fig. 3). Single-channel currents were recorded from excised patches of liposome membrane at pipette potentials ranging from -40 mV to +40 mV and pressures ranging from -50 to -200 mmHg. No channel activity was observed in excised patches from liposomes not containing MscL protein (n = 5 patches). In addition, no activity was observed in patches from liposomes containing either GST-MscL fusion protein (n = 8 patches; protein:lipid ratio of 1:3000) or GST protein alone (n = 4 patches; protein:lipid ratio of 1:5900).

Activation of the MscL by pressure ceased following removal of the stimulus as shown in Fig. 3. As pressure is increased in small steps, the threshold of activation is crossed, and the channel activated. Furthermore, if the applied negative pressure is maintained at a constant level, the channel activity in many patches slowly increased with time (Fig. 3). In patches where the number of active channels was low, MscL activity occurred as a burst of single openings followed by a long inactivation period. Following this inactivation, channel activity was not recovered either by repeated application of voltage (-40 mV to +40 mV) or by application of negative pressure up to 200 mmHg.

Pressure Sensitivity of the MscL—Channels were activated when negative pressure (suction) exceeded a threshold, typically in the range of 50 to 100 mmHg. As the amount of applied pressure increased, channel activity and hence channel open probability also increased (Fig. 4A). We have used a Boltzmann distribution curve to describe the apparent pressure sensitivity of the reconstituted MscL protein. The open probability of the channels in a particular patch was plotted against the applied suction, and the data were fitted to a Boltzmann distribution (Fig. 4B). For reconstituted MscL, an e-fold change in open probability was observed following a change of 4.9 ± 1.4 mmHg (mean ± S.E., n = 3 patches) at a pipette potential of +10 mV, and 3.9 ± 1.1 mmHg at a pipette potential of -10 mV (n = 2 patches). The average applied negative pressure required to induce half-maximal activation of MscL was 72 ± 3 mmHg (n = 3 patches) at a pipette potential of +10 mV, and 71 ± 6 mmHg (n = 2 patches) at a pipette potential of -10 mV.

In response to a more rapid (1–2 s) change in pressure, the MscL exhibited rapid activation, followed by adaptation (Fig. 5). At present, we are unable to examine this phenomenon in more detail because of the relatively slow step change in pressure attainable with our experimental apparatus. This activation following a rapid change in pressure has also been observed for the MscS in in situ recordings from giant E. coli.
Poll versus is the suction (mmHg) at which the channel is open half the conductance of the purified MscL was estimated from the amplitude histograms (giving this particular patch was estimated to be 5.0 mmHg per e-fold change in open probability, and the pressure required for half-activation of the amplitude histograms (giving NPo) divided by the maximum number of channels observed in this particular patch (i.e. n = 3, see panel A, -70 mmHg trace). The curve is a Boltzmann distribution relating applied (negative) pressure and open probability, fitted by nonlinear regression. The Boltzmann distribution has the form:

\[ P_o = \frac{\exp(p - p_{50}S_p)}{1 + \exp(p - p_{50}S_p)} \]  

where \( P_o \) is the channel open probability, \( p \) is the applied suction (mmHg), \( p_{50} \) is the suction (mmHg) at which the channel is open half the time, and \( 1/S_p \) is the slope of the plot of \( \ln(P_o/(1 - P_o)) \) versus suction. From this distribution, the sensitivity to pressure of the channels in this particular patch was estimated to be 5.0 mmHg per e-fold change in open probability, and the pressure required for half-activation (where \( P_o = 0.5 \)) was estimated to be 65.2 mmHg.

Conductance Measurements of Reconstituted MscL—The conductance of the purified MscL was estimated from the amplitude of the single-channel currents and the applied pipette voltage (Fig. 6). The MscL showed slight rectification at positive pipette voltages. The conductance at negative potentials using a linear regression fit to the data was 3,500 pS and, for positive potentials, was 3,300 pS, with the reversal potential close to zero as expected for this nonselective ion channel (9).

Inhibition of the MscL by Gadolinium—In the present study, reconstituted MscL was inhibited by gadolinium in a reversible manner (Fig. 7). Complete blockade of channel activity by 1.0 mM gadolinium was observed even at negative pressures up to 150 mmHg. At a lower concentration (0.2 mM), inhibition by gadolinium was still observed; however, this inhibition could be reversed by increasing the applied pressure (data not shown (9)).

