Characterization of a *Streptococcus pneumoniae* Mutant with Altered Electric Transmembrane Potential

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Received 22 December 1983/Accepted 21 March 1984

It is possible to select transmembrane potential (ΔΨ)-altered mutants in *Streptococcus pneumoniae* on the basis of their resistance to the antifolate methotrexate. Comparison of such a mutant strain (amiA9) with its parent was used to evaluate the role of ΔΨ in the uptake of certain amino acids. The ΔΨ-dependent uptake of isoleucine, leucine, valine, and asparagine showed a reduced maximum velocity of uptake, and decrease in the transport constant of the energy-dependent, ΔΨ-independent uptake of lysine, methionine, and glutamine was observed. No reduction of the intracellular pool of ATP or of lactate excretion could be detected in the mutant strain. Moreover, studies on membrane preparations suggest that the phenotype expressed by the amiA mutation is not a consequence of alteration of its ATPase activity or susceptibility to *N,N*-dicyclohexylcarbodiimide. Therefore, it is unlikely that the amiA mutation affects the H⁺ F₁F₀ ATPase which is involved in the establishment of the proton motive force in anaerobic bacteria. We propose that another function contributes to ΔΨ in *S. pneumoniae*. The amiA gene may be the structural gene of that function.

The molecular mechanism by which metabolic energy is coupled to active transport in bacteria has been of considerable interest. It is now generally accepted that the accumulation of many metabolites requires ATP, a derivative of ATP, or an electrochemical proton gradient (ΔμH⁺) across the cytoplasmic membrane (for a review, see references 12 and 23).

In streptococci, the metabolism of which is primarily fermentative, it has been proposed that excretion of metabolic end products such as lactate contributes to the electrochemical proton gradient created by the F₁F₀ ATPase (EC 3.6.1.3) (20). Ion transports could also be good candidates for assisting the F₁F₀ ATPase.

Isolation and characterization of several classes of ΔμH⁺-altered mutants may be helpful in investigating this problem, as *Escherichia coli* mutants resistant to neomycin have proved to be useful for studying the role of the F₁F₀ ATPase (22, 26, 38) and the energy coupling to transport (14, 17, 18, 30). In this respect, the methotrexate (MTX)-resistant mutant of *Streptococcus pneumoniae* studied here appears to be a good tool for studying the energetics of these bacteria. These mutants, called amiA, are resistant to 10 μM amonoterin or MTX but are as susceptible as the wild-type strain (1 μM) to another folate analog, trimethoprim. Moreover, no detoxifying activity for MTX could be detected in the mutant extracts (M. C. Trombe, Ph.D. thesis, Université P. Sabatier, Toulouse, France, 1972). Nevertheless, in studies on MTX uptake, it has been suggested that the amiA mutants are impaired in the transport of antifolates (28, 30, 32; M. C. Trombe, Ph.D. thesis). In addition, the amiA mutants are pleiotropic, i.e., they are altered in branched amino acid (isoleucine, leucine, and valine) active transport which is mediated by a single common permease with a transport constant (Kₜ) = 1 μM (31). They are also more sensitive than the wild-type strain to some amino acid analogs (32). Such a complex phenotype may express the alteration of several independent transporters resulting from a change in their energy coupling.

Indeed, in the present report we show that the amiA mutants exhibit a reduced electric transmembrane potential (ΔΨ), which leads to an alteration of the velocity for ΔΨ-dependent transports and to a change in the Kₜ for ATP-energized uptakes. The reduction of ΔΨ is not accompanied by a change in chemical transmembrane gradient (ΔpH). Moreover, the shift of ΔΨ does not result from either an alteration of the F₁F₀ ATPase properties of the mutant strain or a reduction of its lactate excretion.

**MATERIALS AND METHODS**

**Media and organisms.** The MTX-sensitive strain of *S. pneumoniae* Cl9 was derived from strain R36 A (29). It is auxotrophic for valine, leucine, isoleucine, arginine, asparagine, histidine, glutamine, and uracil but does not require folic acid for its growth in the synthetic medium (27). Bacterial growth is inhibited by 1 μM MTX in a peptone-yeast extract medium (33) and 0.4 μM MTX in a defined medium (27). MTX inhibition is not overcome by chemicals such as folate, 5-formyltetrahydrofolic, thymine, thiamine, methycytosine, 2-methyadenine, or a mixture of thymidine, glycine, and 2-methyldene even when their concentrations in the growth medium are 20 times higher than the MTX concentration (M. C. Trombe, Ph.D. thesis). Resistant mutants were selected on plates containing 10 μM MTX. Colonies exhibiting pleiotropic properties, i.e., those exhibiting sensitivity to an imbalance in the concentration of branched amino acids, were considered amiA mutants. Two point crosses showed that all such mutations fall in a region of about 7 kilobase pairs (27, 35). One of the mutants, amiA9, bears a nonsense mutation (9) which lies at the beginning of the gene (35). It was isogenized with the wild-type parent by bacterial transformation and was chosen among ~50 mutants of the same locus as being representative of that class of mutants.

