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Characterization of the Na^+/H^+ Antiporter of Alkalophilic Bacilli In Vivo: $\Delta\psi$-Dependent $^{22}Na^+$ Efflux from Whole Cells

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The Na^+/H^+ antiporter of *Bacillus alcalophilus* was studied by measuring $^{22}Na^+$ efflux from starved, cyanide-inhibited cells which were energized by means of a valinomycin-induced potassium diffusion potential, positive out ($\Delta\psi$). In the absence of a $\Delta\psi$, $^{22}Na^+$ efflux at pH 9.0 was slow and appreciably inhibited by *N*-ethylmaleimide. Upon imposition of a $\Delta\psi$, a very rapid rate of $^{22}Na^+$ efflux occurred. This rapid rate of $^{22}Na^+$ efflux was competively inhibited by Li^+ and varied directly with the magnitude of the $\Delta\psi$. Kinetic experiments with *B. alcalophilus* and alkalophilic *Bacillus firmus* RAB indicated that the $\Delta\psi$ caused a pronounced increase in the V_{max} for $^{22}Na^+$ efflux. The K_m values for Na^+ were unaffected by the $\Delta\psi$. Upon imposition of a $\Delta\psi$ at pH 7.0, a retardation of the slow $^{22}Na^+$ efflux rate at pH 7.0 was caused by the $\Delta\psi$. This showed that inactivity of the Na^+/H^+ antiporter at pH 7.0 was not secondary to a low $\Delta\psi$ generated by respiration at this pH. Indeed, $^{22}Na^+$ efflux activity appeared to be inhibited by a relatively high internal proton concentration. By contrast, at a constant internal pH, there was little variation in the activity at external pH values from 7.0 to 9.0; at an external pH of 10.0, the rate of $^{22}Na^+$ efflux declined. This decline at typical pH values for growth may be due to an insufficiency of protons when a diffusion potential rather than respiration is the driving force. Non-alkalophilic mutant strains of *B. alcalophilus* and *B. firmus* RAB exhibited a slow rate of $^{22}Na^+$ efflux which was not enhanced by a $\Delta\psi$ at either pH 7.0 or 9.0.

Obligately alkalophilic bacilli, like other aerobic bacteria and mitochondria, extrude protons concomitantly with electron transport during respiration (12, 14, 15). Nonetheless, alkalophiles are able to maintain their cytoplasmic pH at values no greater than 9.5 at external pH (pH_{out}) values of 10.5 to 11.5 (4, 6). There is considerable evidence that an electrogenic Na^+/H^+ antiporter accomplishes the maintenance of a relatively acidic cell interior by catalyzing the exchange of extracellular protons for intracellular Na^+ (11). Most alkalophilic bacilli require the presence of Na^+ in the growth medium (8), and suspension of such a species in the absence of Na^+ results in the immediate loss of pH homeostasis (10). Activities attributed to the Na^+/H^+ antiporter of alkalophilic bacilli have been studied almost entirely in vitro in isolated membrane vesicles. These activities include Na^+-dependent proton uptake by energized, right-side-out vesicles (12, 15) and Na^+-dependent proton efflux from and $^{22}Na^+$ accumulation by energized everted vesicles (15). Non-alkalophilic mutant derivatives of the alkalophilic bacilli lose the ability to grow at pH values above 9.0, gain the ability to grow at neutral pH, and do not exhibit any of the above activities (12, 13, 15).

The finding of the non-alkalophilic mutants led us to speculate that the functional Na^+/H^+ antiporter was not only required for growth at highly alkaline pH, but might preclude growth of the alkalophiles at neutral pH by excessively acidifying the cytoplasm at lower pH (13, 15). However, more recent studies suggested that the antiporter is largely inactive at pH 7.0; Kitada et al. (10) showed that cells of alkalophilic *Bacillus firmus* RAB actually generated a small transmembrane pH gradient, acid out, in the presence of Na^+. They also remained viable for appreciable time periods at pH 7.0, although they did not grow. Consistently, energized membrane vesicles exhibited only a modest amount of Na^+-dependent proton entry at pH 7.0, not enough to keep pace with proton extrusion during respiration (10). It could not be asserted with certainty, however, whether the apparent lack of Na^+/H^+ antiporter activity at pH 7.0 represented a property of the porter or was secondary to the poor function of the alkalophile respiratory chain at pH 7.0 relative to its ability to generate a driving force at alkaline pH (10, 14).

Therefore, we undertook a study of antiporter function in vivo under conditions in which an artificially induced transmembrane electrical potential ($\Delta\psi$) was used to energize antiport activity.