Effects of Antibodies in Patch-Clamp Experiments—Liposomes containing either purified MscL or MscL which had been preincubated in a 1:1 molecular ratio of affinity-purified anti-MscL antibodies for 1 h, were examined. In 11 of the 16 patches examined containing MscL alone, single-channel currents were observed under standard conditions of voltage and pressure. However, in 16 patches examined where MscL was preincubated with anti-MscL antibody, no single-channel opening events were observed at pressures up to -200 mmHg.

DISCUSSION

In this study, we have used a common method of expressing recombinant proteins in E. coli as fusion proteins with GST (14, 15) to produce substantial amounts of purified MscL protein. A plasmid expression vector was constructed encoding a hybrid protein with fusion of MscL to the C terminus of GST separated by a thrombin cleavage site. Induction of the hybrid gene resulted in strong expression of the fusion protein followed by a rapid single-step purification from E. coli cell lysates using glutathione-coated beads. The recombinant MscL protein was further purified by mild detergent extraction following thrombin cleavage of the fusion protein bound to the beads.

Proteolytic digestion of the fusion protein by thrombin resulted in the presence of several amino acid residues at the N terminus of the recombinant protein that are not found in the wild-type MscL protein, as confirmed by N-terminal amino acid sequence analysis. The experiments with the recombinant protein in E. coli as fusion proteins with GST (14, 15) to produce substantial amounts of purified MscL protein. A plasmid expression vector was constructed encoding a hybrid protein with fusion of MscL to the C terminus of GST separated by a thrombin cleavage site. Induction of the hybrid gene resulted in strong expression of the fusion protein followed by a rapid single-step purification from E. coli cell lysates using glutathione-coated beads. The recombinant MscL protein was further purified by mild detergent extraction following thrombin cleavage of the fusion protein bound to the beads.

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- Fig. 4. A, pressure sensitivity of the MscL in response to normal pressure application recorded from an isolated patch of artificial liposome membrane. Traces are 20-s recordings at a holding potential of -10 mV from the same patch, as pressure applied to the interior of the pipette was increased. C denotes the closed state and O, denotes the open state of n number of channels. B, effect of pressure on the open probability of the large mechanosensitive channel. Data are from the same patch as in A. The open probability was estimated from the area of the amplitude histograms (giving NPo) divided by the maximum number of channels observed in this particular patch (i.e. n = 3, see panel A, -70 mmHg trace). The curve is a Boltzmann distribution relating applied (negative) pressure and open probability, fitted by nonlinear regression. The Boltzmann distribution has the form:

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Proteolytic digestion of the fusion protein by thrombin resulted in the presence of several amino acid residues at the N terminus of the recombinant protein that are not found in the wild-type MscL protein, as confirmed by N-terminal amino acid sequence analysis. The experiments with the recombinant

- Fig. 5. Activation of the MscL following rapid changes in pressure application. Pressure step rise time was approximately 1 s. Upper trace shows pressure applied to the interior of the pipette and the lower trace shows currents. The holding potential was -10 mV. C denotes the closed state and O, denotes the open state of n number of channels. Note, there are up to 14 active channels in this particular patch.

- Fig. 6. Current-voltage plot of mechanosensitive single-channel current amplitude and pipette potential. Recordings of 20-s duration were obtained from isolated patches of liposome membrane in response to normal pressure application, and amplitudes were estimated from the current-amplitude histograms. Data are presented as mean ± S.D. from n patches for the following: +20, +10, -10, -20 mV (n = 7), +30, -30 mV (n = 3), and -40, -40 mV (n = 2).
MscL did not indicate any major effects of these additional amino acid residues on channel properties. However, unlike the native MscL protein examined following gel filtration and reconstitution, which does not exhibit rectification (9), at positive pipette voltages a slight rectification was observed with the recombinant channel. At present, we are unable to explain this observation, but one possibility may be that the additional ten amino acids present on the recombinant MscL interfere with the unidirectional passage of ions through the channel pore. With these amino acids present, it is unlikely that the N-terminal portion of MscL plays a major role in the transduction of mechanical force used for activation of this channel, since the activation pressures for the recombinant MscL were similar to those observed for the native protein reconstituted in liposomes (9).

