Eur Biophys J (2005) 34: 389–395
DOI 10.1007/s00249-005-0468-x

ARTICLE

Thom Nguyen · Brian Clare · Wei Guo · Boris Martinac

The effects of parabens on the mechanosensitive channels of *E. coli*

Received: 12 November 2004 / Revised: 27 January 2005 / Accepted: 30 January 2005 / Published online: 16 March 2005

Abstract Parabens are alkyl esters of *p*-hydroxybenzoic acid used as preservatives in a wide range of food, pharmaceutical, and cosmetic products (Soni et al. *Food Chem. Toxicol.* 39:513–532, 2001). Despite their common use for over 50 years, their mechanism of action is still unclear. In this study we examined the effects of ethyl and propyl paraben, on gating of the *E. coli* mechanosensitive channel of large conductance (MscL) reconstituted into azolectin liposomes. We found that propyl and ethyl paraben spontaneously activate MscL. Moreover, the addition of propyl paraben caused an increase in MscL activity and the lowering of $p_{1/2}$, the pressure at which the MscL was opened 50% of the time, the ΔG_o, the free energy required to open the MscL, and the parameter α, which describes the channel sensitivity to pressure. In addition, in silico studies showed that propyl paraben binds to the channel gate of the MscL. The mechanosensitive channel of small conductance was also found to be spontaneously activated by parabens. In summary, our study indicates that one of the previously unidentified mechanisms of action of parabens as antimicrobial agents is via an interaction with the mechanosensitive channels to upset the osmotic gradients in bacteria.

Keywords Mechanosensitive channels · Parabens · Mechanosensitive channel of large conductance · Mechanosensitive channel of small conductance · *p*-Hydroxybenzoic acid

This revised version was published online in March 2005 with corrections to Figure 6.

T. Nguyen · B. Clare · W. Guo
School of Medicine and Pharmacology, QEII Medical Centre, The University of Western Australia, Crawley, WA, 6009, Australia

B. Martinac (✉)
School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, 4072, Australia
E-mail: b.martinac@uq.edu.au
Tel.: +61-89-3462986
Fax: +61-89-3463469

Abbreviations EDTA: Ethylenediaminetetraacetic acid · HEPES: *N*-(2-Hydroxyethyl)piperazine-*N*′-ethanesulfonic acid · MS: Mechanosensitive · MscL: Mechanosensitive channel of large conductance · MscS: Mechanosensitive channel of small conductance

Introduction

Parabens are alkyl esters of *p*-hydroxybenzoic acid and are a class of antimicrobial agents. Owing to their broad spectrum of antimicrobial activity, and low toxicity, parabens have been used in a wide range of food, pharmaceutical and cosmetic products for over 50 years (Soni et al. 2001). Though the mechanism of action has yet to be elucidated, parabens are hypothesized to act by inhibiting synthesis of DNA and RNA (Nes and Eklund 1983) or of some key enzymes, such as ATPases and phosphotransferases, in some bacterial species (Ma and Marquis 1996) or by disrupting membrane transport processes (Freese et al. 1973).

The mechanosensitive (MS) channel of large conductance (MscL) has become a prototype MS channel for studying structure–function relationships in this class of ion channels.The MscL homologues have commonly been found in Gram-negative and Gram-positive strains forming a subfamily of a larger family of MS class of ion channels encompassing prokaryotes (bacteria and archaea) as well as cell-walled eukaryotes (fungi and plants) (Martinac 2004). The MscL was identified at the molecular level in 1994 (Sukharev et al. 1994) and its 3D crystal structure was solved in 1998 (Chang et al. 1998). The channel conductance has been reported to be in the range 2.5–3.8 nS (Hamill and Martinac 2001).The MscL and its homologues have been found to play an important role in osmoregulation of bacterial cells (Booth and Louis 1999). Bacteria exposed to distilled water (hypoosmotic shock) rapidly release cytoplasmic contents into the surrounding medium, suggesting that the MscL

functions as an osmotically activated emergency valve.The MscL opens in vivo during a hypo-osmotic shock in media of low osmolarity, resulting in osmotically induced effluxes of small osmolytes such as potassium glutamate, trehalose and glycine betaine that serve as osmoprotectants in bacteria. Several site-directed mutations were shown to lead to dramatic changes in the MscL activities examined by the patch-clamp technique (Blount et al. 1997). The same mutations could be associated with a ''slowed or no growth'' phenotype that was partially reversed by increasing the osmolarity of the growth media. In these gain-of-function mutants the correlation could be established between the severity of the phenotype and the severity of electrophysiologically observed abnormalities in ion channel activities of the corresponding MscL mutants. In vivo this disruption of the MS channel function causes slowing or impairment of the bacterial growth. If the gating of the MscL could be affected in a similar way by a pharmacological agent, this would result in a leak of cytoplasmic content and, thus, could also lead to impairment of the bacterial growth.

