Interconversion of Components of the Bacterial Proton Motive Force by Electrogenic Potassium Transport

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The influence of K⁺ ions on the components of the transmembrane proton motive force (ΔμH⁺) in intact bacteria was investigated. In K⁺-depleted cells of the glycolytic bacterium Streptococcus faecalis the addition of K⁺ ions caused a depolarization of the membrane by about 60 mV. However, since the depolarization was compensated for by an increase in the transmembrane pH gradient (ΔpH), the total proton motive force remained almost constant at about 120 mV. Half-maximal changes in the potential were observed at K⁺ concentrations at which the cells accumulated K⁺ ions extensively. In EDTA-treated, K⁺-depleted cells of Escherichia coli K-12, the addition of K⁺ ions to the medium caused similar, although smaller changes in the components of ΔμH⁺. Experiments with various E. coli K-12 K⁺ transport mutants showed that for the observed potential changes the cells required either a functional TrkA or Kdp K⁺ transport system. These data are interpreted to mean that the inward movement of K⁺ ions via each of these bacterial transport systems is electrogenic. Consequently, it leads to a depolarization of the membrane, which in its turn allows the cell to pump more protons into the medium.

The proton motive force plays a primary role in energy transduction in intact bacteria, as well as in mitochondria and chloroplasts (9, 10, 17, 22). Consequently, a lot of effort has been made to measure the proton motive force (29), which can be described as a sum of the transmembrane electrical potential difference (Δψ, internally negative) and a pH difference (ΔpH, pH_out minus pH_in) (22):

$$\frac{Δμ_{H^+}}{F} = -58ΔpH + Δψ$$

(in millivolts).

In bacteria energy transduction takes place at the cytoplasmic membrane, and many groups have reported that various metabolizing bacteria do indeed maintain a large, outwardly-directed ΔμH⁺ across this membrane (3, 12, 13, 21, 25). In addition to pumps which extrude protons, bacteria possess several genetically distinct K⁺ uptake systems (6, 11, 28). It is a matter of debate how the constitutive systems catalyze K⁺ transport (2, 6, 26, 30). To obtain more information on this point, we consider in the present study the effects of K⁺ ions on the two components of ΔμH⁺. Although in streptococci similar measurements have been performed, literature data are controversial. In most studies, addition of K⁺ was found to lead to an interconversion of Δψ into ΔpH (12, 13, 15, 16), and the membrane potential depended on the K⁺ content of the cells (12), whereas experiments with a fluorescent, Δψ-sensitive dye indicated that the latter was not the case (19). Our results, obtained with improved methods (29, 33), show that in both S. faecalis and E. coli K⁺ did cause an interconversion of the components of ΔμH⁺. Moreover, both the concentration dependence of the observed effects and experiments with K⁺ transport mutants lead us to postulate that: (i) a close functional link exists between K⁺ transport, on the one hand, and the observed changes in ΔpH and Δψ, on the other; and (ii) that this is the basis for the observation that the membrane potential depends on cellular K⁺ content (12). We interpret our observations to mean that K⁺ transport is electrogenic.

MATERIALS AND METHODS

Bacterial strains and growth conditions. S. faecalis ATCC 9790 was grown overnight on medium NaTY (sodium-tryptone-yeast extract; 2). The cells were kept for 4 extra h in the stationary phase to deplete them of K⁺ ions. These late-stationary-phase cells were harvested by centrifugation, washed, activated with glucose, and washed again as described previously (1). Finally, the K⁺-depleted NaTY cells, which contained 100 to 200 μmol of K⁺ per g (dry weight) of cells, were suspended at 10 to 20 mg (dry weight) per ml in a medium containing 100 mM so-
The various K⁺ transport mutants of *E. coli* K-12 used are listed in Table 1. All of the strains were grown on a minimal salt medium of varying K⁺ concentration with glucose as a carbon source (5). The strains which contained the constitutive TrkA system (i.e., K-12 and TK1001) were grown on 5 mM K⁺. In strain TK2240, the Kdp system was induced by growing the cells with about 40 μM K⁺. After growth had ceased because of lack of K⁺ ions, 50 μM KCl was added, and growth continued. This procedure was repeated once more before the cells were harvested. Strain TK2242 was grown on 115 mM K⁺ to repress the Kdp system. Under these conditions K⁺ uptake occurred exclusively via the very sluggish TrkF system (Table 1). All cells of *E. coli* were harvested in the late-logarithmic phase and washed once with 120 mM Tris-hydrochloride (pH 8.0). The outer membrane of the cells was disrupted by treatment with EDTA (32) essentially according to Fadán et al. (25), but with the following modifications. The cells were suspended to 10 mg (dry weight) per ml in 120 mM Tris-hydrochloride (pH 8.0) at 37°C, and EDTA was added to a final concentration of 1.0 mM. After 10 min, the cells were collected by centrifugation, washed three times with 200 mM sodium-HEPES buffer (pH 7.5), and suspended at 10 to 20 mg (dry weight) per ml of the same buffer. The cell suspensions were kept aerobic by shaking at 250 rpm in a gyratory shaker bath. The EDTA cells thus prepared contained about 50 μmol of K⁺ per g (dry weight) of cells and were used within 4 h after the EDTA treatment.

