Alkalophilic bacilli maintain a cytoplasmic pH that is lower than optimal external pH values. The current study, using isolated membrane vesicles from *Bacillus alcalophilus*, indicates that respiration results in proton extrusion as in other organisms. Cation/proton antiporters thus catalyze inward proton movements. Upon energization with ascorbate/Na,N,N',N'-tetramethyl-p-phenylenediamine, Na⁺-loaded membrane vesicles from *B. alcalophilus* exhibited a transmembrane pH gradient (ΔpH, inside acid) over a range of external pH values from 8 to 10.5; above pH 11, ΔpH was zero. A transmembrane electrical potential (Δψ, inside negative), increasing from −125 to −135 mV from pH 8 to 11.5, was observed. Over the same pH range, the internal and external pH values of K⁺-loaded vesicles were equal (ΔpH = 0), and the Δψ declined from −125 to −98 mV. Vesicles that were prepared without Na⁺ or K⁺ exhibited a small ΔpH, inside alkaline, and a Δψ, inside negative, of −64 to −100 mV. These observations are compatible with the presence of: a K⁺/H⁺ antiporter which exchanges the protons that are extruded during respiration for K⁺; and a Na⁺/H⁺ antiporter which acidifies the intravesicular space relative to the external medium. Indeed, everted vesicles catalyzed net proton extrusion and Na⁺ accumulation that were dependent upon a Δψ and were half-maximal at a Δψ of about 0.7 mV. Whole cells of a nonalkalophilic strain of *B. alcalophilus* had been found to lack both Na⁺ efflux activity and the ability to grow above pH 9.0. Vesicles from this mutant strain lacked Na⁺/H⁺ antiporter activity, while still exhibiting apparent K⁺/H⁺ antiporter activity. Thus, studies of vesicles from both mutant and wild-type strains indicate that the ability of *B. alcalophilus* to maintain an internal pH that is lower than the external pH is related to the activity of the Na⁺/H⁺ antiporter.

Alkalophilic bacteria present special problems with respect to transmembrane concentration gradients of protons. Specifically, at optimal pH values for growth, alkalophiles such as *Bacillus alcalophilus* (1) and *Bacillus firmus* RAB (2) maintain a cytoplasmic pH that is considerably lower than the external pH. Yet, in accord with the chemiosmotic hypothesis, respiring bacteria have been found to extrude protons (3). This proton extrusion establishes an electrochemical gradient of protons, outside acid and positive. How do the alkalophiles retain a conventionally oriented transmembrane electrical potential (the Δψ component of the electrochemical gradient) while reversing the usual chemical gradient of protons (ΔpH)? In nonalkalophilic bacteria a similar but less extreme problem is apparent. At the alkaline end of their pH ranges for growth, nonalkalophilic bacteria maintain Δψ while exhibiting cytoplasmic pH values no higher than the external pH (4, 5). The activity of monovalent cation/proton antiporters at alkaline pH could account for these observations. Such antiporters have been suggested (4, 6–8) to function in the regulation of cytoplasmic pH in *Escherichia coli*, but unequivocal evidence has not been presented. Since the physiological problem is exaggerated in the alkalophiles, these bacteria have offered a system in which the role of antiporters can readily be tested.

Recently, we reported the isolation of nonalkalophilic mutant strains of *B. alcalophilus* (9) and *B. firmus* RAB (2). The parent strains grew only above pH 8.0, with optimal growth at pH 10.5. By contrast, the nonalkalophilic mutants grew over a range of pH from 5.5 to 9.0. These physiological data correlated with the loss, in the nonalkalophilic mutants, of an energy-dependent Na⁺ efflux activity. Thus a Na⁺/H⁺ antiporter was implicated in the acidification of the cytoplasm relative to the external milieu of the alkalophiles. This acidification apparently facilitates growth at high pH but precludes growth at lower pH. We now report the results of a more extensive study of monovalent cation/proton antiporters in *B. alcalophilus* and its nonalkalophilic mutant derivative. This work, employing isolated membrane vesicles, further documents the presence of these antiporters and supports the proposed role of the Na⁺/H⁺ antiporter in the regulation of cytoplasmic pH.