MATERIALS AND METHODS

Organisms and growth conditions. Two alkalophilic bacilli, *Bacillus alcalophilus* (ATCC 27647) and *B. firmus* RAB (4), were grown with aeration at 30°C in the L-malate-containing medium (pH 10.5) previously described (6). A non-alkalophilic mutant strain of *B. alcalophilus*, strain KM^{er}, carrying a genetic marker for L-ethionine resistance (3), and non-alkalophilic derivative RABN of *B. firmus* RAB (4) were grown under the same conditions as the alkalophiles in L-malate-containing medium at pH 6.8 (4).

Starvation and loading of cells with $^{22}Na^+$. Cells were grown to the late logarithmic phase of growth (ca. 200 Klett units, with a #42 filter) and harvested by centrifugation for 10 min at 12,000 × *g*. The cells were then washed twice with 100 mM potassium phosphate buffer (pH 7.0) and suspended in the same buffer with 10 mM KCN added. These cell suspensions were incubated for 3 h at 30°C on a rotary shaker (shaking at 200 rpm). They were then harvested and equilibrated at the pH desired by incubation, for 30 min at room temperature, in 100 mM potassium phosphate buffer (pH 7.0 or 8.0) or 100 mM potassium carbonate buffer (pH 9.0 or 10.0). After centrifugation at 12,000 × *g*, cells were resuspended in the same buffer to make a concentrated cell suspension (40 to 50 mg of cell protein per ml). Valinomycin was added to a final concentration of 40 μM. After 20 min at room temperature, $^{22}Na^+$ (as $^{22}NaCl$) was added at concentrations indicated for individual experiments at 70 μCi/ml. Equilibration of the $^{22}Na^+$ occurred within 5 min as assessed by calculation of the internal $^{22}Na^+$ concentration ($[^{22}Na^+]_{in}$).

Assays of $^{22}Na^+$ efflux and TPP^+ uptake. Samples (2 μl) of cells that had been equilibrated with $^{22}Na^+$ were diluted 200-fold (unless otherwise noted) into 100 mM choline chloride to establish a diffusion potential or into 100 mM KCl as a control. The choline chloride or KCl solutions were buffered at 10 mM with one of the following: HEPES-Tris [4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid] (pH 7.0 or 8.0); Tris (hydroxymethyl) methylaminopropane sulfonic acid (taps-Tris) (pH 9.0); or cyclohexylaminopropane sulfonic acid (caps-Tris) (pH 10.0). At frequent time intervals, as indicated for individual experiments, suspensions were diluted by the addition of 3.5 ml of 100 mM potassium carbonate buffer (pH 9.0) at room temperature. The samples were immediately filtered through glass fiber filters (Whatman, Inc.; 25 mm), and the filters were washed once with the same buffer. The zero time values were determined by dilution of cells directly into a 3.5-ml volume of 100 mM potassium carbonate buffer (pH 9.0) followed by immediate filtration. The radioactivity retained by the filters was determined by liquid scintillation spectrometry (15).

The diffusion potentials formed were measured by assaying the distribution of [^{3}H]tetraphenylphosphonium (TPP^+) (10 μM) essentially by the method of Schuldiner and Kaback (16), except that glass fiber filters were employed. A cell volume of 10 μl/mg of protein was used for the calculations (10). Corrections were made for TPP^+ bound to cells which were diluted into K^+-containing media. The values obtained for the $\Delta\psi$ from assays with TPP^+ were within 10% of the magnitude of the $\Delta\psi$ that would be predicted by the dilution: −136 mV in most experiments. No TPP^+ uptake was observed when the dilution buffer contained 100 mM KCl. A series of control experiments demonstrated that the magnitude of the diffusion potential was unaffected by the presence versus the absence of the preloaded Na^+.

For experiments in which the inhibitory effect of Li^+ was examined, cells were equilibrated with various concentrations of Li^+ at the same time as Na^+, i.e., the Li^+ was inside the cell at the beginning of the experiment. For experiments in which the effect of *N*-ethylmaleimide (NEM) was studied, starved cells were incubated for 15 min at about 1 mg of cell protein per ml in 100 mM potassium phosphate buffer (pH 7.0) either with or without 2 mM NEM. The cells were then washed, equilibrated to the desired pH, and loaded with $^{22}Na^+$ as described above.