**Internal water space.** For *S. faecalis*, values were taken for the total and internal water space of the cells of 2.6 and 1.75 ml/g (dry weight) of cells, respectively (1). The internal and total water spaces of cells of *E. coli* were determined with 2H₂O and [1⁴C]glucose (14, 29) after centrifugation through silicone oil (1). For this purpose, [1⁴C]glucose (Amersham Büchler, Braunschweig, West Germany) was purified by preincubation with cells. After removal of the cells by centrifugation, the supernatant solution was used for the actual volume determination. The total water space of the cells was 2.7 ± 0.6 ml/g (dry weight) (mean value of the results of 10 experiments). The internal water space was about 55% of that of the total cell water or about 1.45 ml/g (dry weight) of cells.

**Uptake studies.** In the standard protocol three consecutive experiments were carried out in which Δψ, ΔpH, and K⁺ uptake by the cells were measured separately. For this purpose, cells of either *S. faecalis* or *E. coli* were diluted to 1.0 mg (dry weight) per ml in the suspension buffer and supplemented with 10 μM tetrathenylphosphonium (TPP⁺) chloride-10 mM glucose and at time zero (t = 0) with KCl at the concentration indicated. For the determination of Δψ, at t = -5 min, 20 to 40 nCi of [3H]TPP⁺Br⁻ (2,400 Ci/mol) per ml was added to the suspension. For the determination of ΔpH, at t = -5 min, 2H₂O and [1⁴C]benzoic acid (26 or 57 Ci/mol) were added at 1 to 3 μCi/ml and 100 to 200 nCi/ml, respectively. No other additions were made to the suspensions used for K⁺ uptake studies. The cell suspension of *S. faecalis* was mixed thoroughly, but kept without shaking during the experiment. Samples of the *E. coli* cell suspensions (10 ml or less) were placed in 50-ml Erlenmeyer flasks which were shaken throughout the experiment at 250 rpm in a gyratory shaker bath at 20°C. At this temperature, the rate of O₂ uptake by the cell suspensions was slow enough to avoid anaerobiosis during the incubation and the following centrifugation step (see Results). At various times 1.0-ml samples were pipetted into 1.5-ml polypropylene centrifuge tubes (Sarstedt, Nümbrecht, West Germany), which contained 0.2 ml of silicone oil (D = 1.05, Aldrich or Roth). The cells were separated from the medium by centrifugation through the oil in a Beckman B minicentrifuge. For radioactivity measurements, a sample of the supernatant was added directly to 1.0 ml of 0.2 M NaOH in a plastic minival (Zinsser, Frankfurt, West Germany). The remainder of the supernatant, as well as most of the silicone oil, was carefully removed by suction. The tip of the centrifuge tube, containing the pellet, was cut off and also added to a minival containing NaOH. After all of the protein had dissolved, 4.0 ml of Quickzint 212 scintillation fluid (Zinsser) was added, and the radioactivity of the vials was measured in a Packard 2265 Tri-Carb liquid scintillation counter. As judged from the external standard ratio, supernatant and pellet fractions contained approximately the same amount of quencher. For the K⁺ uptake studies, part of the supernatant and the whole pellet were taken from the microcentrifuge tube and treated with 5% trichloroacetic acid as described previously (2). K⁺ concentrations were determined with an Eppendorf flame photometer.