In the present study, we have used the purified recombinant protein to generate specific polyclonal anti-MscL antibodies which showed strong reactivity with both fusion protein and MscL in Western blot and ELISA analyses. When incubated with MscL protein prior to reconstitution, these antibodies abolished channel activity. These anti-MscL antibodies should enable us to study MscL location in the native E. coli cell envelope, as well as to identify cross-reactive proteins in other organisms.

Purified MscL protein was reconstituted into liposomes and found to be fully functional, exhibiting characteristic conductance and pressure sensitivity, similar to that of the native channel (9). In addition, following incorporation into liposomes, the recombinant channel was blocked by the common inhibitor of mechanosensitive channels, gadolinium (4), at concentrations similar to those reported to inhibit the MscL of E. coli following reconstitution of solubilized native membranes (7, 9). Gadolinium appeared to increase the activation threshold of the MscL, suggesting a partial reversal of the inhibition by pressure. However, since in the majority of cases the number of channels observed per patch was relatively high (on average 3 to 6), this result may also reflect that due to the increased open probability, there is an increased likelihood of observing those channels in the patch not inhibited by gadolinium.

The number of channels present in a particular patch of membrane appeared to influence the type of activity displayed by the recombinant protein. Where the number of active channels in the patch was relatively low (1–2), openings occurred as a single burst followed by long inactivation. In the majority of patches where the number of active channels was higher, the MscL exhibited sustained activity, and, furthermore, in many patches channel activity was observed to slowly increase with time. A similar increase in activity of the MscL from native E. coli membranes has been observed, and, typically, channel activity continues to increase with time until the patch ruptures.2 Taken together, the results suggest that upon application of negative pressure there may be cooperativity between MscL molecules, either with regard to activation or association of the channel monomers with one another. It is tempting to speculate that the possible mechanism of MscL activation by lipid bilayer tension consists of assembly of pore-forming multimers from dispersed channel monomers in response to mechanical force. A multimeric form (possibly a tetramer) of the functional channel (9) is suggested from the observation that the native channel purified from the E. coli cell envelope has an approximate molecular mass of 60–80 kDa compared to that of the monomer of 15 kDa. A possible indication of an association mechanism for MscL activation comes from the results of the polyclonal antibody experiments. When mixed with the channel protein prior to incorporation into liposomes, anti-MscL antibodies prevented any channel activity from being observed. However, another more trivial explanation for the effect of the antibodies may simply be that the MscL protein-antibody complex may not insert into the lipid bilayer in a way which allows channel activation by pressure. Further evidence for interaction between functional MscL derives from the observation that the recombinant MscL was more responsive to rapid steps in pressure than in response to a gradual increase in stimulus. A similar phenomenon has been reported for mechanosensitive channels of hair cells of the turtle and stretch-activated channels of Xenopus oocytes (22). A possible physiological role for this rapid activation may be in providing part of a defense mechanism against rapid changes in osmotic pressure (7).

Activation by pressure of mechanosensitive channels in E. coli can be described by a Boltzmann distribution (8). However, for the recombinant MscL, in many patches continuous application of pressure resulted in an increase in channel activity with time, and, furthermore, where channel number was low, channel inactivation was observed. Therefore, in these experiments, the Boltzmann distribution could only be used as an approximate description of the channel quasi-steady-state activity, since the channels were not truly in an equilibrium state. However, the results for this apparent pressure sensitivity of MscL did show that the reconstitution method decreases the pressure required to activate these channels without altering the activation profile with respect to pressure. A similar lowering in activation pressure threshold has been reported for both MscS and MscL when purified native channels were incorporated into liposomes (9).

In conclusion, we have used a common method for expressing recombinant proteins in E. coli to produce significant amounts of purified MscL protein, and the recombinant channel isolated was found to be fully functional when reconstituted into artificial liposomes. Furthermore, the rapid protein purification method described in this paper will enable us to examine mutagenized MscL proteins and hence explore the role of specific amino acid residues on channel properties.

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1. C. C. Häse, A. C. Le Dain, and B. Martínez, unpublished observation.
regions of the protein molecule involved in the mechanotransduction process.

Acknowledgments—We thank Dr. C. Kung and Dr. P. Blount, University of Wisconsin-Madison, for helpful discussions and the generous donation of the *E. coli* strain AW737-KO and mscL carrying plasmid p5-2-2. We would also like to thank Dr. B. Chang for the donation of *E. coli* strain DH5a and plasmid pGEM11Zf (+), and Dr. T. Ratajczak for the donation of plasmid pGEX-2T, both of The University of Western Australia.

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