**Growth and preparation of cells.** The bacteria were grown to the exponential phase (absorbance at 550 nm, 0.7) in
peptone-yeast extract medium (33). The cells were centrifuged at 3,000 \times g for 10 min at 4°C, washed three times, and suspended to an appropriate protein concentration in the buffer used in the uptake experiments (see below).

**Transport assays.** Unless otherwise stated, bacteria were diluted to 0.03 to 0.3 mg of protein per ml in salt-buffered medium containing 120 mM NaCl, 52 mM NH4Cl, 7.5 mM KCl, 20 mM Na2HPO4, 40 mM Trizma base (Sigma), and 0.4% (wt/vol) glucose (pH 7.55) (27). They were allowed to undergo glycolysis at 20°C for 2 min. Uptake was initiated by adding the labeled substrate solution, and 0.1-ml samples were taken at intervals, filtered through glass microfiber filters (GF/B; Whatman, Inc.), and washed three times with 3 ml of salt-buffered medium without glucose. The ΔpH (interior alkaline) and ΔΨ (interior negative) were determined respectively with [14C]benzoic acid and [14C]tetraphenylphosphonium (TPP+) distributions at the steady state. Kinetics of accumulation showed that under our experimental conditions, the steady state was obtained from a 2-min incubation at 20°C.

For [14C]TPP+ accumulation, the filtration technique with Millipore cellulose acetate filters (type EH, 0.45-μm pore size) was routinely performed since it has been shown to give similar results as flow dialysis in the gram-positive bacteria *Micrococcus lysodeikticus* (8).

The data were quantified, assuming that the amount of [14C]benzoic acid which was retained when the external pH was adjusted to 8 represented no ΔpH. The amount of [14C]TPP+ which was retained by valinomycin-treated cells in the presence of 100 mM KCl was taken as the amount of TPP+ which bound to cellular components in nonenergized cells and represented 5 to 10% of the total amount.

Unless otherwise specified, the final concentrations of the radioactive substrates used were 10 μM (20 fmol/cpm) for the amino acids, 3 μM (0.4 nmol/cpm) for benzoic acid, and 5.5 μM (4.3 fmol/cpm) for TPP+.

Concentration gradients were calculated by using a value of 3.4 μl of intracellular volume per mg of cell proteins as determined with [14C]hydroxymethyliminulin and [3H]H2O (2). The ΔpH and ΔΨ were calculated with steady-state accumulation values of the reciprocal probes (24).

**Energy deprivation and creation of an artificial ΔΨ by K+ efflux.** *S. pneumoniae* relies upon glycolysis as a source of energy. Therefore, sodium arsenate, an analog of phosphate, was used to deplete the cells of ATP (15). The bacteria were resuspended in either 100 mM KCl–10 mM arsenate–0.4% glucose (pH 7) or 50 mM Tris–10 mM arsenate–0.4% glucose (pH 7.9). They were incubated at 20°C for 10 min. Arsenate was also present in the uptake media.

Artificial ΔΨ (inside negative) was created by diluting arsenate-treated bacteria preloaded with KCl in media containing low amounts of KCl. Control experiments with flame photometry indicated that osmotic K+ efflux occurs spontaneously in energy-depleted cells. Valinomycin was therefore not needed in those experiments.

**ATP pool determination.** Cell extracts from freshly grown bacteria were prepared by the method of Forrest and Walker (7). The ATP contents of the extracts were measured by the luciferin-luciferase procedure of Scheer et al. (25).

**Lactate titration.** Lactate production from glucose was determined with a lactate electrode (4). The bacterial suspension contained 3 mg of protein per ml. Glycolysis was initiated in a phosphate-containing medium by the addition of glucose to a final concentration of 22 mM, and incubation was then carried out for 30 min at 37°C.