**MATERIALS AND METHODS**

**Organism and Growth Conditions—** *B. alcalophilus* (ATCC 27647) and its nonalkalophilic derivative (strain KM 23) were grown on L-malate-containing medium at pH 10.5 and pH 6.8, respectively, as described previously (1, 9).

**Preparation of Membrane Vesicles—** Right-side-out membrane vesicles were prepared from L-malate-grown cells of both strains using a modification of Kaback's method (10). Protoplasts were prepared from washed whole cells in 30 mM Tris, 20 mM MgSO₄, 10% mannitol at pH 6.0 in the presence of 300 μg of lysozyme/ml, with shaking for 30 min at 30°C. Sodium-loaded (Na⁺-loaded) vesicles were prepared by lysing protoplasts in 100 mM sodium carbonate buffer, pH 9.0. Potassium-loaded (K⁺-loaded) vesicles were formed by lysis in 100 mM potassium carbonate buffer, pH 9.0. Vesicles containing neither Na⁺ nor K⁺ were prepared by shocking protoplasts in 100 mM amiodiol, pH 9.0. MgSO₄, 10 mM, was present in all buffers. The concentrations of protons, outside acid and positive. How do the alkalophiles retain a conventionally oriented transmembrane electrical potential (the Δψ component of the electrochemical gradient) while reversing the usual chemical gradient of protons (ΔpH)?

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1. The abbreviations used are: ammediol, 2-amino-2-methyl-1,3-propanediol; DMO, 5,5-dimethyl-2,4-oxazolidinedione; PMF, proton motive force; TMPD, N,N,N',N'-tetramethyl-p-phenylenediamine; TPMP⁺, triphenylmethylphosphonium bromide.
internal volume of the vesicles was determined by the method of Stock et al. (11) to be 1.2 ± 0.1 μl/mg of vesicle protein. 1C-Ultilin was used as a marker for extravesicular space.

Everted membrane vesicles were prepared from B. alcalophilus cells by a modification of the procedure of Kobayashi et al. (12). Protoplasts, prepared as described above, were lysed in 100 mM potassium carbonate buffer, pH 9.0, or in 10 mM Tris-HCl, 100 mM choline chloride, pH 9.0, with DNase (10 μg/ml) and 10 mM MgSO₄ present. The membranes were collected, passed through a French pressure cell (8,000 p.s.i.), and washed with the same buffer used for lysis except that the enzymes were omitted (12). The internal volume of these vesicles, determined by the method of Stock et al. (11) was 0.6 ± 0.1 μl/mg of vesicle protein. Protein concentrations were determined by the method of Lowry et al. (13) using egg white lysozyme as a standard. All assays were performed at 25°C.

Measurement of the ΔpH and Δψ in Right-Side-Out Membrane Vesicles—The ΔpH was determined from the distribution of the weak base, [14C]methylamine, or the weak acid, [3H]DMO, using the flow dialysis assay of Ramos et al. (14). The assay technique was essentially the same as that described for whole cells of B. alcalophilus (1), except that right-side-out membrane vesicles were energized with 20 mM ascorbate (neutralized with the appropriate base) plus 2 mM TMPD, and everted vesicles were energized with 10 mM NADH (appropriate salt). Vesicle concentrations of approximately 10 and 20 mg of vesicle protein/ml were used for right-side-out and everted vesicles, respectively. The internal pH was calculated from the distribution of weak base or weak acid using the method of Waddell and Butler (15); ΔpH is the internal pH-external pH. The equation used to calculate the internal pH contains a term which takes into account the extent of dissociation of the probe. While pH 9.0, the approximate pH used in most of the experiments, is relatively far from the pK values of both methylamine and DMO, a ΔpH of 0.3 unit could be measured quantitatively under the conditions employed. Much smaller gradients could be demonstrated qualitatively. The methods were tested in a series of experiments in which the suspending medium of unenergized vesicles was subjected to a known sudden change of pH. The ΔpH calculated from weak acid or base uptake was within 10% of the expected value. Further evidence for the validity of the methods employed to determine the ΔpH included: 1) the ΔpH measured was the same at several different concentrations of the probe; 2) treatment of vesicles with 10 μM gramicidin abolished any observed DMO or methylamine uptake; and 3) methylamine uptake by right-side-out vesicles correlated with DMO uptake by everted vesicles (and vice versa).