The uptake of labeled compounds or of K⁺ ions by the cells was measured as a function of time. Usually in about 10 min, a steady state was reached. The data given are mean values of data points taken at 15, 25, and 35 min after the addition of KCl at t = 0. If the standard deviation was larger than the symbols used in the figures, it is given as a vertical bar as well.

Gradients of K⁺ and K⁺ ions, ΔpH across the cytoplasmic membrane was determined with 2H₂O and [1⁴C]benzoic acid (15, 29). The use of this acid seems to be justified, since no indication was found that it was metabolized by the cells, and it gave results identical to those obtained with 5,5-dimethyloxazolidine-2,4-dione. Δψ was calculated from the [3H]TPP⁺ distribution across the cytoplasmic membrane by means of the Nernst equation (29, 33). K⁺ uptake by the cells was expressed in micromoles of K⁺ taken up per gram (dry weight) of cells, or sometimes as the K⁺ distribution ratio across the cytoplasmic membrane, Δψ/k = 58 log (K⁺in/K⁺out). The activity coefficients of TPP⁺ and K⁺ ions were taken to be 1.0 for the calculation of Δψ and Δψ/k, and it was assumed that no binding of these cations occurred to cellular components. In one experiment the membrane potential was measured with the fluorescent dye 3,3'-dihexyloxacarbocyanine [dio-C₃(3)] (19). The experimental conditions were as described elsewhere (1, 15).

**Reagents.** The "good" buffers 2-(N-morpholino)ethanesulfonic acid (MES), N-(2-acetamido)-2-aminoethane sulfonic acid (ACES), HEPES, tris-[hydroxymethyl]methylamino propane sulfonic acid (TAPS), and N-tris(hydroxymethyl)methylglycine (Tricine) were from Sigma Chemical Co., St. Louis,
Mo. TPP⁺ Cl⁻ was from Fluka, Buchs, Switzerland. [¹⁴C]benzoic acid was either from Amersham Büncher, Braunschweig, West Germany, or from New England Nuclear Corp., Boston, Mass. H₂O and [¹⁴C]glucose were from Amersham. We gratefully acknowledge H. R. Kaback for his gift of [³H]TPPP⁺.

RESULTS

*S. faecalis.* Figure 1A shows the components of Δµ₄H⁺ as a function of the amount of K⁺ added to glycolyzing, K⁺-depleted cells of *S. faecalis.* Saturating amounts of K⁺ ions depolarized the membrane from 160 to 100 mV (internally negative). Concomitantly, ΔpH increased from ~35 to +20 mV, with the result that the total Δµ₄H⁺ was almost constant at about 120 mV. A possible explanation for this effect is that the K⁺ cation is transported electrogenically into the cell (11, 12, 15). If this is the case, the cells should extensively accumulate K⁺ ions at concentrations affecting the components of Δµ₄H⁺. Figure 1B shows that this was indeed the case: metabolizing cells accumulated maximally about 800 μmol of K⁺ per g (dry weight); half-maximal effects on Δψ, ΔpH, and the extent of K⁺ uptake were observed at 550, 450, and 300 μmol of K⁺ added per g, respectively. Figure 1 also shows that cells with different K⁺ contents did develop different membrane potentials. This was originally described elsewhere (12), but could not be confirmed with the fluorescent dye diO-C₁₀-(3) (19).

In the experiment of Fig. 2 we employed this dye to measure K⁺-induced changes in Δψ on a faster time scale. It has been shown that the fluorescence intensity of diO-C₁₀-(3) reflects the membrane potential of cells in suspension fairly rapidly (2, 15, 19). Addition of increasing amounts of KCl to the suspension of glycolyzing cells led to a rapid depolarization (Fig. 2). At 2 mM K⁺ and above, overshoots were observed before the potential reached a new steady-state level. The extent of the final depolarization and its affinity for K⁺ ions are in agreement with the results presented in Fig. 1, in which Δψ was measured by [³H]TPPP⁺ distribution.

