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Electrochemical Proton Gradient in *Micrococcus lysodeikticus* Cells and Membrane Vesicles

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Using the distribution of weak acids to measure the pH gradient (ΔpH; interior alkaline) and the distribution of the lipophilic cation [^{3}H]tetraphenylphosphonium$^+$ to monitor the membrane potential (ΔΨ; interior negative), we studied the electrochemical gradient of protons ($\Delta\bar{\mu}_{H^+}$) across the membrane of *Micrococcus lysodeikticus* cells and plasma membrane vesicles. With reduced phenazine methosulfate as electron donor, intact cells exhibited a relatively constant $\Delta\bar{\mu}_{H^+}$ (interior negative and alkaline) of −193 mV to −223 mV from pH 5.5 to pH 8.5. On the other hand, in membrane vesicles under the same conditions, $\Delta\bar{\mu}_{H^+}$ decreased from a maximum value of −166 mV at pH 5.5 to −107 mV at pH 8.0 and above. This difference is related to a differential effect of external pH on the components of $\Delta\bar{\mu}_{H^+}$. In intact cells, ΔpH decreased from about −86 mV (i.e., 1.4 units) at pH 5.5 to zero at pH 7.8 and above, and the decrease in ΔpH was accompanied by a reciprocal increase in ΔΨ from −110 mV at pH 5.5 to −211 mV at pH 8.0 and above. In membrane vesicles, the decrease in ΔpH with increasing external pH was similar to that described for intact cells; however, ΔΨ increased from −82 mV at pH 5.5 to only −107 mV at pH 8.0 and above.

Chemiosmotic phenomena, as postulated by Mitchell (6, 8, 18–21), play a central, obligatory role in the active transport of many solutes across the bacterial cytoplasmic membrane (9, 10, 27–30, 38). Accordingly, oxidation of electron donors via a membrane-bound respiratory chain or hydrolysis of ATP by the Ca^{2+}, Mg^{2+}-stimulated adenosine triphosphatase complex is accompanied by the expulsion of protons into the external medium, leading to the generation of an electrochemical gradient of protons ($\Delta\bar{\mu}_{H^+}$, interior negative and alkaline) across the membrane that is the immediate driving force for transport.

The proton electrochemical gradient is composed of electrical and chemical parameters according to the following relationship:

$$\Delta\bar{\mu}_{H^+} = \Delta\Psi - \frac{2.3RT}{F}\Delta pH \qquad (1)$$

where ΔΨ represents the electrical potential across the membrane and ΔpH is the chemical difference in proton concentrations across the membrane (2.3RT/F is equal to 58.8 mV at 25°C).

Measurement of ΔpH and ΔΨ in microscopic systems that are not amenable to a direct electrophysiological approach is frequently based on determination of the equilibrium distribution of permeant weak acids or bases and permeant lipophilic ions, respectively (see references 32 and 33). Traditionally, the internal concentration of the permeant species is measured after separation of the cells or membrane vesicles from the bathing medium by filtration or centrifugation techniques. However, the manipulations involved in the separation step may result in significant and sometimes complete loss of accumulated solute, leading to an underestimate of the concentration gradient and thus to artifactually low values of $\Delta\bar{\mu}_{H^+}$. Recently (30), this problem has been resolved through the use of flow dialysis, a technique devised originally to measure ligand binding to enzymes (2). The technique allows continuous monitoring of the external concentration of any dialyzable solute under conditions that require no manipulation of the experimental system (31), and over the past few years, it has been utilized to measure $\Delta\bar{\mu}_{H^+}$ and steady-state levels of accumulation of a variety of transport substrates in a number of systems.

Strong support for the quantitative validity of the ΔpH and ΔΨ measurements has been obtained. Electrophysiological techniques have been applied to *Escherichia coli* giant cells induced by growth in 6-amidinopenicillanic acid (3), and the ΔΨ values measured agree to within 10 mV with those obtained from [^{3}H]tetraphenylphosphonium$^+$ ([^{3}H]TPP$^+$) distribution studies (J. S. Porter, C. L. Slayman, H. R. Kaback,

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and H. Felle, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, p. 145). It has also been demonstrated that the distribution of this lipophilic cation provides an excellent quantitative assessment of $\Delta\Psi$ in cultured neuroblastoma/glioma NG108-15 hybrid cells (15, 16) and isolated guinea pig synaptosomes (26). Finally, Navon et al. (22) and Ogawa et al. (23) have utilized high-resolution ^{31}P nuclear magnetic resonance spectroscopy to measure ΔpH in *E. coli*, and the results are very similar to those obtained from distribution studies with permeant weak acids in cells (24) and right-side-out membrane vesicles (27, 30, 38).