The Δψ generated by right-side-out vesicles upon energization with 20 mM ascorbate/2 mM TMPD was determined from the distribution of 25 μM [3H]TMPD, as described by Schuldiner and Kaback (16). Vesicles with 10 μM gramicidin were used as controls for nonspecific binding of TPMP⁺ to vesicle membranes and filters. The Δψ, the electrochemical potential, was calculated from: Δψ = ΔpH - 60 ΔpH.

Fluorimetric Studies with B. alcalophilus Membrane Vesicles—Energy-dependent generation of a ΔpH, interior acid, was monitored in right-side-out membrane vesicles using quenching of quinacrine dye fluorescence (17), measured in a Perkin-Elmer model 250-105 Fluorospectrophotometer coupled with a Perkin-Elmer model 025 chart recorder. The assay mixture contained 200 μg of K⁺-loaded or Na⁺-loaded membrane vesicle protein, 100 mM potassium or sodium carbonate buffer, pH 9.0, 10 mM MgSO₄, and 1 μM quinacrine dye in a volume of 1 ml. Vesicles were energized with 10 mM ascorbate, 1 mM TMPD, and correction was made for nonspecific quenching effects of ascorbate/TMPD on the quinacrine dye by using vesicles which had been pretreated with 10 μM gramicidin or by using heat-killed vesicles for the reaction.

Assays of 22Na⁺ Uptake by Everted Membrane Vesicles—22Na⁺ uptake by everted membrane vesicles was measured using a flow dialysis assay, as described for the ΔpH determinations. Vesicles (at approximately 20 mg of vesicle protein/mg) were incubated in the upper chamber with oxygenation, and 100 mM potassium carbonate buffer, 10 mM MgSO₄, at pH 8.7, was pumped through the apparatus. After 5 min, the experiment was initiated by the addition of 22Na⁺, at 250 μCi/ml, at the concentrations indicated; fractions of 1.7 ml were collected. NADH (potassium salt) was added after 10 min (Fraction 30) to a concentration of 10 mM.

Chemicals—Ascorbic acid, ammediol, DNase I, N-ethylmaleimide, gramicidin, lysozyme (egg white), NADH (Tris-salt), quinacrine dihydrochloride, RNase, TMPD, and valinomycin were purchased from Sigma Chemical Co. [3H]N-bromosuccinimide (carrier-free) (4.6 mCi/ml), [3H]triphenylphosphonium (3.59 Ci/mmol), [14C]methylamine (52.2 mCi/mmol), 5,5'-dimethylyl-2,4-dione (6.8 mCi/mmol), and [2H]triphenylphosphonium (2.1 mCi/g) were purchased from New England Nuclear Corp. Nonradioactive TPMP was supplied by ICN K&K Laboratories. All other chemicals were obtained commercially at the highest purity available.

RESULTS

The PMF in Membrane Vesicles—The PMF generated by energized, right-side-out membrane vesicles from L-malate-
**Na⁺/H⁺ and K⁺/H⁺ Antiporters in B. alcalophilus**

TABLE II

<table>
<thead>
<tr>
<th>Vesicles</th>
<th>Diluting and assay buffer</th>
<th>Gramicidin present</th>
<th>Percent quenching of quinacrine fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>KCO₃</td>
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<td></td>
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<tr>
<td></td>
<td>NaCO₃</td>
<td>+</td>
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</tr>
</tbody>
</table>

Right-side-out vesicles from wild type *B. alcalophilus* and KM 23 were prepared in 100 mM KCO₃ buffer and 10 mM MgSO₄. Before the beginning of the experiment, these vesicles were diluted 10-fold with either 100 mM KCO₃ or 100 mM NaCO₃ buffer, pH 9.0, containing 10 mM MgSO₄. The suspensions were incubated at 0°C for 10 min, and then warmed to room temperature. The vesicles were energized with 10 mM potassium ascorbate and 1 mM TMPD in the presence or absence of 10 μM gramicidin. Quinacrine fluorescence was monitored as described under "Materials and Methods."