**Membrane preparations.** One liter of bacteria in the late-exponential phase was centrifuged, and the cells were washed twice with 100 ml of buffered salt medium (27). The cells were resuspended in 5 ml of 10 mM Tris–10 mM MgCl2 (pH 7.7) and sonicated for 3 min (six times for 30 s, with 1 min of cooling between each sonication). The crude sonic extract was centrifuged at 6,000 \times g for 15 min, and the supernatant was centrifuged at 30,000 \times g for 1 h. The pellet of this centrifugation, taken as the membrane fraction, was resuspended in 50 mM Tris–10 mM MgCl2 (pH 7.2) and kept at −80°C until use.

**ATPase activity of the membrane.** The ATPase assays were carried out by the method of Evans (5). The released P i was quantified by a modified technique described previously (3, 6). The optimal activity was observed at pH 7.5. No significant amount of P i was released at pH 9 (less than 10% of the signal value at pH 7.5). The susceptibility of the membrane ATPase to N,N′-dicyclohexylcarbodiimide (DCCD) was determined in wild-type and mutant membrane fractions. Extracts were incubated for 10 min with 1 mM DCCD at 20°C before the addition of ATP. The ATPase activity of both extracts was then measured as described above.

**Protein determination.** Protein was measured as described by Lowry et al. (19), with bovine serum albumin used as a standard. Intact cells were treated with 10% (wt/vol) trichloroacetic acid. The precipitate was dissolved in 0.1 M NaOH. The bovine serum albumin used as the standard was also dissolved in 0.1 M NaOH.

**Chemicals.** MTX was kindly provided by Specia Laboratories, Paris, France. 14C-labeled amino acids, [14C]TPP+, and [14C]benzoic acid were from Commissariat à l’Energie Atomique, GIF-Yvette, France. Luciferin and luciferase were from Boehringer-Mannheim. The other chemicals were of grade A quality.

**RESULTS**

**Specific reduction of ΔΨ in the amiA9 strain.** We compared the ability of both wild-type and amiA9 strains to produce and use energy. Lactate excretion was taken as an indicator

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lactate excreted (nmol)</th>
<th>Membrane ATPase activity</th>
<th>DCCD inhibition of the ATPase (%)</th>
<th>ATP pool (mM)</th>
<th>ATP pool in arsenate-treated bacteria (mM)</th>
<th>ΔΨ (MV)</th>
<th>ΔpH (MV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>10.8 ± 0.3</td>
<td>10.8 ± 0.5</td>
<td>65</td>
<td>1.3 ± 0.2</td>
<td>0.3</td>
<td>130 ± 5</td>
<td>47.2 ± 3</td>
</tr>
<tr>
<td>Mutant</td>
<td>10.4 ± 0.3</td>
<td>10.6 ± 0.5</td>
<td>59</td>
<td>1.5 ± 0.2</td>
<td>0.5</td>
<td>100 ± 5</td>
<td>46 ± 3</td>
</tr>
</tbody>
</table>

*a* Lactate concentration in the medium was monitored after allowing the bacteria to undergo glycolysis for 30 min at 37°C.

*b* ATPase activity is expressed as micromoles of P i released at 37°C in 1 h by an amount of membrane equivalent to 1 mg of protein.

*c* ATPase activity of membranes pretreated with 1 mM DCCD for 10 min at 20°C was measured under standard conditions.

*d* For ATP pool determination, see the text.

*e* ΔΨ was measured at pH 7.7; the [14C]TPP+ concentration was 5.5 μM.

*f* ΔpH was measured at pH 7; the [14C]benzoic acid concentration was 3 μM.
of the energetic state of the bacteria, since lactate production is directly linked to glycolysis. The ATP content of cells and its reduction by arsenate treatment, as well as the specific activity of the membrane ATPase and its susceptibility to the inhibitor DCCD, were also determined. Moreover, the two components of Δ\(\mu\)H\(^+\), i.e., Δ\(\Psi\) and Δ\(\phi\), were measured (see above).

No difference was noted between the wild-type and the mutant strains, except for their Δ\(\Psi\) values, which were estimated to be 130 ± 5 mV in the wild type and 100 ± 5 mV in amiA9 (Table 1). These values correspond to a threefold difference in \(\Delta \mu \text{H}^+\) accumulation by the two strains and can be correlated to the difference in isoleucine accumulation in the wild-type and mutant cells, i.e., ~100-fold accumulation of the amino acid in the wild type and ~30-fold accumulation in the mutant (31).