RESULTS

In the absence of a $\Delta\psi$, the efflux of $^{22}Na^+$ from cells of *B. alcalophilus* at pH 9.0 was slow and linear with time over a 20-s period (Fig. 1A); treatment of the cells with NEM caused about a 50% reduction in $^{22}Na^+$ efflux from the unenergized cells. In the presence of a $\Delta\psi$ at pH 9.0, $^{22}Na^+$ efflux was extremely rapid until ca. 60% of the $^{22}Na^+$ had been released (Fig. 1A). Efflux continued at a declining rate until almost all of the $^{22}Na^+$ had effluxed. The initial, rapid $^{22}Na^+$ efflux observed upon imposition of a $\Delta\psi$ at pH 9.0 was not inhibited as much as the $\Delta\psi$-independent flux by NEM treatment. As shown in Fig. 1B, generation of a $\Delta\psi$ at pH 7.0 did not stimulate $^{22}Na^+$ efflux but, rather, retarded efflux of the cation. Although data are not shown, the presence of 10 mM non-radioactive Na^+ in the dilution medium had no effect on the patterns of $^{22}Na^+$ efflux shown in Fig. 1A. Nor did the addition of carbonate or bicarbonate to the buffer at pH 7.0 or omission of carbonate from the buffer at alkaline pH alter the $^{22}Na^+$ efflux patterns. A kinetic analysis of the data from Fig. 1A showed clearly that the major and probably sole effect of the $\Delta\psi$ was a dramatic increase in the V_{max} for $^{22}Na^+$ efflux (Fig. 2); this parameter was somewhat variable in dozens of independent experiments in the range of 0.35 to 1.0 μmol Na^+ efflux per min/mg of protein. Apparently, very minor variations in starvation conditions affected the V_{max}, whereas the K_m values were quite reproducible. It should be noted that the data from this and other experiments were not corrected for a small passive diffusion component which slightly distorts efflux values at low velocities. The K_m for Na^+ calculated from the kinetic data obtained in the presence of a $\Delta\psi$ was ca. 1 mM. The observed K_m was essentially the

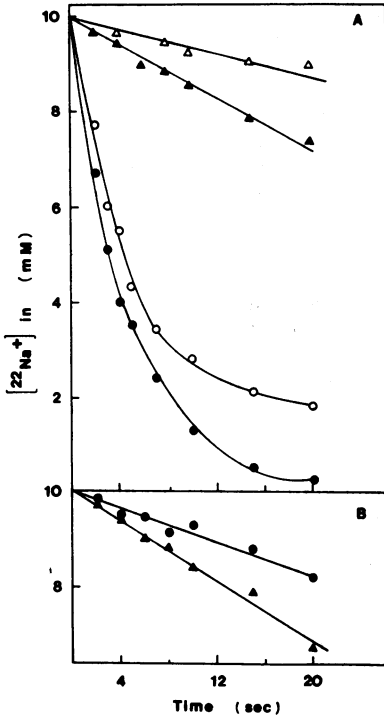

FIG. 1. $^{22}Na^+$ efflux from starved cells of *B. alcalophilus* at pH 9.0 and 7.0. Cells of *B. alcalophilus* were starved in the presence of KCN as indicated in the text and resuspended in either 100 mM potassium carbonate buffer (pH 9.0) (A) or 100 mM potassium phosphate buffer (pH 7.0) (B). The cell suspensions were treated with valinomycin and equilibrated with 10 mM $^{22}NaCl$ at room temperature. Efflux of $^{22}Na^+$ was measured after dilution into either 100 mM choline chloride (●, ○) or 100 mM KCl (▲, △) containing 10 mM taps-Tris (pH 9.0) (A) or 10 mM HEPES-Tris (pH 7.0) (B). At the indicated times, samples were diluted and filtered as described in the text. Where indicated by open symbols in (A), identical experiments were carried out with cells that had been treated, after starvation, with 2 mM NEM as described in the text.

same in the absence of a Δψ and, assuming a small diffusion component, was probably identical. The presence of Li^+ in the cells caused substantial inhibition of $^{22}Na^+$ efflux from cells of *B. alcalophilus* in the presence of a Δψ at pH 9.0. A Dixon plot of the inhibition data (Fig. 3) suggested that Li^+ is a competitive inhibitor of $^{22}Na^+$ efflux with a K_i of ca. 1 mM.

The rate of $^{22}Na^+$ efflux was linear with the magnitude of the Δψ in a range from 50 to 200 mV and extrapolates to a rate of zero at zero mV (Fig. 4). The deviation from linearity at zero mV presumably reflects the passive diffusion component. Attempts were made to monitor Na^+_{in}-dependent proton influx upon generation of a Δψ. The distribution of methylamine, assayed by a filtration assay (10), was used to assess proton movements in cells that had or had not been loaded with 10 mM Na^+ before generation of a diffusion potential. Although Δψ-dependent methylamine uptake was observed, Na^+ dependence could not be demonstrated (data not shown).