Wild type

<table>
<thead>
<tr>
<th>Vesicles</th>
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<tr>
<td></td>
<td>NaCO₃</td>
<td>+</td>
<td>0</td>
</tr>
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</table>

Fig. 2. A model illustrating possible antiporter involvement in generation of the observed PMF patterns. A/T = ascorbate/TMPD, KHA = K⁺/H⁺ antiporter; NHA = Na⁺/H⁺ antiporter; and the respiratory chain is indicated as the site of primary proton extrusion.

Fig. 3. Acidification of the intravesicular space of right-side-out vesicles as a function of Na⁺ concentration. K⁺-loaded vesicles of wild type *B. alcalophilus* (-----) and KM 23 (----) were suspended in 100 mM KCO₃ buffer, 10 mM MgSO₄, pH 9.0, at 10 mg of vesicles protein/ml. NaCO₃, pH 9.0, was added (to the indicated final concentrations) just prior to energization with 20 mM potassium ascorbate/2 mM TMPD. The ΔpH was determined from the distribution of 25 μM [14C]methylamine (50 μCi/ml) as described under "Materials and Methods." Each point represents the average of several determinations.

grown cells of wild type *B. alcalophilus* was examined; vesicles loaded with sodium carbonate, potassium carbonate, or ammonium buffer at pH 9.0 were compared. As shown in Fig. 1 (left), Na⁺-loaded vesicles exhibited a reversed ΔpH over a range of external pH from 8.0 to 10.5. At pH 11.0 and 11.5, ΔpH was zero. The Δψ increased only very slightly over the pH range studied, so that the ΔψΔpH pattern essentially followed that of the ΔpH. By contrast, K⁺-loaded vesicles exhibited no acidification of the intravesicular space, i.e. the internal and external pH values were equal over the range examined (Fig. 1, right). Thus, the Δψ and ΔψΔpH were equivalent, declining slightly as the pH was raised. Vesicles loaded with ammonium buffer generated both Δψ and ΔpH values of the conventional orientation (inside negative and alkaline) (Table I). The ΔpH was relatively constant, from -35 to -44 mV, with increasing external pH from 8.0 to 9.5. The Δψ increased somewhat as the pH was raised, but was in a lower range than the Δψ observed in Na⁺- or K⁺-loaded vesicles. Similar results were obtained using vesicles prepared in choline/Tris buffer (data not shown).

A working model, which would account for the observed PMF patterns, is shown in Fig. 2. In the absence of both K⁺ and Na⁺, primary proton extrusion would establish an electrochemical gradient of protons, outside acid and positive. In K⁺-loaded vesicles, a K⁺/H⁺ antiporter is shown to catalyze an electroneutral exchange subsequent to proton extrusion. This
results in abolition of the $\Delta p$H, but a $\Delta p_i$ outside positive, is maintained. Na$^+$-loaded vesicles might have an antiporter which, in exchange for Na$^+$, inwardly translocates more protons than are extruded during respiration. The activity could be energized, at least in part, by the $\Delta p_i$. Depending upon the actual stoichiometries and rates, the net result of respiration and such Na$^+/H^+$ antiporter activity could be the observed acidified intravesicular space and a $\Delta p_i$ outside positive. For example, this result would be obtained if 2 protons were extruded during respiration, and the antiporter then catalyzed an exchange of 3 H$^+$ inward/2 Na$^+$ outward.