The MS channel of small conductance (MscS) cloned by the group of Booth (Levina et al. 1999), is a 286-residue membrane protein encoded at the *yggB* locus.The MscS is gated at pressures approximately half of that at which the MscL is activated and has a conductance of approximately 1 nS (Martinac et al. 1987; Sukharev et al. 1993). Its 3D structure has revealed that the functional channel is a homoheptamer (Bass et al. 2002). The MscS, like the MscL, is also involved in osmoregulation and the two channels are capable of compensating the absence of each other (Berrier et al. 1992; Levina et al. 1999).

In the present work we describe a mechanism of action previously unidentified of parabens, that is, via MS channels in bacteria.

Experimental

Materials

Escherichia coli strains AW737KO pGEX1.1 (Häse et al. 1995) and AW737 WT (Sukharev et al. 1994) were used. *p*-Hydroxybenzoic acid, ethyl 4-hydroxybenzoate and *n*-propyl *p*-hydroxybenzoate were purchased from Sigma-Aldrich, Australia. Thrombin, isopropyl-1-thio-*β*-D-galactopryranoside, *n*-octyl-*β*-D-glucopyranoside and cephalexin were purchased from Sigma, Deisenhofen, Germany. Bacto-tryptone and yeast extract were from Oxoid (Hampshire, UK). Ampicillin, chloramphenicol and kanamycin were from Boehringer Mannheim (Mannheim, Germany). Glutathione-Sepharose 4B beads were obtained from Pharmacia Biotech (Sweden) and Calbiosorb beads were purchased from Calbiochem-Novabiochem (USA). Bio-Beads SM-2 adsorbent was from Bio-Rad Laboratories (CA, USA). All other materials were of analytical grade.

Purification of the MscL

The purification of the MscL protein followed basically the method previously described (Häse et al. 1995). *E. coli* AW737 KO cells harbouring the plasmid pGEX1.1, encoding a continuous glutathione-*S*-transferase–MscL (GST–MscL) protein, were used for expression of the GST–MscL fusion protein. The purity of the MscL protein was examined using 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Reconstitution of the MscL

The reconstitution of the MscL into an artificial membrane followed essentially the methods of Häse et al. (1995). Phosphatidylcholine was dissolved in chloroform and dried under nitrogen. Lipids were then resuspended in dehydration/rehydration buffer [200 mM KCl, 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid, HEPES, pH 7.2] to achieve a final concentration of 10 mg/ml and bath-sonicated (5 min or until the solution was clear). The purified MscL was added at the required protein to lipid ratio and rocked at room temperature for 1 h before Bio-Beads (approximately 10 mg) were added to the lipid/protein to remove the detergent (octylglucoside). After 3 h, the Bio-Beads were allowed to settle and the supernatant was centrifuged (100,000 *g*, 30 min, T-100, Beckman Instruments, USA). The pellet was resuspended in 40 µl dehydration/rehydration buffer and spotted onto glass slides (cleaned with 100% ethanol) and dehydrated for 4–6 h (vacuum desiccator, 4°C), followed by rehydration overnight with dehydration/rehydration buffer under humid conditions. Liposome preparations were used on the second day.

Preparation of giant spheroplasts from *E. coli*

Giant spheroplasts from *E. coli* AW737WT were prepared as described previously (Martinac et al. 1987; Sukharev et al. 1997). Briefly, the bacterial culture was treated with cephalexin, which prevented septation, and thus cells grew into snakelike filaments of 50–150 µM in length after incubation for approximately 2 h. The addition of ethylenediaminetetraacetic acid (EDTA) and lysozyme weakened the cell wall and giant spheroplasts large enough to be used for patch-clamp experiments were formed.

