Buffering capacity and $H^+$ membrane conductance of Gram-negative bacteria

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Abstract

Buffering capacity and membrane $H^+$ conductance were examined in seven Gram-negative species: *Aquaspirillum serpens*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Proteus mirabilis* and *Aeromonas hydrophila*. All strains of Enterobacteriaceae studied here showed a decrease in both parameters as the external pH increased, over the pH range studied. The other four species presented an increase in buffering capacity and membrane conductance to protons as the external pH increased from 5.5 to 7.0.

Keywords: Buffering capacity; $H^+$ membrane conductance; Gram-negative bacteria

1. Introduction

Bacteria must respond to environmental stress during life in the biosphere. In particular, they can adapt to changes in external pH [1,2]. The ability of food-borne pathogens to adapt to acidic conditions is a concern in food safety [3-6]. It has also been reported that the susceptibility of some enterobacteria to antibiotics change under acidic conditions [7,8]. Foster and Hall [9] proposed that acid adaptation may alter metabolism and enhance internal pH homeostasis. Krulwich et al. [10] examined the buffering capacity of *E. coli* and of Gram positive bacilli that grow at different pH ranges, but there are no comparative measurements of buffering capacity or membrane conductance to protons of Gram-negative bacteria.

We report here measurements of buffering capacity and passive $H^+$ conductance over a wide range of pH, from 3.5 to 7.5, for the following seven Gram-negative species: three Enterobacteriaceae, *E. coli*, *S. typhimurium* and *P. mirabilis*, and four bacterial species distributed in water and the environment, *A. serpens*, *P. aeruginosa*, *A. faecalis* and *A. hydrophila*. We used the method in which the decay of an acid pulse is measured to determine both parameters [11-13].

2. Materials and methods

2.1. Bacterial strains and growth conditions

Bacterial strains, media and growth conditions used in this study are listed in Table 1. All the
Fig. 1. $H_0$ and $B_0$ values for *Escherichia coli*, *Salmonella typhimurium* and *Proteus mirabilis* over a range of external pH values. *Escherichia coli* cell protein was 10.4 mg ml$^{-1}$, *Salmonella typhimurium* cell protein was 13.3 mg ml$^{-1}$ and *Proteus mirabilis* cell protein was 10.1 mg ml$^{-1}$. The smooth curves were obtained from a polynomial regression.
Fig. 2. B<sub>a</sub> and B<sub>b</sub> values for Aquaspirillum serpens, Pseudomonas aeruginosa, Alcaligenes faecalis and Aeromonas hydrophila over a range of external pH values. Aquaspirillum serpens cell protein was 1.2 mg ml<sup>-1</sup>, Pseudomonas aeruginosa cell protein was 2.1 mg ml<sup>-1</sup>, Alcaligenes faecalis cell protein was 6.6 mg ml<sup>-1</sup> and Aeromonas hydrophila cell protein was 6.9 mg ml<sup>-1</sup>. The smooth curves were obtained from a polynomic regression.
Table 1
Bacterial species used and their growth conditions

<table>
<thead>
<tr>
<th>Organism</th>
<th>Source</th>
<th>Temp. (°C)</th>
<th>Medium</th>
<th>ref</th>
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<tbody>
<tr>
<td>A. serpens</td>
<td>ATCC 11335</td>
<td>27</td>
<td>MPSS</td>
<td>[16]</td>
</tr>
<tr>
<td>P. aeruginosa</td>
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<td>37</td>
<td>TSB</td>
<td></td>
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<tr>
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<td>NB</td>
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<td>TSB</td>
<td></td>
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<td>S. typhimurium</td>
<td>Mutton</td>
<td>37</td>
<td>TSB</td>
<td></td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>NCTC 5887</td>
<td>37</td>
<td>TSB</td>
<td></td>
</tr>
<tr>
<td>A. hydrophila</td>
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<td>27</td>
<td>TSB</td>
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</tbody>
</table>

TSB: Trypticase Soy Broth (BBL, Becton Dickinson and Co., Cockeysville, USA); NB: Nutrient Broth (Oxoid, Unipath Ltd., Basingstoke, UK).

bacteria were grown with shaking except A. serpens and A. faecalis. The cells, grown to early stationary phase, were collected and washed three times with 300 mM KCl. The washed cells were treated with 3 mM EDTA and resuspended in 300 mM KCl [12]. The final concentration of cell protein was 1–13 mg ml⁻¹ (1 x 10¹⁰–8 x 10¹⁰ cfu ml⁻¹).

2.2. Measurement of buffering capacity and membrane H⁺ conductance

Experiments were conducted on 7-ml samples of cell suspensions in 10-ml glass vials, which were magnetically stirred at room temperature. The pH of such suspensions was between 6.2 and 7.2. The buffering capacity and membrane conductance to H⁺ of these bacteria were measured by an acid-pulse technique, according to [13].