*E. coli.* Unlike *S. faecalis,* genetically defined mutants of *E. coli* K-12 which are impaired in K⁺ uptake are available (5, 28). Therefore, we focused our attention on this organism. First, the influence of K⁺ ions on the rate of metabolism by EDTA-treated cells of *E. coli* K-12 was investigated. At 20°C the K⁺-depleted cells oxidized glucose at a constant rate of about 30 to 50 μatoms of O min⁻¹ g⁻¹ (dry weight). Addition of 2 mM KCl stimulated the rate of O₂ uptake by a factor of 2 to 3 (31), but after several minutes the rate of uptake decreased to a value of 1.0 to 1.5 times that before K⁺ was added. Similar

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**Table 1. K⁺ transport mutants of *E. coli* K-12 and their properties**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>K⁺ transport system(s) present</th>
<th>Energetic requirement for K⁺ transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12</td>
<td>Y-met (λ) F⁻</td>
<td>TrkA, TrkD (TrkF)</td>
<td>ATP and Δµ₄H⁺</td>
</tr>
<tr>
<td>TK1001</td>
<td>F⁻ trkD1 kdpABC5 lacZ rha thi gal</td>
<td>TrkA (TrkF)</td>
<td>ATP and Δµ₄H⁺</td>
</tr>
<tr>
<td>TK2240</td>
<td>F⁻ trkD1 trkA405 nagA lacZ rha thi</td>
<td>Kdp (TrkF)</td>
<td>ATP</td>
</tr>
<tr>
<td>TK2242</td>
<td>F⁻ trkD1 trkA405 kdp-42 nagA lacZ rha thi</td>
<td>TrkF</td>
<td>Δψ</td>
</tr>
</tbody>
</table>

*Genetic data after references 5 and 28, and energetics of K⁺ transport after reference 26. Strain TK2242 has a Kdp system with much decreased affinity for K⁺ ions (7). All TK strains were kindly donated by W. Epstein.*
effects were observed with cells of mutants TK1001 or TK2240. The time course of the temporary stimulation of O₂ uptake was very similar to that of K⁺ uptake by the cells (data not shown). Therefore, we conclude that net K⁺ uptake does require metabolic energy. In the steady state K⁺ ions may stimulate cellular metabolism to some extent without, however, affecting the magnitude of Δµ⁺ (see below).

We then investigated the influence of K⁺ ions on the components of the proton motive force in the various E. coli K⁺ transport mutants. Table 2 shows that at pH 7.5 and with glucose as a substrate all of these mutants developed a large Δµ⁺, which consisted almost exclusively of a Δψ component. The addition of 2 mmol of K⁺ per g to cells that contained at least the constitutive TrkA K⁺ transport system (strains K-12 or TK1001, Table 1), or the induced Kdp system (strain TK2240, Table 1), decreased Δψ by about 20 mV and increased ΔpH by about the same amount (Table 2). Under these conditions the cells of these strains all accumulated K⁺ ions rapidly (data not shown; 28). By contrast, in cells which only contained the sluggish TrkF system, (i.e., strain TK2242, not induced for Kdp [7]), the addition of as much as 60 mmol of K⁺ per g caused only minimal conversion of Δψ into ΔpH (Table 2). This correlated well with the limited K⁺ transport capacity of system TrkF (28).

These results indicate that in E. coli also the small effects of K⁺ ions on the components of Δµ⁺, although smaller than those in S. faecalis, are related to the ability of the cells to take up K⁺. Further support for this notion was sought in mutant TK1001, which only contains the constitutive TrkA system. Figure 3 shows at pH 7.5 the dependence on the amount of K⁺ added of the components of Δµ⁺, on the one hand (Fig. 3A), and of the K⁺ contents of cells and of Δψ, on the other (Fig. 3B). The results obtained were very similar to those shown in Fig. 1 and 2 for S. faecalis, except that the E. coli cells only accumulated maximally about 250 μmol of K⁺ per g (dry weight). Half-maximal effects on K⁺ accumulation, ΔpH, and Δψ were observed when K⁺ was added in amounts of 150, 250, and 350 μmol/g, respectively (Fig. 3). Almost identical results were obtained with cells which only contained the Kdp instead of the TrkA system (data not shown).