Electrophysiology

Single-channel currents of the MscL were recorded using the improved patch-clamp techniques of Hamill et al. 1981). Aliquots of the rehydrated liposomes (4 µl) were added to an approximately 800 µl patch-clamp chamber containing recording solution (200 mM KCl, 40 mM $MgCl_2$, 5 mM HEPES, pH 7.2 (Häse et al. 1995).

Liposomes incubated in a solution containing millimolar Mg^{2+} collapsed to form unilamellar blisters, which are amenable to the patch-clamp technique (Delcour et al. 1989). Liposomes were viewed under a phase-contrast microscope (IMT-2, Olympus Optical Co., Japan). Pipettes were filled with either recording solution or a paraben solution at the indicated concentration. The pipette holder was soaked in 100% ethanol for approximately 10 min between each different paraben to prevent cross contamination. A Ag/AgCl electrode was inserted into the pipette solution and the reference Ag/AgCl electrode was in contact with the chamber solution. A Leitz micromanipulator (Ernst Leitz Wetzlar, Wetzlar, Germany) was used to position the pipette against unilamellar blisters. Once a seal had been formed, the excised patches were then observed for any spontaneous activity. Currents were filtered at 1 kHz using a patch-clamp amplifier (AxoPatch 1D, Axon Instruments, USA) and digitized at 5 kHz for recording to a computer using the pCLAMP 6 software package (Axon Instruments, USA). The applied pipette pressure was converted to a voltage signal using a piezoelectric pressure transducer (Omega, Stamford, USA).

Spontaneous channel activity of the MscS in giant spheroplasts was recorded following essentially the same protocol except that the chamber solution contained spheroplast recording solution (250 mM KCl, 90 mM $MgCl_2$, 10 mM $CaCl_2$, 0.1 mM EDTA and 5 mM HEPES) only. Application of negative pressure (by mouth) was required for formation of a seal of gigaohm levels of 2–3 GΩ. Different paraben solutions were then added to the recording chamber and the patches were then examined for spontaneous channel activity.

Measurement of the MscL conductance

Conductance was estimated from the least-squares regression line fitted to the plot of single-channel currents at different applied pipette potentials (current–voltage plot). Suction was applied to activate single channels and the potential was held constant for each measurement, and then changed as required for the next measurement. Currents were measured as the peaks of the current amplitude using the Axoscope program (Axon Instruments, USA).

Determination of the Boltzmann distribution function of the MscL

Suction was applied stepwise until saturation in channel activity was reached. The negative pressure was held constant for 20 s at the different levels of negative pressure for measurement of channel activity.

Activation of the MscL by pressure can be described by the Boltzmann distribution function for the channel open probability (P_o)

$$P_o/(1-P_o) = \exp\left[\alpha\left(p - p_{1/2}\right)\right],$$

where P_o is the single-channel open probability, α is the slope of the plot of $\ln[P_o/(1-P_o)]$ against negative pressure, and $p_{1/2}$ is the negative pressure (suction) applied to the patch at which the channel is open half the time (i.e. $P_o = 0.5$).

Autodock experiments

The genetic algorithm optimization technique, modelled on biological evolution, was used. Genetic algorithms were basically a Monte Carlo approach, with the ligand starting at many random locations, and the energy minimization was based on a "fitness function", which is a measure of the lowness of the interaction energy, and is a function of the "genome", which is a coding of the variables of the conformation and location of the ligand (Morris et al. 1998).

Results and discussion

Spontaneous MscL activity was induced with 1 mM propyl and ethyl paraben

To study the pharmacological effects of propyl and ethyl paraben on the MscL, patch-clamp experiments were conducted on reconstituted MscL liposomes. Patch-clamp pipettes were backfilled with each paraben solution and used to form gigaohm seals with blisters that appeared as faint spheres under the phase-contrast microscope, as they were more likely to be unilamellar. The patches were then simply observed for spontaneous channel activity without prior application of negative pressure. The spontaneous activity induced by 1 mM propyl paraben was observed to be greater than that induced by 1 mM ethyl paraben as illustrated by the more frequent openings (Fig. 1). In the presence of propyl and ethyl paraben spontaneous MscL activity was observed in a high percentage of the patches examined, that is, 60% and 58%, respectively (Table 1). These results evidently show there is an interaction between the MscL and parabens to initiate the opening of the channel. The opening of a single MscL channel for 1 ms would suffice to upset osmotic gradients in a bacterial cell.