In a number of bacterial systems, it has been demonstrated that ΔpH varies with external pH (1, 7, 14, 24, 27, 30, 38). With intact *E. coli* and right-side-out membrane vesicles from this organism (27, 30) and *Salmonella typhimurium* (38), in particular, ΔpH exhibits maximal values of about 2 pH units (i.e., −120 mV) at pH 5.5 to 6.0 and decreases to zero at about pH 7.5. On the other hand, $\Delta\Psi$ has been reported in some instances (24, 27, 30, 38) to increase slightly with increasing external pH and in others (40; Porter et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K2, p. 145) to increase much more markedly with pH. This difference in $\Delta\Psi$ values at alkaline pH is critical to the putative increase in H^+-solute stoichiometry with certain transport substrates at relatively alkaline pH (see references 28, 29, 40).

In this paper, the relationship between ΔpH and $\Delta\Psi$ is studied as a function of external pH in intact cells and membrane vesicles from *Micrococcus lysodeikticus*, a gram-positive obligate aerobe. The experiments demonstrate that respiring cells generate a large and relatively constant $\Delta\bar{\mu}_{H^+}$ over a range of pH values. At acidic pH, both ΔpH and $\Delta\Psi$ contribute to $\Delta\bar{\mu}_{H^+}$, whereas at alkaline pH, $\Delta\Psi$ is the sole component of $\Delta\bar{\mu}_{H^+}$. Thus, ΔpH and $\Delta\Psi$ respond reciprocally as a function of external pH. The magnitude of ΔpH as a function of external pH in isolated membrane vesicles is similar to that observed for intact cells, and the magnitude of $\Delta\Psi$ is comparable in both systems at acidic pH; however, the vesicles exhibit a significantly lower $\Delta\Psi$ at alkaline pH relative to intact cells.

MATERIALS AND METHODS

Growth of cells and preparation of membrane vesicles. *M. lysodeikticus* ATCC 4698 (Fleming) was grown on a defined medium (4) at 30°C with shaking to late logarithmic phase. Cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C, washed and suspended 1:10 (wet weight/vol) in fresh growth medium, frozen, and stored in liquid N_2. The viability of cells treated in this manner was essentially 100% relative to freshly grown, unfrozen cells. Moreover, measurements of ΔpH and $\Delta\Psi$ with freshly grown cells, cells that were stored at 4°C for 3 days, and cells that were frozen and stored in liquid N_2 yielded the same values.

Membrane vesicles were prepared essentially as described by Konings et al. (12, 13). Cells were harvested, washed twice in 0.05 M KPO_4 (pH 7.0) containing 5 mM $MgSO_4$, suspended 1:200 (wet weight/vol) in the same medium, and equilibrated to 30°C. Lysozme, DNase, and RNase were then added to final concentrations of 100, 2, and 2 μg/ml, respectively, and incubation was continued at 30°C. The turbidity of the cell suspension decreased from 300 to 30 Klett units (Klett-Summerson colorimeter with a no. 54 filter) within 3 min. After an additional 10 min of incubation at 30°C, the vesicles were harvested by centrifugation at 40,000 × *g* for 30 min at 4°C, washed five times in 0.05 M KPO_4 (pH 7.0) containing 5 mM $MgSO_4$, resuspended in the same medium, and frozen and stored in liquid N_2.

For studies at various pH values, cell or membrane suspensions containing 6 to 7.5 mg of protein per ml were thawed at 30°C, diluted at least 100-fold in 0.1 M KPO_4 buffer at the desired pH containing 2 mM $MgSO_4$, and incubated at room temperature for 15 min. Cell suspensions were centrifuged at 10,000 × *g* for 10 min, membrane suspensions were centrifuged at 40,000 × *g* for 30 min, and the pellets were suspended to an appropriate protein concentration in 0.1 M KPO_4 at the same pH containing 2 mM $MgSO_4$.

Measurement of ΔpH. ΔpH (interior alkaline) was determined from the equilibrium distribution of [7-^{14}C]benzoic acid, 5,5′-dimethyl-[2-^{14}C]oxazolidine-2,4-dione ([2-^{14}C]DMO), or [1-^{14}C]acetic acid using flow dialysis as described (27, 30, 31, 38). For intact cell measurements, radioactive solute was added initially to the upper chamber, and after equilibrium was achieved, a sample of a concentrated cell suspension, potassium ascorbate, and phenazine methosulfate (PMS) were added to the upper chamber to final concentrations of 1.58 mg of protein per ml, 20 mM, and 0.1 mM, respectively. After 20 fractions were collected, 2.5 μM valinomycin was added, and after another 10 fractions, 5 μM nigericin was added. Measurement of ΔpH in membrane vesicles was performed in a similar manner, except that the vesicles were present in the upper chamber (3.7 mg of protein per ml) before the addition of radioactive weak acid, and the reaction was initiated by adding ascorbate and PMS (31). In addition, valinomycin and nigericin were used at final concentrations of 1 μM each. Where indicated, the equilibrium distribution of [^{14}C]methylamine was studied using the same procedures in an effort to detect the presence of ΔpH (interior acid).