Figure 4 shows the pH dependence of the effects of saturating amounts of K⁺ on the components of Δµ⁺ in mutant TK1001. Between pH 6.1 and 8.4, K⁺ ions depolarized the membrane and increased ΔpH to about the same extent. In

![Figure 2. Time course of the depolarization by K⁺ in S. faecalis. Cells of S. faecalis were preincubated at 0.5 mg/ml in 100 mM sodium-HEPES (pH 7.5)-0.1 mM MgCl₂-1.25 μM dio-C₂(3) (1). Samples of this suspension were transferred to cuvettes, and the fluorescence intensity was measured as a function of time. At t = 3 min the cells were supplemented with 20 mM glucose, and at t = 6 min the cells were supplemented with KCl at the concentrations indicated in the figure. The fluorescence intensity was converted into values of Δψ by means of a calibration curve determined for the same batch of cells. (1, 15).](http://jb.asm.org/)

### Table 2. Influence of K⁺ ions on the components of Δµ⁺ in various E. coli K⁺ transport mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>K⁺ transport system(s) present</th>
<th>K⁺ added (mmol/g of cells)</th>
<th>−58ΔpH (mV)</th>
<th>Δψ (mV)</th>
<th>Δµ⁺ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12</td>
<td>TrkA, TrkD, TrkF</td>
<td>—</td>
<td>−3</td>
<td>158</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>+16</td>
<td>134</td>
<td>150</td>
</tr>
<tr>
<td>TK1001</td>
<td>TrkA, TrkF</td>
<td>—</td>
<td>−1</td>
<td>145</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>+20</td>
<td>126</td>
<td>146</td>
</tr>
<tr>
<td>TK2240 (Kdp induced)</td>
<td>Kdp, TrkF</td>
<td>—</td>
<td>−25</td>
<td>168</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>+9</td>
<td>140</td>
<td>149</td>
</tr>
<tr>
<td>TK2242 (Kdp not induced)</td>
<td>TrkF</td>
<td>—</td>
<td>−7</td>
<td>152</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5</td>
<td>−7</td>
<td>150</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>−1</td>
<td>142</td>
<td>141</td>
</tr>
</tbody>
</table>

* —, None.
TrkA separate between indicated which intact all conditions the linear K+ contents on concomitant with this observations. Therefore, mV.

*a_v ml)* were added.

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FIG. 3. Components of Δμ_H+ (A) and the K+ content (B) in E. coli TK1001 as a function of the amount of K+ added. Metabolizing, EDTA-treated cells (1 mg/ml) were incubated in the presence of the indicated amount of K+ ions. For further details, see the text.

a separate experiment (data not shown), it was established that over the pH range studied, the TrkA system of the cells was active and allowed the cells to maintain K+ gradients of about 230 mV. Therefore, it can be concluded that, under all conditions tested, the K+-induced interconversion between the components of Δμ_H+ was concomitant with K+ uptake by the cells.

DISCUSSION

In this paper we describe the effect of K+ ions on the components of the proton motive force in intact bacteria. In S. faecalis, K+ depolarized the membrane by 60 mV, and cells with different K+ contents developed different membrane potentials (Fig. 1 and 2). This confirms previous observations with this organism (2, 12, 13), but it contradicts a study with the dye diO-C6-(3), which indicated that Δψ was independent of the cellular K+ content (19). A possible explanation for the latter results may be that under the given conditions fluorescence quenching exceeded the linear region of the calibration curve (1), so that any difference in Δψ may have been missed.

Alternatively, it may be that the cell types tested by Laris and Pershad-singh (19) did not differ very much in their K+ contents.

Both in S. faecalis (Fig. 1) and in E. coli (Table 2, Fig. 3 and 4), as well as in S. lactis (15, 16), K+ caused an interconversion between the components of Δμ_H+. Two lines of evidence indicate that this effect is functionally related to K+ transport activity of the cells. First, in both S. faecalis and E. coli, the amount of K+ causing half-maximal changes in ΔpH and Δψ was close to that at which cells became half-maximally filled with K+ (Fig. 1 and 3). Second, the experiments with the various K+ transport mutants of E. coli indicated that the cells had to contain at least one functional K+ transport system to show the observed effects (Table 2).

