Propyl paraben induces potassium efflux in *Escherichia coli*

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Received 27 October 2004; returned 22 December 2004; revised 15 February 2005; accepted 27 February 2005

**Objectives:** Parabens are currently used as antibacterial preservatives in pharmaceutical, cosmetic and food products but there are no precise data concerning their activity on bacterial membranes.

**Methods:** We analysed the cytoplasmic potassium release during propyl paraben addition by using a selective electrode. Various conditions were assayed to investigate the bacterial paraben susceptibility. We compared the activity of propyl paraben with the activities of colicin A and polymyxin B.

**Results:** Propyl paraben induced potassium efflux that was related to the porin expression in the bacterial outer membrane. In addition, the presence of spermine, previously described as an efficient OmpF channel-blocker, protected susceptible cells against paraben activity.

**Conclusions:** Propyl paraben induced potassium release in susceptible *Escherichia coli* cells similar to that observed with polymyxin B. Moreover, this efflux depended on porin channel activity. This permeabilizing effect is probably related to antibacterial properties of paraben molecules.

**Keywords:** outer membrane proteins, membrane channels, porins, pore forming molecules

**Introduction**

Parabens are commonly added in pharmaceutical, cosmetic and food products according to their wide antibacterial properties, low toxicity, inertness and chemical stability. Although this family of molecules is largely used for its antibacterial capacity, our knowledge about its activity and bacterial target is quite unclear. Some authors reported that paraben molecules could induce an alteration of cell membrane properties. Moreover, at the moment only few data report the existence of bacterial resistance against this compound: Valkova et al. recently reported the characterization of an esterase that is involved in the hydrolysis of parabens in *Enterobacter cloacae* and *Enterobacter gergoviae*. Despite these results, the first steps of paraben activity, i.e. penetration and targeting, remain quite unclear and the aim of this study is to decipher these key points.

In the present work, we used an in vivo approach based on K+ efflux measurements as a reporter of membrane alteration after paraben addition. We established that propyl paraben alters the integrity of bacterial membranes, favouring potassium release, and that this mechanism is accelerated by the presence of functional OmpF porin in the outer membrane. In addition, this potassium release caused by propyl paraben was compared with the effect of colicin A and polymyxin B, two antibacterial agents that induce bacterial membrane permeabilization.

**Materials and methods**

**Bacterial strains and susceptibility tests**

The strain used in this work was *Escherichia coli* SM1005 strain (F−, ΔlacU169, rpsL, relA, thiA, fibB, gyrA, ompC, ompF14) which does not express OmpF or OmpC, and the derivative strain containing the plasmid pNLF encoding OmpF. Immunodetection assays with specific porin antiserum were used to check the level of porin synthesis in the strain as previously described. The standard disc diffusion method on Mueller–Hinton (MH) agar was used. Approximately 10⁶ cells were inoculated onto plates of MH agar. Various amounts of propyl paraben or polymyxin B were loaded and the corresponding zones of inhibition were measured after 18 h at 37°C.

**Evaluation of potassium efflux**

The potassium efflux measurements were carried out after addition of propyl paraben, colicin A or polymyxin B as previously described. Briefly, cells grown to an A₆₀₀ of 0.6 in Luria-Bertani broth were collected by centrifugation and washed with 100 mM...
sodium phosphate buffer, pH 7. After washing, the pellet was resuspended in 1/100 of culture volume in the same buffer containing 5% glycerol. Cells (5 × 10^9) were suspended in a glass cup filled with 6 mL of the same buffer at 37°C and under agitation. Propyl paraben was injected when temperature and potassium fluxes were equilibrated. Potassium concentration measurements were performed with a K+-specific electrode and recorded. Colicin A was obtained from our laboratory stock. The plotted values, presented as extracellular K+ increases as previously described, are the means of three independent experiments.

Results

Effect of propyl paraben on bacterial membranes

Potentiometric measurements performed with an ion-selective electrode have been developed to follow membrane permeabilization in turbid bacterial suspensions; the potassium electrode, allowing fast, easy and continuous measurements, is especially appropriate for analysing the action of antibacterial compounds on the membrane. To investigate the effect of propyl paraben on bacterial membranes, we added various concentrations to OmpF-producing E. coli. A major concern with propyl paraben solubility is an appropriate medium showing no adverse activity on bacterial membranes. We thus tested propyl paraben concentrations up to 0.5 mg/mL. The results are presented in Figure 1(a).

At this concentration, the addition of propyl paraben induced a significant linear K+ release. No lag time was observed after the addition of paraben and the curve was quite linear with a slope of about 10 μM K+/min for 0.5 mg/mL, whereas no significant release was obtained with lower amounts of propyl paraben.