The Na$^+/H^+$ Antiportor—First, the apparent requirement for Na$^+$ for acidification of the intravesicular space was further studied using right-side-out vesicles that had been prepared in potassium carbonate buffer, pH 9.0. In the experiments shown in Fig. 3, Na$^+$ (as carbonate) was added to such vesicles just prior to energization with ascorbate/TMPD. Identical results were obtained when the vesicles were preloaded with Na$^+$ for 30 min. $\Delta p$H, inside acid, was observed with concentrations of Na$^+$ ions as low as 1 mM; the magnitude of the $\Delta p$H increased as the Na$^+$ concentration was raised. Linear regression analysis of the data indicated that a half-maximal effect of Na$^+$ occurred at 0.7 mM Na$^+$. As shown by the dashed line at the bottom of Fig. 3, right-side-out vesicles from the nonalkalophilic strain KM 23 failed to exhibit acidification of the intravesicular space upon addition of Na$^+$.

The Na$^+$-dependent acidification of the intravesicular space of wild type vesicles was also demonstrated with an assay in which the quenching of quinacrine was used as an indicator of proton influx. Quinacrine fluorescence, in suspensions of vesicles that were energized by ascorbate/TMPD, was markedly quenched in the presence of Na$^+$ (Table II). The quenching was prevented by gramicidin. Vesicles from KM 23 failed to exhibit a similar quenching of quinacrine fluorescence.

Both proton and Na$^+$ movements were then studied in everted membrane vesicles that were prepared in potassium carbonate buffer. Proton movements were monitored using the uptake of weak acids and bases. Upon energization with NADH, everted K$^+$-loaded vesicles exhibited no methylamine uptake, and little or no DMO uptake, depending upon the preparation. The transient DMO uptake sometimes observed in the absence of added Na$^+$ (e.g. Fig. 4A) correlated with somewhat variable contamination of solutions with Na$^+$. The levels of contamination were in a range from 10 to 100 $\mu$M Na$^+$. Treatment of vesicles with 10 $\mu$M gramicidin prior to energization completely abolished DMO uptake. As shown in Fig. 4, addition of 10 $\mu$M Na$^+$ or Li$^+$ to energized K$^+$-loaded vesicles resulted in DMO uptake, i.e. net proton efflux. This was consistent with the Na$^+$-dependent methylamine uptake ob-

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FIG. 5. Accumulation of $^{22}$Na by everted K$^+$-loaded membrane vesicles. Everted vesicles of wild type B. alcalophilus (Curves A to G) and KM 23 (Curve H) were suspended in 100 mM KCl buffer, 10 mM MgSO$_4$, pH 8.7, at approximately 20 mg of protein/ml. Uptake of $^{22}$Na was assayed by flow dialysis. $^{22}$NaCO$_3$, pH 8.7, was added to initiate the experiment at the indicated concentrations along with the other indicated additions: A, 10 mM $^{22}$NaCO$_3$, no addition at $\Delta p$H; B, 0.5 mM $^{22}$NaCO$_3$, 10 mM NADH (K$^+$-salt) at $\Delta p$H; C, 10 mM $^{22}$NaCO$_3$, 10 mM NADH at arrow; D, 2.5 mM $^{22}$NaCO$_3$, 10 mM NADH at arrow; E, 10 mM $^{22}$NaCO$_3$, 10 mM NADH at arrow; F, 10 mM $^{22}$NaCO$_3$, added to vesicles preincubated with 10 mM KSCN (also in dialysate buffer) for 30 min, 10 mM NADH was added at arrow; G, 10 mM $^{22}$NaCO$_3$, added to vesicles preincubated with 10 mM valinomycin, 10 mM NADH added at arrow; H, 10 mM $^{22}$NaCO$_3$, 10 mM NADH added at arrow to KM 23 everted vesicles.