Propyl paraben increases the mechanosensitivity of the MscL

For a more quantitative analysis, the Boltzmann characteristics and conductance of the MscL in the presence of parabens was examined. The α, $p_{1/2}$ and ΔG_o values of the MscL in the presence of 1 mM propyl paraben was found to be significantly different ($p < 0.05$) from values for the control (Fig. 2). The α, $p_{1/2}$ and ΔG_o values were,

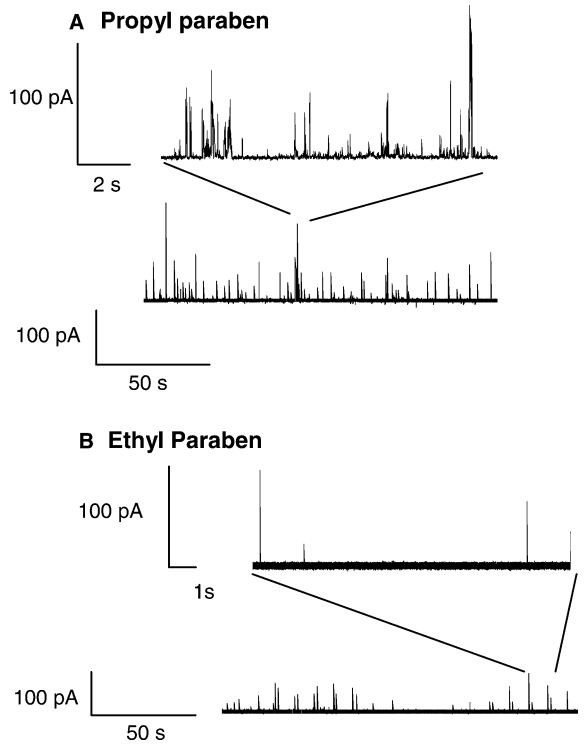

Fig. 1 Traces of spontaneous mechanosensitive channel of large conductance (*MscL*) activity reconstituted in liposomes, in the presence of 1 mM propyl paraben (**a**) and 1 mM ethyl (**b**) paraben. Patch-clamp pipettes were backfilled with each paraben and patches were observed for spontaneous activity at +30 mV. The *upper traces* are expanded traces of the corresponding *lower trace*

respectively, 53%, 9% and 59% lower than the values for the control (Table 2). The decrease in these parameters indicates that the mechanosensitivity is increased in the presence of propyl paraben. Therefore, the MscL of bacteria exposed to propyl paraben could be expected to gate at a lower activation threshold, thus allowing the leakage of cytoplasmic contents essential for survival. Also, the single-channel conductance of the MscL was found to be consistently higher in the presence of 1 mM propyl paraben than for the control (Fig. 3, Table 2), although, this increase in conductance was within the range of values reported for the MscL (Hamill and Martinac 2001; Häse et al. 1995).

It has been shown that the antimicrobial activity of the parabens increases as the alkyl chain length increases (Giordano et al. 1999). The inability of ethyl paraben to

Table 1 The percentage of liposome patches showing spontaneous activity (SA) of the mechanosensitive channel of large conductance (*MscL*) in the presence of 1 mM propyl paraben and 1 mM ethyl paraben

Paraben	Percentage of patches showing SA	*n*
1 mM propyl	60	23
1 mM ethyl	58	20

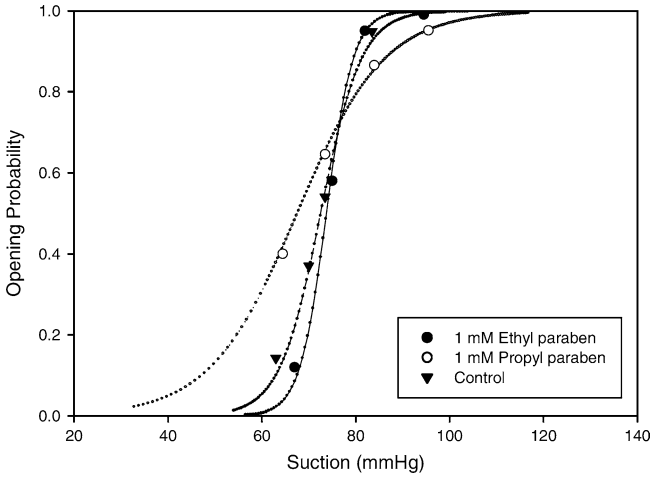

Fig. 2 The effect of 1 mM propyl paraben and 1 mM ethyl paraben on the Boltzmann distribution curve of the MscL