This change in Δ\(\Psi\) was not accompanied by changes in Δ\(\phi\), ATP hydrolytic activity, or sensitivity to DCCD of the ATPase (Table 1). This phenotype is different from the well-studied F\(_o\)F\(_{\text{atp}}\) ATPase mutants of E. coli, which exhibit either an alteration of their capacity to hydrolyze ATP (uncA) or a resistance to DCCD (uncB) (26), and we conclude that it is unlikely that the amiA mutants are altered at the level of their membrane ATPase.

Lactate excretion can generate a proton motive force (\(V_{\text{max}}\)) in fermentative bacteria (20). In the present case, if S. pneumoniae can derive some energy from lactate, it is unlikely that this factor was involved in the amiA mutant, since the amount of lactate excreted by the wild-type and the mutant strains were similar (Table 1). Nevertheless, a change in the stoichiometry of lactate to protons might explain the phenotype of the amiA mutant and cannot be excluded.

Selective alteration of the kinetic parameters of amino acid transports in the amiA9 strain. The kinetic parameters \(K_T\) and \(V_{\text{max}}\) of uptake in the wild-type and the amiA9 mutant strain for several amino acids, including isoleucine, leucine, and valine, were determined by Lineweaver-Burk plots (Table 2). It is possible to distinguish two classes of amino acids on the basis of the change in their kinetic parameters in response to the amiA mutation. In class A, isoleucine, leucine, valine, and asparagine exhibited a reduced \(V_{\text{max}}\) in the amiA strain without detectable change of the \(K_T\) for uptake. In class B, glutamine, methionine, and lysine showed no change in \(V_{\text{max}}\) in the mutant strain, but the \(K_T\) values were lowered significantly by the amiA mutation.

### Table 2. Kinetic parameters of amino acid uptake in the wild-type and amiA9 strains

<table>
<thead>
<tr>
<th>Class</th>
<th>Amino acid</th>
<th>(K_T) (µM) in:</th>
<th>(V_{\text{max}}) (nmol/min per mg of protein) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>amiA9</td>
<td>Wild type</td>
</tr>
<tr>
<td>A</td>
<td>Isoleucine</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>3.7</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Asparagine</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>Glutamine</td>
<td>8</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Methionine</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td>0.8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Thirty-second uptake values were taken as the initial velocity of transport. \(K_T\) and \(V_{\text{max}}\) values were determined by the Lineweaver-Burk plots. Values are the averages of two determinations that differed by less than 10%.

These data indicate that the amiA mutation altered the active transport of several amino acids and that its effect varied, depending on the amino acid, since it was the \(V_{\text{max}}\) of certain transport processes which was altered but the \(K_T\) of others.

One important question to be addressed is whether asparagine and the branched amino acids share the same transporter, since they all show a reduced \(V_{\text{max}}\) of uptake in the amiA mutant. Although leucine and valine inhibited the uptake of isoleucine, neither asparagine nor lysine had any effect in the wild-type and the amiA9 mutant strain (Table 3), a result which suggests that their transport does not occur via the branched amino acid permease. Therefore, it is likely that more than one transporter was modified by the amiA mutation. The different response of the amino acid transports to amiA mutation may indicate a difference in their coupling to metabolic energy.

### Table 3. Effect of asparagine, leucine, lysine, and valine on isoleucine uptake

<table>
<thead>
<tr>
<th>Competitor and concn (mM)</th>
<th>Isoleucine accumulation (%) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Asparagine (2)</td>
<td>103</td>
</tr>
<tr>
<td>Leucine (7)</td>
<td>7</td>
</tr>
<tr>
<td>Lysine (3)</td>
<td>83</td>
</tr>
<tr>
<td>Valine (11)</td>
<td>3</td>
</tr>
</tbody>
</table>

* Standard uptake conditions were used. \(^{14}\)C]Isoleucine (10 µM, 0.6 mCi/mmol) and the competing amino acids were added at time zero, and accumulation of radioactivity at 1 min was measured. The wild-type strain accumulated 1.96 nmol of isoleucine per µl of intracellular free space; the mutant accumulated 0.98 nmol/µl of intracellular free space in the absence of competitor.

### Table 4. Inhibition of amino acid uptake by arsenate, DCCD, and valinomycin

<table>
<thead>
<tr>
<th>Treatment substrate</th>
<th>Inhibition of uptake (%) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenate (20 mM)(^a)</td>
<td>DCCD (0.1 mM)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>65</td>
</tr>
<tr>
<td>Glutamine</td>
<td>95</td>
</tr>
<tr>
<td>Asparagine</td>
<td>45</td>
</tr>
<tr>
<td>Lysine</td>
<td>90</td>
</tr>
</tbody>
</table>

* \(^a\) Inhibition was calculated by comparison with untreated control bacteria.