FIG. 6. Effect of K$^+$ on the acidification of the intravesicular space of everted membrane vesicles. Everted membrane vesicles from wild type B. alcalophilus were prepared in either 10 mM Tris, 10 mM MgSO$_4$, and 100 mM choline chloride buffer, pH 8.7 (Curves A to D), or 100 mM KCl, 10 mM MgSO$_4$, buffer, pH 8.7 (Curves E and F). Acidification of the intravesicular space was monitored by the accumulation of methylamine using a flow dialysis assay as described under "Materials and Methods." In all runs [$^{14}$C]methylamine, 25 $\mu$g (50 $\mu$Ci/ml), was present from the beginning of the experiment under the following conditions: A, energized choline/Tris-loaded vesicles; B, 10 mM NADH (Tris salt) added to choline/Tris-loaded vesicles at arrow; C, 10 mM NADH plus 50 mM KCl added to choline/Tris-loaded vesicles at arrow; D, 50 mM KCl added to choline/Tris-loaded vesicles at arrow; E, 10 mM NADH added to K$^+$-loaded vesicles at arrow; F, 10 mM NADH added at arrow to K$^+$-loaded vesicles preincubated with 10 mM KSCN for 30 min (also in dialysate buffer).
Na⁺/H⁺ and K⁺/H⁺ Antiporters in B. alcalophilus

erved in energized right-side-out vesicles. A ΔpH of -53 mV was calculated for everted vesicles energized by NADH in the presence of 10 mM Na⁺. Treatment of everted vesicles with either thiocyanate or valinomycin, to prevent the generation of a Δψ (inside positive), abolished Na⁺-dependent proton efflux (Fig. 4, D to G). No DMO was taken up by everted vesicles from KM 23 upon addition of NADH and Na⁺ (Fig. 4H); no methyamine uptake was observed under these conditions, either as discussed, below.

The pattern of 32Na⁺ uptake by everted vesicles correlated nicely with the above experiments. 32Na⁺ uptake was dependent upon energization (Fig. 5A) and increased with increasing concentrations of Na⁺ up to about 10 mM (Fig. 5, B to E). A ΔpH, of -70 mV (a 15-fold concentration gradient) was calculated for energized everted vesicles incubated with 10 mM Na⁺. Treatment of the vesicles with thiocyanate (Fig. 5F) or valinomycin (Fig. 5G) abolished NADH-dependent 32Na⁺ uptake. No uptake of 32Na⁺ was observed upon energization of everted vesicles from KM 23 (Fig. 5H).

The K⁺/H⁺ Antipporter—The presence of a K⁺/H⁺ antipporter was suggested by the difference in ΔpH patterns between K⁺-loaded right-side-out vesicles and right-side-out vesicles loaded with either ammendiol or choline/Tris (Fig. 1 and Table I). It proved difficult to study both Na⁺/H⁺ and K⁺/H⁺ antipporter activities in the latter buffers because of apparent inhibitory effects of buffer constituents. However, at least some K⁺-dependent movement of protons could be demonstrated. As shown in Fig. 6, everted vesicles, loaded with and assayed in choline/Tris buffer, exhibited energy-dependent methyamine uptake. In many separate experiments, the addition of KCl together with the NADH caused at least a small reduction in the magnitude and duration of the observed ΔpH (Fig. 6, B to D). That is, in the presence of K⁺, the respiratory-coupled acidification of the intravesicular space was diminished. As expected from experiments with right-side-out vesicles, K⁺-loaded everted vesicles exhibited neither DMO uptake (Fig. 4) nor methyamine uptake (Fig. 6E) upon energization with NADH. Treatment of the vesicles with KSCN to prevent the generation of a Δψ did not result in methyamine uptake upon energization (Fig. 6E). Thus, K⁺-dependent ablation of the ΔpH did not appear to require the Δψ. K⁺-loaded vesicles from KM 23 exhibited the same absence of DMO and methyamine uptake as those from the wild type.