The rate of Δψ-dependent $^{22}Na^+$ efflux from starved cells of *B. alcalophilus* did not vary appreciably at pH_{out} values from 7.0 to 9.0 when the internal pH (pH_{in}) was 9.0 and only pH_{out} was varied (Fig. 5). However, with pH_{in} still equal to 9.0, the rate did decline appreciably at a pH_{out} value of 10.0. In a different experimental protocol, the pH_{in} and pH_{out} were equilibrated to the same pH values over a range from 7.0 to 10.0. Under these conditions, the rate of $^{22}Na^+$ efflux increased over the entire range of pH from 7.0 to 10.0 (Fig. 5). As also indicated in Fig. 5, with pH_{in} = 10.0, the rate of $^{22}Na^+$ efflux was much greater when the pH_{out} was 7.0 than when it was 10.0.

Efflux of $^{22}Na^+$ was then examined in starved cells of the non-alkalophilic strain of *B. alcalophilus*, KM^{er}. As shown in Fig. 6, a relatively slow rate of $^{22}Na^+$ efflux was observed at both pH 7.0 and 9.0, with no effect of the Δψ. A second pair of alkalophilic-non-alkalophilic *Bacillus* strains showed similar differences. Higher

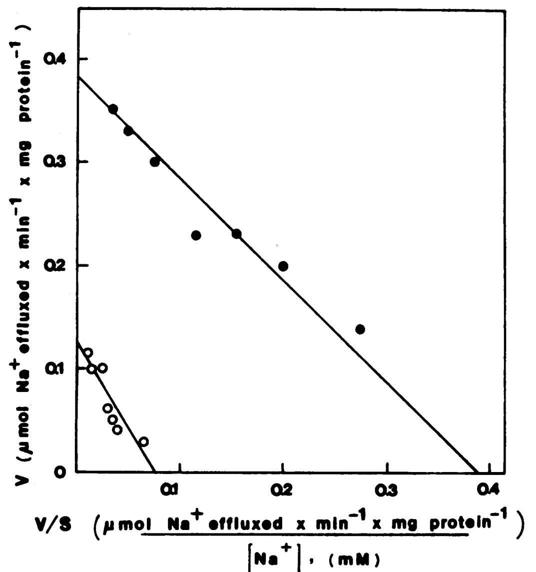

FIG. 2. Kinetic analysis of $^{22}Na^+$ efflux from *B. alcalophilus* cells at pH 9.0. Rates of $^{22}Na^+$ efflux were determined from time points taken during the first 4 s (initial rate conditions) in experiments such as those depicted in Fig. 1A. Starved, $^{22}Na^+$-loaded (0.5 to 10 mM range of concentration) cells of *B. alcalophilus*, at pH 9.0, were diluted 200-fold into 100 mM choline chloride–10 mM taps-Tris (pH 9.0) (●) or 100 mM KCl–10 mM taps-Tris (pH 9.0) (○).

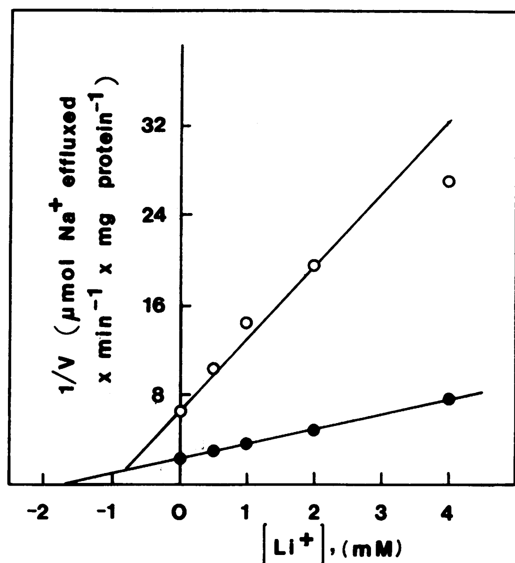

FIG. 3. Dixon plot analysis of the inhibition of $\Delta\psi$-dependent $^{22}Na^{+}$ efflux from *B. alcalophilus* cells at pH 9.0 by Li^{+}. Starved cells of *B. alcalophilus* were suspended in 100 mM potassium carbonate buffer (pH 9.0) and treated with valinomycin as described in the text. The cells were loaded with either 1 mM $^{22}NaCl$ (○) or 10 mM $^{22}NaCl$ (●) and equilibrated with the indicated concentrations of LiCl before 200-fold dilution into 100 mM choline chloride–10 mM taps-Tris (pH 9.0). The reactions were terminated as described in the text. Rates of $^{22}Na^{+}$ efflux were calculated from points taken during the first 3 s after dilution.