In this experiment, ATP was reduced by 75%. For other experimental conditions, see the text.

* Valinomycin treatment was carried out in the presence of 10 mM KCl.
collapse $\Delta \Psi$ when sufficient $K^+$ ions are present in the medium. In such bacteria, a lower accumulation of isoleucine and asparagine can be observed with no significant reduction of lysine or glutamine accumulation (Table 4). In addition, when valinomycin inhibition was measured at different pHs, its effect was more pronounced at high pH than at low pH (34), a result which is expected for $\Delta \Psi$-requiring transport systems. These data suggest that $\Delta \Psi$ energizes isoleucine and asparagine uptake.

**Accumulation of class A amino acids in energy-depleted bacteria in response to an artificial $\Delta \Psi$.** The bacteria were incubated for 10 min at 20°C in media containing 100 mM KCl, 0.4% glucose, and 10 mM arsenate (energy-depleted cells) at pH 7. The cells were then tested for uptake in incubation medium with low $K^+$. The $K^+$ diffusion potential was varied by the concentration of $K^+$ in the external medium. A transient accumulation of isoleucine was observed in depleted bacteria, a magnitude of which correlated with the potential diffusion of $K^+$ (Fig. 1).

The kinetics of uptake of amino acids by energy-depleted bacteria were compared with controls which consisted of nondepleted bacteria. Amino acids of class A were accumulated in energy-depleted bacteria, with initial rates comparable with that of control cells (Fig. 2). In contrast, amino acids belonging to class B (methionine, lysine, and glutamine)

**FIG. 1.** Accumulation of $[^{14}C]$isoleucine in energy-depleted bacteria in response to $K^+$ diffusion potential. Bacteria were preloaded with $K^+$ by incubation for 10 min in a medium containing 100 mM KCl, 10 mM arsenate, and 0.4% glucose (pH 7). The $K^+$ diffusion gradient was obtained by diluting these cells into the uptake medium which contained 10 mM arsenate, 0.4% glucose, $[^{14}C]$isoleucine (10 $\mu$M, 0.25 $\mu$Ci/ml), and KCl to a final concentration of 30 mM (●), 50 mM (▲), and 100 mM (○). The ion concentration was kept constant with NaCl.

**FIG. 2.** Accumulation of amino acids in response to $K^+$ potential diffusion. Wild-type bacteria (round symbols) were preloaded with KCl, as described in the legend to Fig. 1, in phosphate- (○) or arsenate- (●) containing medium. Amino acid uptake was initiated by diluting the cells in the uptake medium which contained the $[^{14}C]$-labeled amino acid, 30 mM KCl, and either 10 mM arsenate (●) or 10 mM phosphate (○). In (B), diamonds represent the amiA9 mutant. NaCl was used to keep the ion concentration constant. (A) Methionine; (B) lysine; (C) glutamine; (D) isoleucine; (E) leucine; (F) valine (20 $\mu$M, 0.25 $\mu$Ci/ml); (G) asparagine (1 $\mu$M, 0.25 $\mu$Ci/ml).
were accumulated more slowly and to a lesser extent in starved bacteria than in the controls. It is unlikely that the residual uptake of these amino acids results from ATP synthesis in response to \( \Delta \Psi \), since all the experiments with starved cells were performed in the presence of a large excess of arsenate and without added phosphate. One possibility is that the low level of methionine, lysine, and glutamine accumulation in arsenate-treated cells indicates that there is a residual pool of ATP present after arsenate treatment. Such residual accumulation may also be due to the activity of other \( \Delta \Psi \)-dependent transporters that exist for these amino acids. However, these transporters should be much less efficient compared with the ATP-dependent uptake systems.

Decreasing the \( \Delta \Psi \) in the wild-type cells reduces the \( V_{\text{max}} \) of isoleucine uptake and the \( K_T \) of lysine uptake. To check whether the reduced \( \Delta \Psi \) seen in the amiA strain was responsible for the decrease in \( V_{\text{max}} \) for the transport of class A amino acids and \( K_T \) in these others, these parameters were determined in wild-type bacteria preincubated with the permeant cation triphenylmethylphosphonium (TPMP\(^+\)) to partially reduce their \( \Delta \Psi \). Controls were bacteria preincubated without TPMP\(^+\). Cells incubated with TPMP\(^+\) for 10 min showed a lower \( V_{\text{max}} \) for isoleucine transport without a significant change in its \( K_T \) (Table 5). In contrast, the \( K_T \) of lysine was significantly reduced in TPMP\(^+\)-treated bacteria, whereas the \( V_{\text{max}} \) of uptake was not altered. This suggests that the pleiotropic effect of the amiA mutation on amino acid uptake is a consequence of an alteration of \( \Delta \Psi \) in the mutant strain.