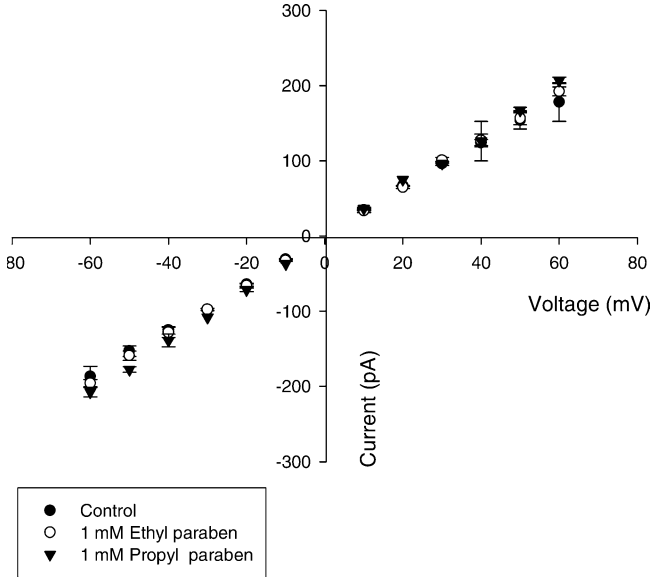

Fig. 3 The effect of 1 mM propyl paraben and 1 mM ethyl paraben on the conductance of the MscL

Table 2 Single-channel conductance values and Boltzmann characteristics of the MscL in the absence (control) of parabens and in the presence of 1 mM ethyl paraben or 1 mM propyl paraben

Paraben	Conductance (nS)	α (mmHg)	$p_{1/2}$ (mmHg)	ΔG_o (kT)	*n*
Control	3.1 ± 0.1	0.28 ± 0.05	77.9 ± 7.1	22.7 ± 5.0	5
1 mM ethyl	3.2 ± 0.05	0.35 ± 0.07	77.9 ± 7.6	26.6 ± 6.0	6
1 mM propyl	3.4 ± 0.07*	0.13 ± 0.02*	71.0 ± 4.2*	9.34 ± 1.8*	5

The conductance, α, $p_{1/2}$ and ΔG_o values of the MscL in the presence of 1 mM propyl paraben were found to be higher compared with the values for the control. Ethyl paraben was not observed to alter the conductance or Boltzmann characteristics of the MscL.
*$p < 0.05$ from the control as determined by Student's t tests. No significant difference, in any parameter measured, was observed between the 1 mM ethyl paraben treatment and the control treatment.

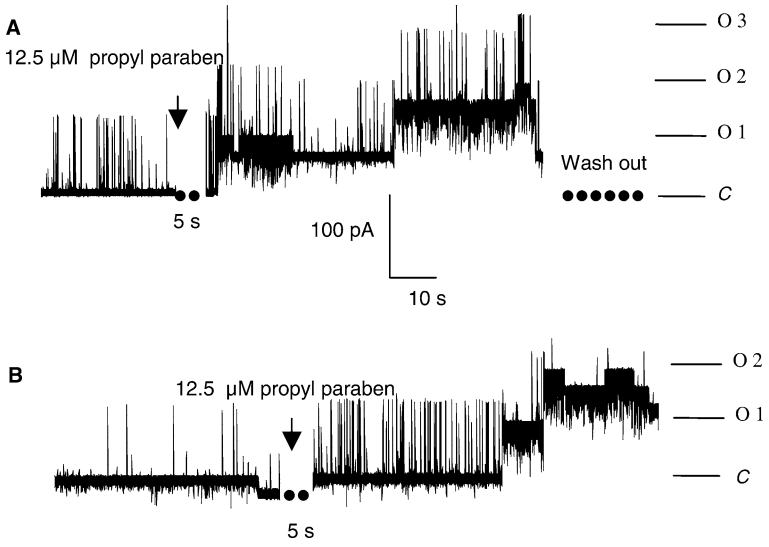

Fig. 4 Current traces showing the effects of 12 μM propyl paraben on channel activity of the MscL in *E. coli*. The two traces are of one continuous trace. A negative pressure of −20 mmHg was maintained throughout the recording. **a** The initial activity at −20 mmHg in the control solution. The *arrow* indicates the addition of 12.5 μM (final concentration in bath solution) propyl paraben directly to the recording chamber, which resulted in an increase in channel activity. **b** Following washout, the channel activity returned to control levels. Washout of the recording chamber involved removing 100 μl of the bath solution containing paraben, and replacing it with 100 μl of clean recording solution. This procedure was carried out three times. The second addition of propyl paraben also resulted in an increase in channel activity. *C* denotes the closed state; *O1*, *O2* and *O3* denote the open state of one, two and three channels, respectively