concentrations of Na^{+} were employed with the *B. firmus* strains because previous work indicated a higher K_m for Na^{+} for the antiporter of *B. firmus* RAB than for that of *B. alcalophilus* (12). Alkalophilic *B. firmus* RAB exhibited $\Delta\psi$-dependent $^{22}Na^{+}$ efflux at pH 9.0, but the slower $^{22}Na^{+}$ efflux at pH 7.0 was retarded by imposition of a $\Delta\psi$ (Fig. 7). Kinetic analysis indicated that the $\Delta\psi$ increased the V_{max} for $^{22}Na^{+}$ efflux at pH 9.0, whereas the K_m for Na^{+} was 24 mM and was unaffected by the $\Delta\psi$ (Fig. 8). The nonalkalophilic strain *B. firmus* RABN exhibited a relatively slow rate of $^{22}Na^{+}$ efflux at pH 9.0 that was unaffected by the $\Delta\psi$ and an even slower rate of $^{22}Na^{+}$ efflux at pH 7.0 that was retarded by the $\Delta\psi$ (Fig. 9).

DISCUSSION

The kinetic properties of $^{22}Na^{+}$ efflux from starved alkalophile cells were in striking accord with those found with in vitro assays of Na^{+}-dependent proton movements. In the current study, K_m values for Na^{+} were 1 and 24 mM for *B. alcalophilus* and *B. firmus* RAB, respectively, as compared with estimates of 0.7 and ca. 20 mM for the two species from earlier studies of proton movements (12, 15). Also in accord with prior findings was the absence of $\Delta\psi$-dependent $^{22}Na^{+}$ efflux in wild-type alkalophile cells equilibrated at pH 7.0 and in cells of non-alkalophilic mutant strains at either pH 7.0 or 9.0. Finally, the finding of competitive inhibition of $^{22}Na^{+}$ efflux by Li^{+} was consistent with the prior finding that Li^{+} could substitute for Na^{+} in causing $\Delta\psi$-dependent acidification of the interior of membrane vesicles (15). All these positive correlations with in vitro assays and physiological observations supported the expectation that $\Delta\psi$-dependent $^{22}Na^{+}$ efflux would represent activity of the electrogenic Na^{+}/H^{+} antiporter. Unfortunately, Na^{+}-dependent proton movements could not clearly be demonstrated by using the current experimental protocol because appreciable proton influx, as monitored by methylamine uptake, occurred upon generation of a diffusion potential even in the absence of added intracellular Na^{+}. Comparable voltage-dependent proton influx has been observed in liposomes under similar conditions (11) and has been suggested in at least some studies utilizing a diffusion potential in membrane vesicles (17). In the absence of a $\Delta\psi$ at pH 9.0, a slow but

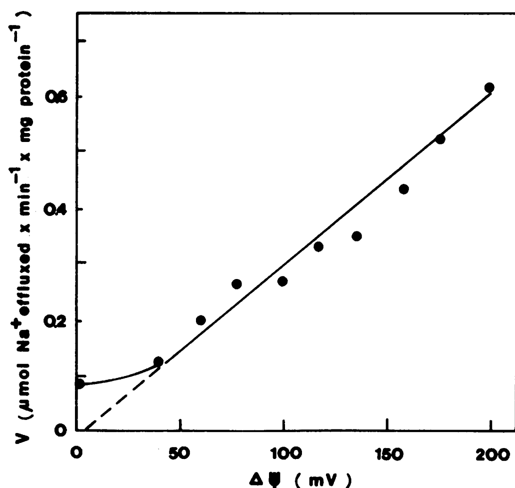

FIG. 4. The rate of $^{22}Na^{+}$ efflux from *B. alcalophilus* cells at pH 9.0 as a function of the magnitude of the $\Delta\psi$. Starved cells of *B. alcalophilus* were suspended in potassium carbonate buffer (pH 9.0) treated with valinomycin and loaded with 10 mM $^{22}NaCl$ as described in the text. Samples (2 μl) were diluted into media containing various proportions of choline and potassium chloride (final choline + potassium concentrations of 100 mM) and 10 mM taps-Tris (pH 9.0). Initial rates of $^{22}Na^{+}$ efflux were plotted as a function of the $\Delta\psi$ calculated from the distribution of $[^{3}H]TPP^{+}$ under each condition; these values for the $\Delta\psi$ agreed within 10% with the theoretical values.