FIG. 4. Components of Δμ_H+ in E. coli TK1001 as a function of pH_out. The appropriate amounts of cells were isolated from the suspension buffer by centrifugation and suspended at 1.0 mg/ml in 200 mM of one of the following buffers: sodium-MES (pH 6.1), sodium-ACES (pH 6.8), sodium-HEPES (pH 7.5) sodium-Tricine (pH 8.1), sodium-TAPS (pH 8.4). The buffers were chosen such that the Na+ concentration in the system was the same at all pH values and amounted to about 90 mM. Further additions were made as indicated in the text, except that no KCl was added. Glucose was added at t = 0, and KCl (2 μmol/ml) was added at t = 35 min. ΔpH and Δψ were determined as a function of time. Open symbols, steady-state values before the addition of KCl; closed symbols, steady-state values after the addition of KCl.
The simplest interpretation of these observations is that each of the K⁺ transport systems introduces an apparent permeability of the membrane towards a charged species, which is most likely K⁺ itself. This means that K⁺ transport via all these systems is electrogenic (11, 12, 15). In the absence of other permeant ions the proton pumps of the cytoplasmic membrane tend to develop a large, outwardly directed membrane potential and a small pH gradient (22). Inward movement of K⁺ will decrease Δψ, which allows more protons to be pumped outwards, with the result that in the steady state Δψ is partially converted into ΔpH. Such interconversions are well known in energy-transducing membranes: the lipid-soluble cation triphenylmethylphosphonium (TPP⁺) causes an extensive conversion of Δψ into ΔpH in illuminated cell suspensions of Halobacterium halobium (3), and valinomycin plus K⁺ causes similar effects in mitochondria (24). The pattern emerging from the latter cases is that increasing concentrations of permeant cations continue to decrease Δψ. Up to a certain point this depolarization can be compensated for by an increase of ΔpH, until the membrane becomes leaky for protons, due to secondary effects like swelling (24).

By contrast, the depolarization of bacteria by K⁺ saturated at about 2 mM K⁺ out (Fig. 1–3). It seems unlikely that this saturation was an artifact, due to the methods used to monitor Δψ, since TPP⁺ and dio-C₆-(3) gave similar results (Fig. 1 and 2). Moreover, the protonophore carbonylcyanide-p-trifluoro-methoxyphenylhydrazone caused a further depolarization to 70 mV (data not shown).

Most likely, the saturation phenomenon is caused by the characteristics of the K⁺ transport systems themselves. An important factor may be that the K⁺ transport systems all have KM values of 1 mM and below (2, 28). Consequently, all of these systems operated close to or under Vₖₑₐₚ conditions at the concentration of K⁺ out at which the saturation started. By contrast, transport of K⁺ by the carrier valinomycin saturates only at very high K⁺ concentrations (20). An additional cause of the saturation phenomenon may be that K⁺ in exerts feedback pressure on the unidirectional rate of 42K⁺ influx via the constitutive K⁺ transport systems of E. coli and S. faecalis (2, 18). In the steady state, at high K⁺ in, the permeability of the membrane towards K⁺ will then be much lower than that expected on the basis of the kinetic parameters, which were determined at low K⁺ in (2, 27, 28).

One of the reviewers of this manuscript has suggested that the observed interconversion of the components of ΔµH⁺ may be triggered by some unspecified effects of K⁺ in. This hypothesis requires that K⁺ in increases the permeability of at least one other ion. We cannot exclude this possibility. However, the initial overshoots in the depolarization observed early after the addition of KCl to the medium (Fig. 2) suggest that, at least in S. faecalis, but presumably also in E. coli, electrogenic K⁺ transport is the major factor for the depolarization of the membrane in the steady state.

The value of the total ΔµH⁺ of 120 mV in the glycolytic bacterium S. faecalis (Fig. 1) was very similar to that reported for the related S. lactis (15, 16). Similarly, a ΔµH⁺ of 140 to 160 mV in E. coli (Table 2; Fig. 3 and 4) is close to values reported for this organism by others (4, 8, 25, 33). It should, however, be reemphasized that the measured values of ΔµH⁺ and its components depend very much on the method used. For instance, in S. faecalis we have reported considerably lower values for ΔpH and Δψ when [14C]methylamine and TPP⁺ were employed instead of benzoic acid and TPP⁺, respectively. The reason for this discrepancy is unclear. However, recent evidence for E. coli (8) indicates that the values of Δψ calculated from TPP⁺ distribution are very close to those measured directly with microelectrodes. In addition, the values for ΔpH given in Fig. 4 are almost identical to those measured independently with 31P nuclear magnetic resonance (23). For these reasons we consider the data in this study to be quite reliable, and especially for S. faecalis we prefer them above the range of values for ΔµH⁺ reported in previous communications (1, 2, 12, 19).

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LITERATURE CITED