affect the Boltzmann characteristics of the MscL may reflect the lesser effect of the shorter alkyl chain of ethyl paraben. Since the MscL is localized in the inner cytoplasmic membrane with the carboxyl and amino terminal of the transmembrane domains situated on the cytoplasmic side (Blount et al. 1996), it would be likely that a more hydrophobic compound could reach the channel gate in our recording configuration at concentrations sufficient to activate the channel. The greater spontaneous MscL activity observed with propyl paraben (Fig. 1a) is also consistent with this view.

The effect of propyl paraben on the MscL activity is reversible

The next approach was to examine the effects of propyl paraben on an already activated MscL. The basal activity of the MscL was activated and maintained with 20 mmHg negative pressure. With the addition of propyl paraben, resulting in a final concentration of 12.5 μM, the channel activity increased to two fully open channels with the third channel gating between open and closed states (Fig. 4a). Gradual washouts of the bath solution resulted in a gradual decrease in channel activity. The third washout resulted in a decrease in channel activity to control levels (Fig. 4b). The washout determined two things: that the increased channel activity observed was a paraben effect and not an artefact, and that the paraben effect was reversible. The addition of propyl paraben was repeated, after which channel activity increased (Fig. 4b), further demonstrating that the addition of propyl paraben resulted in the increased channel

Fig. 5 Traces of spontaneous activity of the mechanosensitive channel of small conductance in spheroplasts in the presence of **a** 1 mM methyl paraben, **b** 0.5 mM ethyl paraben and **c** 0.1 mM propyl paraben. After the formation of a seal between the pipette and spheroplast, the different parabens were added and patches were observed for spontaneous activity at +30 mV. *C* denotes the closed state; *O1* denotes the open state of one channel

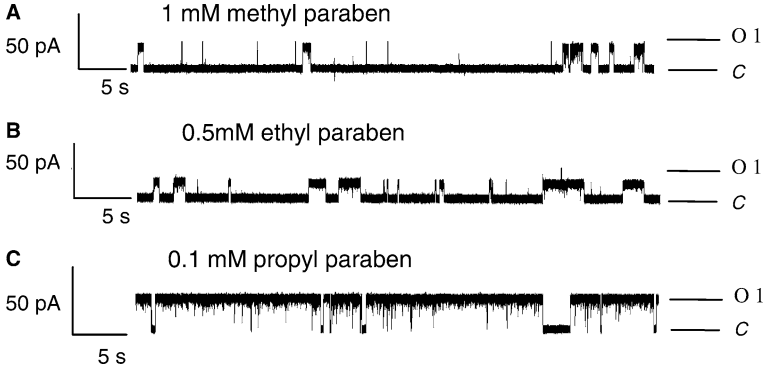

Table 3 The percentage of spheroplast patches in which spontaneous activity (SA) of the mechanosensitive channel of small conductance was observed with the addition of different parabens

Paraben	Percentage of patches showing SA	*n*
1 mM methyl	50	8
0.5 mM ethyl	40	5
0.1 mM propyl	67	6

activity initially observed. This means that for already osmotically challenged bacteria, such as those exposed to rain, the activity of the MscL would be in excess of that required to maintain osmotic balance.

Propyl paraben and ethyl paraben induce spontaneous MscS activity

The MscS was also observed to be spontaneously activated in the presence of parabens. It is interesting to note that similar to its effects on the MscL, propyl paraben caused a greater spontaneous MscS activity than ethyl paraben or methyl paraben (Fig. 5, Table 3) denoted by longer opening times. The spontaneous activity observed with the MscS consisted of fully open channels with longer opening times compared with the activity observed with the MscL, which predominantly consisted of brief openings. This suggests that parabens could have a greater effect on the MscS than on the MscL. This result has been reinforced by preliminary in vivo data which showed that the growth of the bacterial strain containing only the MscS was dramatically reduced compared with that of the bacterial strain having only the MscL (data not shown).