The pH recordings were analyzed graphically, as described elsewhere [11–13]. Measurements of buffering capacity and the observed half-time of approach to final equilibrium were used to calculate membrane H⁺ conductance [11]. Buffering capacity and passive H⁺ conductance are presented as functions of external pH.

2.3. Measurement of cell protein

Protein content was determined according to [14].

3. Results and discussion

Fig. 1 and Fig. 2 show the measurements of buffering capacity of the cell surface (B₀) and total buffering capacity (B₁) of the seven bacterial species studied. Both the B₀ and the B₁ values were quite different from species to species. Three of the organisms exhibited B₀ values up to about 1200 nmol H⁺/pH unit per mg of protein, whereas the four others exhibited values that were at least double. It is also interesting that the Enterobacteriaceae showed different curves that described the behaviour of B₀ and B₁, compared with the other Gram-negative species studied. The B₀ and B₁ of E. coli, S. ty-
Fig. 4. Membrane conductance vs. pH of E. coli, Salmonella typhimurium, and Proteus mirabilis.
Fig. 5. Membrane conductance to $H^+$ of *Aquaspirillum serpens*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Aeromonas hydrophila*.
phimurium and P. mirabilis generally increased with decreasing pH in the acid range of pH studied (Fig. 1). P. mirabilis presented higher values of these parameters than E. coli and S. typhimurium.

The cell wall composition of P. mirabilis cells differs from that of other Enterobacteriaceae in that (i) a large percentage of the N-acetylmuramic residues of the peptidoglycan are substituted by O-acetyl groups and (ii) the lipopolysaccharide molecules have negatively charged components [15]. These differences can explain the high $B_o$ and $B_i$ values of P. mirabilis cells.

The other four bacterial species are oxidase positive Gram-negative rods that are distributed in water and the environment. Aquaspirillum serpens, Pseudomonas aeruginosa and Alcaligenes faecalis are aerobic, with a strictly respiratory type of metabolism, with oxygen as a terminal electron acceptor. P. aeruginosa can also use nitrate as an alternate electron acceptor. A. hydrophila is a facultatively anaerobic bacterium physiologically, genetically and taxonomically related to the enterobacteria studied here but their buffering capacity and $H^+$ membrane conductance were comparable to the other three bacterial species found in water and the environment. All of them presented higher values of $B_i$ at pH 6.5 than at pH 5 (Fig. 2). With all the species, the $B_o$ values were appreciable and varied over the range of external pH values examined. Except for A. serpens, the $B_o$ of the cells decreased as external pH increased.

There were no significant differences between cytoplasmic buffering capacity values for the Enterobacteriaceae and A. faecalis studied here and the values found for Serratia marcescens [12] or for E. coli cells permeabilized with n-butanol [10], over the pH range from 5.0 to 7.5 (Fig. 3). A. serpens, P. aeruginosa and A. hydrophila presented high values of $B_i$ at their optimal pH for growth, but A. serpens showed $B_i$ values that were 3-fold greater than those of the other two species.

 Passive $H^+$ conductance of these species, as well as buffering capacity, was sensitive to proton concentration at the external surface, over the pH range studied. The membrane conductance to protons of the three enterobacteria increased as pH became more acidic at pH below 5.5 (Fig. 4). The smooth curves that described the behavior of $C_m^H$ were comparable to the curve reported for a pigmented strain of Serratia marcescens [12]. However, this pigmented strain showed $C_m^H$ values that were 2-fold greater than E. coli and S. typhimurium. The membrane $H^+$ conductance and buffering capacity of P. mirabilis was higher than those found for the other Enterobacteriaceae.

$C_m^H$ values of the rest of the species studied were markedly different from data reported here for the Enterobacteriaceae. Our results of $C_m^H$ values of A. faecalis were comparable to those found for a non-pigmented strain of Serratia marcescens [12]. A. serpens and P. aeruginosa exhibited a maximum value of $C_m^H$ at pH 6.5 and the $C_m^H$ of A. serpens was 2-fold greater than that of P. aeruginosa. A. hydrophila had a maximum at pH 4 and a minimum at pH 5.5 (Fig. 5).

The experiments described in this paper show that buffering capacities and membrane $H^+$ conductance of Enterobacteriaceae were higher at acidic external pH than at neutral pH, and that non-Enterobacteriaceae species presented higher values of $C_m^H$ at pH near neutrality than at pH 5.5. These two groups of bacteria can be found in different natural habitats, intestinal and urinary tract and water respectively, where they adapt to environmental changes. It has been reported that adaptive responses to acid for E. coli and S. typhimurium, involve changes in cell surface properties in addition to the enhancement of intracellular pH homeostasis [1,2,9]. Our results of buffering capacity for the Enterobacteriaceae agree well with these reports. There are no reports about pH-response or pH-homeostasis of chemo-heterotrophic Gram-negative bacteria, other than Enterobacteriaceae, over the pH range from 4 to 7.5, available in the literature. The results reported here contribute to the study of the response of bacteria to external pH changes.

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References