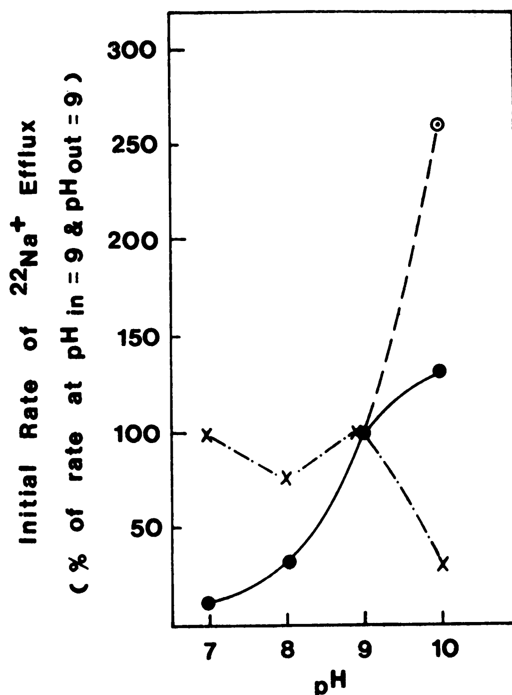

FIG. 5. The pH dependence of $^{22}Na^+$ efflux from *B. alcalophilus* cells. In one experimental protocol (×), starved cells were equilibrated in potassium carbonate buffer (pH 9.0) treated with valinomycin and loaded with 10 mM $^{22}NaCl$. Efflux of $^{22}Na^+$ was measured after dilution of those cells into 100 mM choline chloride buffered to various indicated pH_{out} values with the buffers listed in the text. In a second protocol (●), starved cells were equilibrated in potassium phosphate buffers (pH 7.0 or 8.0) or potassium carbonate buffers (pH 9.0 or 10.0) treated with valinomycin and loaded with 10 mM $^{22}NaCl$. Samples of each suspension were diluted into 100 mM choline chloride buffered to the same pH value employed for equilibration (with the buffers listed in the text). The initial rates of $^{22}Na^+$ efflux are presented relative to the rate observed when both pH_{in} and pH_{out} were 9.0. The single point (⊙) represents the rate observed when cells equilibrated so that pH_{in} = 10.0 were diluted into 100 mM choline chloride–10 mM HEPES-Tris (pH 7.0).

significant rate of $^{22}Na^+$ efflux occurred from alkalophile cells. Importantly, this component exhibited a K_m for Na^+ that was characteristic of the particular alkalophile species. Moreover, at least in *B. alcalophilus*, this component was inhibited substantially by NEM treatment. Thus, there is support for the possibility that the $^{22}Na^+$ efflux that is observed at pH 9.0 in the absence of a $\Delta\psi$ is carrier mediated and may even involve the same porter that is stimulated by the $\Delta\psi$.

Previous studies of proton motive force patterns of *B. firmus* RAB cells and vesicles at pH 7.0 indicated that the Na^+/H^+ antiporter of the wild-type alkalophile is inactive at that pH. The results of the current experiments clearly show that the inactivity of the antiporter is not secondary to the relatively low $\Delta\psi$ values generated by the alkalophile respiratory chain at neutral pH (10, 14). A diffusion potential of identical magnitude was established in starved cells at pH 9.0 and 7.0, resulting in a pronounced stimulation of $^{22}Na^+$ efflux only at the alkaline pH. Indeed, although the rate of $^{22}Na^+$ efflux varied in a direct linear fashion with the magnitude of the $\Delta\psi$, the relationship of the efflux rate to both pH_{in} and pH_{out} was more complex. When pH_{in} was 9.0, there was little variation in the rate of $^{22}Na^+$ efflux at pH_{out} values from 7.0 to 9.0. On the other hand, when pH_{in} was varied over the same range (with pH_{in} = pH_{out}), there was a pronounced and sigmoidal increase in the rate of $^{22}Na^+$ efflux with increasing pH_{in}. The rate of $^{22}Na^+$ efflux continued to increase when the