Propyl paraben was shown in silico to bind to the MscL channel gate

Using the structure of the MscL from *Mycobacterium tuberculosis*, which is homologous to the *E. coli* MscL (Chang et al. 1998), in silico studies were conducted to examine the interaction of propyl paraben with the MscL. Using the Autodock program, the algorithm grid was centred on the interior pore of the channel and showed that propyl paraben bound to the channel gate of *Tb*–MscL with energy of −4.91 kcal/mol. Figure 6 shows a propyl paraben molecule bound to the channel gate at the residue alanine 20. This binding energy corresponds to a concentration of approximately 0.25 mM, which is comparable to the concentration used in patch-clamp experiments that was sufficient to induce spontaneous activity of the MscL.

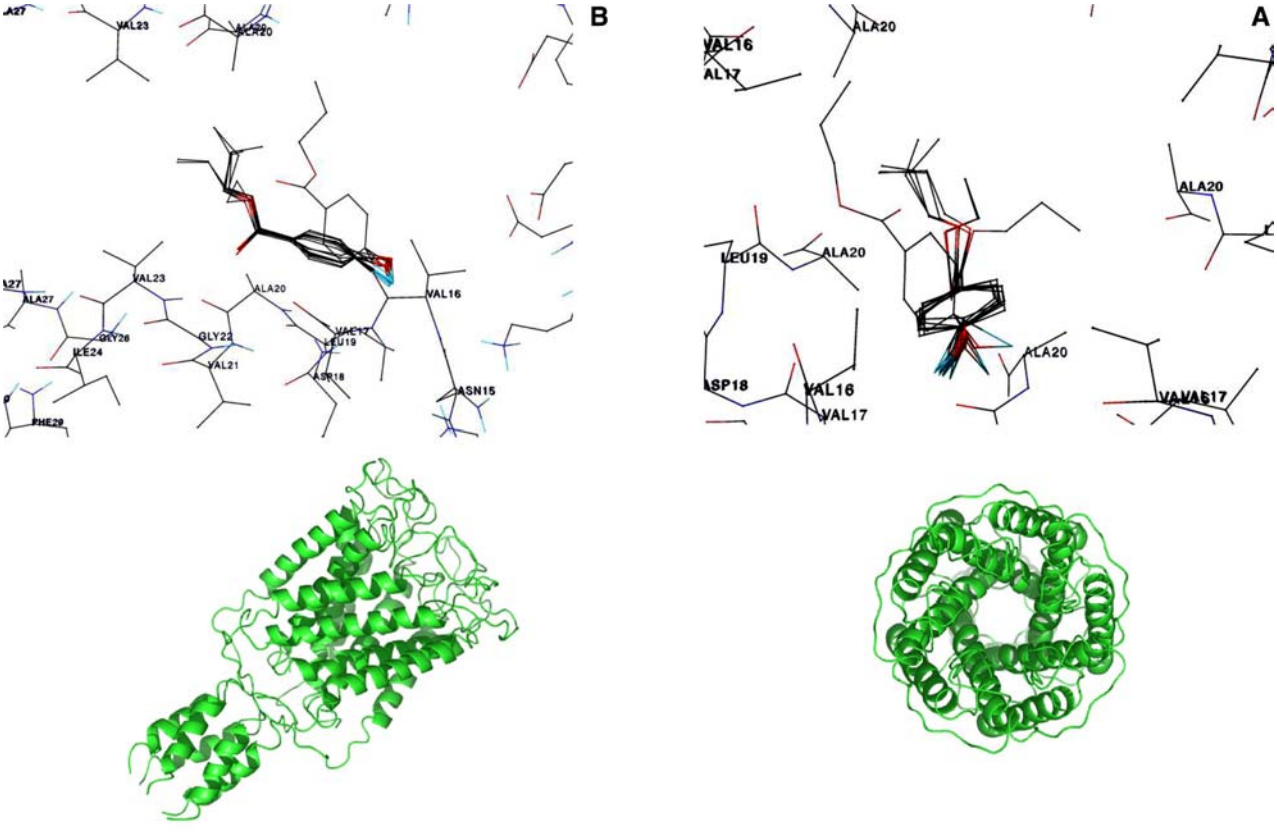

Fig. 6 In silico studies showed propyl paraben was able to bind to the channel gate of *Tb*–MscL at −4.91 kcal/mol. Viewed from the top of the channel looking down into the channel gate (**a**) and from the side of the channel (**b**). Green channel schematics have been added to indicate the orientation of the adjacent channel structures

In summary, the results shown in this study strongly indicate an interaction between parabens and bacterial MscL and MscS channels to inhibit the growth of bacteria by opening the channels and thus collapsing the cell turgor and allowing the leakage of cytoplasmic contents.