DISCUSSION

Cation/proton antiporters have been described in several species of bacteria (7, 18-26), and may well prove to be ubiquitous among prokaryotes. In addition to the regulation of cytoplasmic pH, functions ascribed to these antiporters include active extrusion of potentially cytotoxic Ca²⁺ and Na⁺ (21, 24, 25), and generation of a sodium-motive force for energization of certain transport systems (27). The results reported here clearly support the specific role for a Na⁺/H⁺ antiporter in acidification of the cytoplasm in an alkalophilic bacterium. This antiporter, in vesicles from B. alcalophilus, translocates protons in exchange for Na⁺ such that chemical gradients of the two ions are established on opposite sides of the membrane. The orientation of the proton gradient is the reverse of that established by primary proton extrusion during respiration. Thus, the antiporter could account for the lower pH of the cytoplasm (or intravesicular space of right-side-out vesicles) relative to the external milieu. That this is indeed its function, is indicated by the absence of the Na⁺/H⁺ antiporter in vesicles from the nonalkalophilic strain KM 23.

It is of interest that a ΔpH, inside acid, was observed in B. alcalophilus vesicles at external pH values from 8.0 up to 10.5. Whole cells exhibited a ΔpH at pH values from 10.0 to 11.5 only; at pH 9.0 and 9.5, ΔpH was zero (1). The differences between whole cells and vesicles suggest a role for cytoplasmic constituents in buffering the protons taken up via the antiporter or affecting the activity of the antiporter. It is likely that a limited ability of the cytoplasm to buffer the inwardly translocated protons below pH 8.0 is the basis for the obligately alkalophilic nature of B. alcalophilus; i.e. below pH 8.0, the antiporter may lower the cytoplasmic pH below the minimum for viability. This hypothesis is substantiated by the properties of KM 23, which gains the ability to grow from pH 5.5 to 9.0, while exhibiting loss of antiporter activity and the ability to grow above pH 9.0 (9).

The Na⁺/H⁺ antiporter of B. alcalophilus is Δψ-dependent, as indicated by the effects of valinomycin and thiocyanate in everted vesicles. The dependence on the Δψ could reflect an electrogenic transport of >1 H⁺/Na⁺. Neither cells nor right-side-out vesicles show any dependence on a sodium gradient, ΔpH, for acidification of the interior, but only require the presence of Na⁺. Therefore, the antiporter probably catalyzes an electrogenic exchange that is energized by the Δψ. The Δψ could be required, in addition, because of a gating effect; a gating of the Na⁺/H⁺ antiporter in B. alcalophilus, at approximately -95 mV, is suggested by whole cell experiments on $^{32}$Na efflux. Similar gating has been reported for the Na⁺/H⁺ antiporter of Halobacterium halobium (28); the critical potential in that species was ~130 to ~155 mV. There are other possible bases for Δψ dependence, as discussed by Brey and Rosen (25). An electrogenic Na⁺/H⁺ antiporter would be expected to convert the Δψ (at least in part) to a ΔpH, and Δψ, inside acid, whereas the Δψ in Na⁺-loaded vesicles is as high as, or higher than, in vesicles prepared without Na⁺. Perhaps the activity of the Na⁺/H⁺ antiporter, e.g. the presence of the reversed ΔpH, facilitates the generation of a much higher Δψ than is produced in the absence of Na⁺ by stimulating respiration. This possibility is being investigated. It is notable that as the external pH was raised, none of the vesicle preparations exhibited the marked increase in the Δψ that was observed in whole cells (1); this difference between vesicles and whole cells has also been observed in E. coli (9).

The presence of an electroneutral (Δψ-independent) K⁺/H⁺ antipporter is consistent with the absence of a ΔpH in K⁺-loaded vesicles (including thiocyanate-treated everted vesicles). In the absence of both Na⁺ and K⁺, right-side-out vesicles exhibited a ΔpH, inside alkaline, and everted vesicles exhibited a ΔpH, inside acid. The physiological role of the K⁺/H⁺ antipporter is unclear. It might serve as a back-up system for proton reentry in this alkalophile or as one of several processes regulating intracellular K⁺ levels. Since KM 23 appears to possess the same activity as the wild type, the K⁺/H⁺ antipporter is not implicated in facilitating growth at very high pH. However, this antiporter could be involved in the regulation of cytoplasmic pH in a lower pH range, as suggested by Rosen and his colleagues (8, 22) for the K⁺/H⁺ antiporter in E. coli.

REFERENCES


³ H. R. Kaback, personal communication.