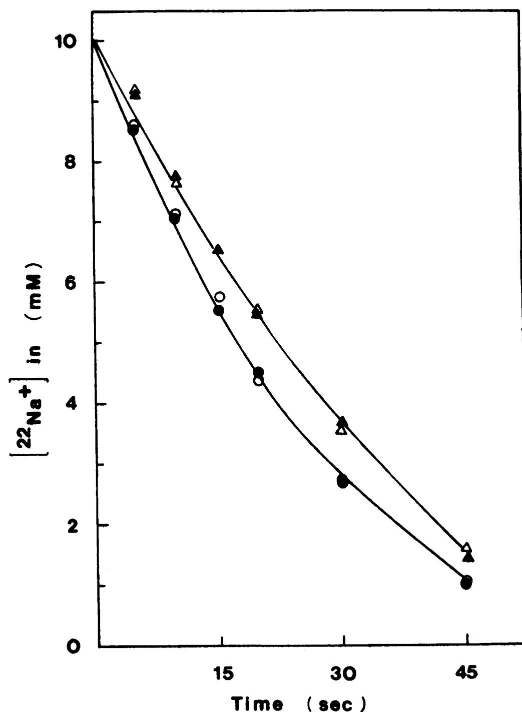

FIG. 6. $^{22}Na^+$ efflux from cells of non-alkalophilic strain KM^{er}. Starved cells of *B. alcalophilus* KM^{er} were suspended in potassium carbonate buffer (pH 9.0) (●, ○) or 100 mM potassium phosphate buffer (pH 7.0) (▲, △) treated with valinomycin and loaded with 10 mM $^{22}NaCl$ as described in the text. Efflux was measured after diluting samples into either 100 mM choline chloride (●, ▲) or 100 mM KCl (○, △) containing 10 mM taps-Tris (pH 9.0) (●, ○) or 10 mM HEPES-Tris (pH 7.0) (▲, △), as in the experiment depicted in Fig. 1.

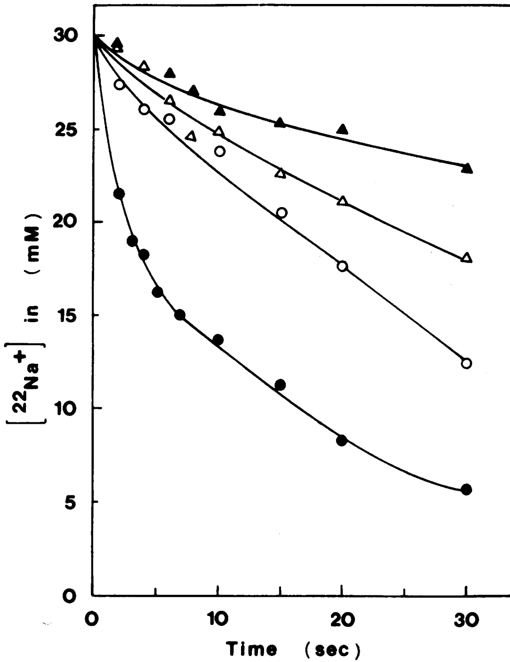

FIG. 7. $^{22}Na^+$ efflux from starved cells of *B. firmus* RAB at pH 9.0 and 7.0. Starved cells of *B. firmus* RAB were suspended in either 100 mM potassium carbonate buffer (pH 9.0) (●, ○) or 100 mM potassium phosphate buffer (pH 7.0) (▲, △) treated with valinomycin and loaded with 30 mM $^{22}NaCl$. $^{22}Na^+$ efflux was measured after dilution into 100 mM choline chloride (●, ▲) or 100 mM KCl (○, △) buffered to pH 9.0 (●, ○) or pH 7.0 (▲, △) as described in the legend to Fig. 1.

pH_{in} was raised to 10.0; the increase was especially great if the pH_{out} was acidified. Thus, the activity of the Na^+/H^+ antiporter, as measured by $^{22}Na^+$ efflux, appears to be inhibited by the presence of a high intracellular concentration of protons and unaffected by the external proton concentration in a range from pH 7.0 to 9.0. Interestingly, Aronson and his colleagues (1, 2) studying the electroneutral Na^+/H^+ antiporter of renal cell membranes have found that both H^+ and Li^+ compete for the Na^+ binding site, whereas the presence of protons on the other side of the membrane has a stimulatory effect on antiport activity.

In alkalophiles, the antiporter is normally functioning at optimal pH_{out} values for growth (pH 10 to 11). It was, therefore, notable that in this study, the rates of $^{22}Na^+$ efflux at pH_{out} values of 10.0 were reduced at a given pH_{in} over those observed at lower values of pH_{out}. For example, at a pH_{in} of 9.0—a typical physiological cytoplasmic pH for the alkalophiles (4, 6, 10)—$^{22}Na^+$ efflux was much more rapid at pH_{out} = 9.0 than at pH_{out} = 10.0. These results differ from antiporter activities inferred from proton motive force patterns of respiring cells, which suggested that the antiporter in such cells is very active at pH_{out} = 10.0 (4, 6). Presumably, the mode of energization-diffusion potential versus respiration may account for the differences. We would speculate that under the current experimental conditions, external protons may become limiting at very high pH_{out} values. Respiring cells may supply the requisite protons. Importantly, though, neither Na^+-loaded cells nor vesicles of *B. alcalophilus* or *B. firmus* RAB exhibit transient acidification of the exterior in highly buffered alkaline media such as those used here (4, 6, 12, 13). Indeed, even lightly buffered cells of *B. firmus* RAB, incubated in the absence of Na^+, failed to exhibit rapid extrusion of protons into the bulk phase unless valinomycin was added (14). It has been suggested by Kell and Hitchens (9) and Haines (7) that these kinds of findings reflect the existence of a more localized pathway than the bulk phase for the movement of respiration-derived protons to the porters or enzymes which utilize them.