Acknowledgements This work was supported by the Australian Research Council and a UWA Postgraduate Award to T.N.

References

Bass RB, Strop P, Barclay M, Rees DC (2002) Crystal structure of Escherichia coli MscS, a voltage-modulated and mechanosensitive channel. Science 298:1582–1587

Berrier C, Coulombe A, Szabo I, Zoratti M, Ghazi A (1992) Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. Eur J Biochem 206:559–565

Blount P, Sukharev SI, Moe PC, Schroeder MJ, Guy HR, Kung C (1996) Membrane topology and multimeric structure of a mechanosensitive channel protein of Escherichia coli. EMBO J 15:4798–4805

Blount P, Schroeder MJ, Kung C (1997) Mutations in a bacterial mechanosensitive channel change the cellular response to osmotic stress. J Biol Chem 272:32150–32157

Booth IR, Louis P (1999) Managing hypoosmotic stress: aquaporins and mechanosensitive channels in Escherichia coli. Curr Opin Microbiol 2:166–169

Chang G, Spencer RH, Lee AT, Barclay MT, Rees DC (1998) Structure of the MscL homolog from Mycobacterium tuberculosis: a gated mechanosensitive ion channel. Science 282:2220–2226

Delcour AH, Martinac B, Adler J, Kung C (1989) Modified reconstitution method used in patch-clamp studies of Escherichia coli ion channels. Biophys J 56:631–636

Freese E, Sheu CW, Galliers E (1973) Function of lipophilic acids as antimicrobial food additives. Nature 241:321–325

Giordano F, Bettini R, Donini C, Gazzaniga A, Caira MR, Zhang GG, Grant DJ (1999) Physical properties of parabens and their mixtures: solubility in water, thermal behavior, and crystal structures. J Pharm Sci 88:1210–1216

Hamill OP, Martinac B (2001) Molecular basis of mechanotransduction in living cells. Physiol Rev 81:685–740

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflug Arch 391:85–100

Häse CC, Le Dain AC, Martinac B (1995) Purification and functional reconstitution of the recombinant large mechanosensitive ion channel (MscL) of Escherichia coli. J Biol Chem 270:18329–18334

Levina N, Totemeyer S, Stokes NR, Louis P, Jones MA, Booth IR (1999) Protection of Escherichia coli cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. EMBO J 18:1730–1737

Ma Y, Marquis RE (1996) Irreversible paraben inhibition of glycolysis by Streptococcus mutans GS-5. Lett Appl Microbiol 23:329–333

Martinac B (2004) Mechanosensitive ion channels: molecules of mechanotransduction. J Cell Sci 117:2449–2460

Martinac B, Buechner M, Delcour AH, Adler J, Kung C (1987) Pressure-sensitive ion channel in Escherichia coli. Proc Natl Acad Sci USA 84:2297–2301

Morris G, Goodsell D, Halliday R, Huey R, Hart W, Belew R, Olson A (1998) Automated docking using a lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 19:1639–1662

Nes IF, Eklund T (1983) The effect of parabens on DNA, RNA and protein synthesis in Escherichia coli and Bacillus subtilis. J Appl Bacteriol 54:237–242

Soni MG, Burdock GA, Taylor SL, Greenberg NA (2001) Safety assessment of propyl paraben: a review of the published literature. Food Chem Toxicol 39:513–532

Sukharev SI, Martinac B, Arshavsky VY, Kung C (1993) Two types of mechanosensitive channels in the Escherichia coli cell envelope: solubilization and functional reconstitution. Biophys J 65:177–183

Sukharev SI, Blount P, Martinac B, Blattner FR, Kung C (1994) A large-conductance mechanosensitive channel in E. coli encoded by mscL alone. Nature 368:265–268

Sukharev SI, Blount P, Martinac B, Kung C (1997) Mechanosensitive channels of Escherichia coli: the MscL gene, protein, and activities. Annu Rev Physiol 59:633–657