**DISCUSSION**

In this report we give evidence for a reduction of \( \Delta \Psi \) in an MTX-resistant mutant of *S. pneumoniae*. That alteration was not accompanied by an alteration of the \( \Delta \Psi \) and did not result from changes in the \( \Delta \Psi \) for the transport of class A amino acids and \( K_T \) in the others, these parameters were determined in wild-type bacteria preincubated with the permeant cation triphenylmethylphosphonium (TPMP\(^+\)) to partially reduce their \( \Delta \Psi \). Controls were bacteria preincubated without TPMP\(^+\). Cells incubated with TPMP\(^+\) for 10 min showed a lower \( V_{\text{max}} \) for isoleucine transport without a significant change in its \( K_T \) (Table 5). In contrast, the \( K_T \) of lysine was significantly reduced in TPMP\(^+\)-treated bacteria, whereas the \( V_{\text{max}} \) of uptake was not altered. This suggests that the pleiotropic effect of the amiA mutation on amino acid uptake is a consequence of an alteration of \( \Delta \Psi \) in the mutant strain.

**TABLE 5.** Kinetic parameters of \(^{14}\)C]isoleucine and \(^{14}\)C]lysine transport in wild-type bacteria which accumulated the permeant cation TPMP\(^+\)

<table>
<thead>
<tr>
<th>Amino acid transported</th>
<th>TPMP(^+) present</th>
<th>TPMP(^+) not present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_T ) (( \mu M ))</td>
<td>( V_{\text{max}} ) (nmol/min/mg of protein)</td>
</tr>
<tr>
<td>(^{14})C]isoleucine</td>
<td>1.7</td>
<td>19.8</td>
</tr>
<tr>
<td>(^{14})C]lysine</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

\(^{a}\) Kinetic parameters of \(^{14}\)C]isoleucine and \(^{14}\)C]lysine transport were determined in bacteria which underwent glycolysis in the absence or presence of 10 mM TPMP\(^+\) for 2 min before the addition of the labeled amino acid. The values of the transport parameters were determined as described in footnote a of Table 2.

conformation of some membrane proteins or their accessibility to external solutes. Such a response is reminiscent of previous observations on the influence of energization (21) and surface charge potential (36, 37) on the properties on membrane-bound enzymes.

From the present results, it appears that under our experimental conditions lysine, methionine, and glutamine uptakes were selectively affected for their \( K_T \) values, whereas a change of \( V_{\text{max}} \) was measured for the \( \Delta \Psi \)-dependent uptakes of asparagine, leucine, isoleucine, and valine. Therefore, the energy state of the membrane appears to modulate \( \Delta \Psi \)-energized and ATP-dependent transports, suggesting a fine regulation of metabolite accumulation by the energetics of the cells.

In any case, these results altogether show that the amiA mutants appear to be good tools for studying the influence of \( \Delta \Psi \) on membrane-linked biological activities. However, little is known about the molecular alterations which are responsible for the reduction of \( \Delta \Psi \) in these mutants. Since the amiA mutant strain bears a nonsense mutation (9) which is located at the beginning of the amiA locus (27, 35), a protein is probably missing in that strain. From our data, it is unlikely that the amiA gene codes for a \( H^+\)F\(_{\text{ATPase}} \)like ATPase in *S. pneumoniae*. As another possibility, it is suggested rather that the amiA function might be involved in another cation transport system. Indeed, there is evidence for several cation transporters in streptococci (for a review, see reference 13). In addition, the role of the \( K^+ \) content of the cells in the membrane potential value has been established in bacteria (1, 16), and several reports point to the importance of several cation-translocating ATPases in streptococci (13). Therefore, the absence of such a transporter is a good candidate to account for the alteration of \( \Delta \Psi \) in the amiA mutant strain. Experiments are in progress to characterize the products and the function of the amiA gene.

**ACKNOWLEDGMENTS**

We thank E. Schechter for helpful discussion during the course of this work, J. Lever for advice in editing the manuscript, and F. M. Harold for critical reading of the manuscript.

This work was supported by the Centre National de la Recherche Scientifique (LP 006201).

**LITERATURE CITED**