At pH 7.0, there was a slow but significant rate of $^{22}Na^+$ efflux in all four of the strains examined, even though no $\Delta\psi$-dependent $^{22}Na^+$ efflux was observed at that pH. In all but strain KM^{er}, $^{22}Na^+$ efflux at pH 7.0 was inhibited by the generation of a $\Delta\psi$ as would be expected, for

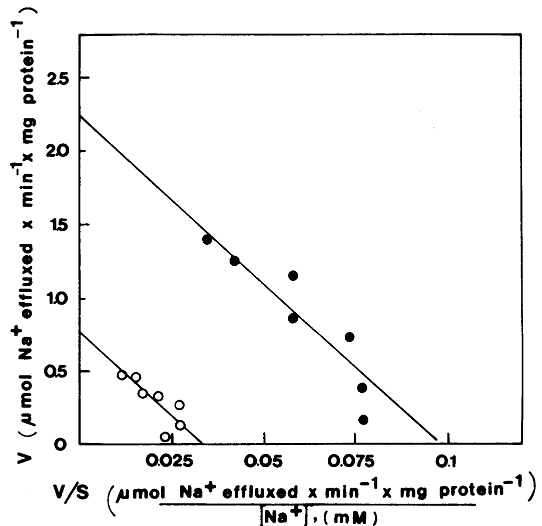

FIG. 8. Kinetic analysis of $^{22}Na^+$ efflux from *B. firmus* RAB cells at pH 9.0. The details are as in the legend to Fig. 2, except that *B. firmus* RAB cells were loaded with a range of $^{22}NaCl$ concentrations from 2 to 40 mM before dilution into 100 mM choline chloride–10 mM taps-Tris (pH 9.0) (●) or 100 mM KCl–10 mM taps-Tris (pH 9.0) (○).

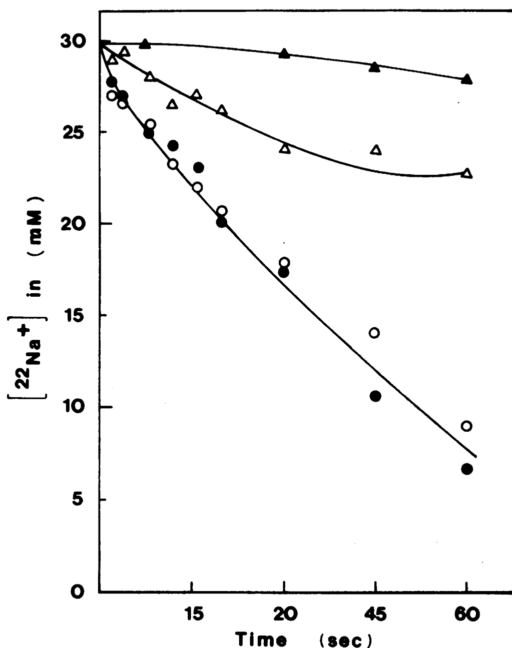

FIG. 9. $^{22}Na^{+}$ efflux from starved cells of non-alkalophilic strain RABN. The conditions were the same as described in the legend to Fig. 7. Symbols: ○, ● and △, ▲, measurements at pH 9.0 and 7.0, respectively; ○ and △, samples diluted into KCl; ● and ▲, samples diluted into choline chloride.

example, if Na^{+} was moving electrogenically down its chemical gradient. It is not clear how KM^{er} might differ from the other strains with respect to this property. No increase in the $^{22}Na^{+}$ efflux rate or Δψ dependence occurred in any of the strains when changes in anion composition were made, e.g., the addition of carbonate or bicarbonate. Thus, it is likely that the slow rates of $^{22}Na^{+}$ efflux at pH 7.0 truly reflect the capacity of the system at that pH. With respect to alkalophile cells, which transport solutes in symport with Na^{+} (e.g., 3–6, 8), the possibility is raised that one factor in precluding growth of wild-type alkalophiles at pH 7.0 is an insufficient ability to extrude the Na^{+} that would be taken up by multiple solute symporters. Non-alkalophilic mutants, which couple solute to protons rather than Na^{+} (3, 5, 13), would escape this limitation.

ACKNOWLEDGMENTS

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