It has been previously reported that polymyxin and colicin induce an intracellular potassium efflux from E. coli cells. We compared the K+ release induced by propyl paraben with the curves resulting from polymyxin B or colicin A addition. Under the same conditions, we observed propyl paraben-induced potassium release kinetics that could be compared to those generated by polymyxin B (Figure 1a). These two release kinetics contrasted with the kinetics obtained with colicin A. Concerning this pore-forming protein, we observed a short lag time followed by a linear rapid release, a typical curve of colicin activity, as previously reported.

This suggests that propyl paraben may induce a K+ release with a profile and an efficiency close to that obtained with polymyxin B.

To correlate these results with the antibacterial activity of propyl paraben, we measured the inhibition of E. coli growth. The results presented in Table 1 indicate that only the porin-producing cells were susceptible to propyl paraben under the conditions used. In addition, the propyl paraben MIC for this OmpF-producing strain was about 1 mg/mL. In contrast, no significant change was observed for polymyxin B susceptibility whatever the strain tested (Table 1).

OmpF channel participation in propyl paraben activity

A major concern with agents that destabilize the membrane is the involvement of membrane proteins during the uptake and travel to the inner target, e.g. the role of OmpF porin in colicin A activity. To clarify this point, we analysed the outer membrane proteins that can modulate the activity of propyl paraben by two independent methods. We measured the potassium leakage in: (i) an OmpF- strain and in OmpF-producing cells; and (ii) the presence or absence of spermine with the OmpF-producing cells. This polyamine has previously been demonstrated to jam the OmpF channels and generate a protection against β-lactams and fluoroquinolones using this penetration route through the outer membrane.

Under these conditions, a weak K+ release was observed after the addition of propyl paraben to E. coli cells devoid of porin while the expression of OmpF porin restored a significant potassium efflux (Figure 1b). In OmpF-producing bacteria, the K+ release was severely decreased by the addition of spermine. These results indicate that the level of propyl paraben activity on the bacterial membrane depended on the presence of functional porin channels in the outer membrane and are in accordance with the inhibition measurements.

Discussion

The widespread nature of multidrug bacterial resistance mechanisms strongly affects the activity of all antibiotic families. Consequently, it is interesting to investigate the effect of propyl
Propyl paraben alters bacterial membranes

Table 1. Susceptibilities of E. coli to propyl paraben and polymyxin B

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition diameter (mm)</th>
<th>SM1005</th>
<th>SM1005, pNLF10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propyl paraben</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg</td>
<td>6'</td>
<td>6'</td>
<td></td>
</tr>
<tr>
<td>0.5 mg</td>
<td>6'</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1 mg</td>
<td>6'</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Polymyxin B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg</td>
<td>21</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>5 mg</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

*E. coli* cells, producing OmpF (SM1005, pNLF10) or devoid of porins (SM1005) were used. Values are means of two independent determinations.

No inhibition, corresponds to disc/well diameter.

paraben on bacterial membranes since this product is widely used as an antimicrobial preservative. Despite various studies carried out on eukaryotic and prokaryotic cells, there has been no clear evidence to explain its antibacterial properties. This is a key point since at this moment, several bacterial resistance mechanisms against these products have been reported, including enzymic degradation or efflux pumps.

In this study, we report that propyl paraben can induce a destabilization of bacterial membranes. The profile of potassium release is similar to that obtained with polymyxin B, a drug inducing outer membrane permeabilization. In addition, the OmpF porin participates in the activity of propyl paraben. In OmpF mutant cells or in the presence of spermine, an efficient channel blocker, a drastic reduction in potassium release is observed. These results are close to those previously reported with colicin A: pore-forming colicin uses the OmpF channel to penetrate and this process is altered by spermine. However, the absence of OmpF only reduced the potassium efflux induced by propyl paraben. The contribution of OmpF porin in propyl paraben activity is especially important taking into account the widespread use of parabens in cosmetics. This molecule family may induce potassium release from the target cell. This potassium efflux is the first clue of bacterial membrane leakage induced by propyl paraben. In this context, it is worth considering the paraben content in various standard cosmetic products. Rastogi et al. have determined that a maximum of 0.32% propyl paraben was detected in paraben-containing cosmetics, an amount significantly above the concentration used in this study.

We can hypothesize that propyl paraben firstly induces the permeabilization of bacterial membranes causing the release of intracellular molecules and the de-energization of membranes. This cascade of processes may support the antibacterial activity of this family of preservatives.

Acknowledgements

We thank C. Bollet and J. Chevalier for discussions. J. B. was funded by the Fondation pour la Recherche Médicale. This study was supported by the Université de la Méditerranée.

References