Measurement of $\Delta\Psi$. $\Delta\Psi$ (interior negative) was determined from the equilibrium distribution of [^{3}H]-TPP^+ (S. Ramos, L. Patel, and H. R. Kaback, manuscript in preparation) using flow dialysis (27, 30, 31, 38) or filtration (4, 35). Flow dialysis was performed as described above for measurement of ΔpH. For filtration assays, 20 mM ascorbate and 0.1 mM PMS were added to 1 ml of a cell or membrane suspension in 0.1 M KPO_4 at the desired pH plus 2 mM $MgSO_4$ in a 50-

ml Erlenmeyer flask that was gassed with oxygen. The reaction was initiated immediately thereafter by adding 10 μM [^{3}H]TPP$^+$ (400 mCi/mmol). Samples (100 μl) were withdrawn at given times, filtered through Millipore cellulose acetate filters (type EH, 0.5-μm pore size) (35), and immediately washed with 2 ml of 0.1 M KPO_4 at the same pH plus 2 mM $MgSO_4$ at room temperature.

Calculations. Concentration gradients established by intact cells or membrane vesicles were calculated from flow dialysis and filtration experiments using values of 4.74 (4) and 2.2 μl of internal volume per mg of protein for intact cells and membrane vesicles, respectively. The internal volume of isolated membrane vesicles was determined with ^{3}H-labeled water and [^{14}C]sorbitol (34). Internal pH was calculated from the distribution of given weak acids as described (31, 33, 36), and ΔpH was calculated from the difference between internal and external pH (alterations in external pH due to ascorbate oxidation were taken into account) (27). The electrical potential (ΔΨ) was calculated from the Nernst equation ($\Delta\Psi = 58.8 \log [TPP^+]_{in}/[TPP^+]_{out}$) using steady-state concentration values obtained from flow dialysis and filtration experiments as indicated. In all flow dialysis experiments with intact cells, corrections were made for changes in the volume of the upper chamber on addition of cell suspensions. The proton electrochemical gradient ($\Delta\bar{\mu}_{H^+}$) was calculated by substituting values of ΔΨ and ΔpH in equation (1).

Protein determinations. Protein was measured as described by Lowry et al. (17) using bovine serum albumin as a standard. Intact cells were lysed by incubation with 20 μg of lysozyme per ml at 30°C before the assay.

[^{3}H]TPP$^+$ (bromide salt) was synthesized by the Isotope Synthesis Group of Hoffmann-La Roche, Inc. under the direction of Arnold Liebman. Other isotopically labeled materials were purchased from New England Nuclear Corp. and Amersham/Searle. Valinomycin and carbonylcyanide-*m*-chlorophenylhydrazone were obtained from Calbiochem. Nigericin was the generous gift of John Wesley of Hoffmann-La Roche Inc.

RESULTS

Determination of ΔpH (interior alkaline). Figure 1A shows the results of a typical flow dialysis experiment for determination of ΔpH in *M. lysodeikticus* cells. At the inception of the experiment, [^{14}C]benzoic acid was added to the buffer in the upper chamber of the flow dialysis cell, and after about 4 min (i.e., fraction 7, or 12 ml), the level of radioactivity appearing in the dialysate reached a maximum and then decreased at a constant rate. When cells, ascorbate, and PMS were added (fraction 20), the level of radioactivity in the dialysate decreased rapidly to a new steady state. This effect is due both to dilution of [^{14}C]benzoate in the upper chamber on addition of the cell suspension and to uptake of the permeant weak acid by the cells. The contributions of both factors were easily resolved. Thus, the broken line in Fig. 1A represents the calculated dilution of [^{14}C]benzoate, and as described below, the quantity of benzoate taken up by the cells was readily ascertained when ΔpH was collapsed at the end of the experiment. In any event, addition of valinomycin resulted in additional accumulation of [^{14}C]benzoate (fraction 40), and when nigericin was added (fraction 50), the accumulated acid was released from the cells, and the level of radioactivity in the dialysate returned to precisely the level expected after correcting for the dilution of [^{14}C]benzoate in the upper chamber.

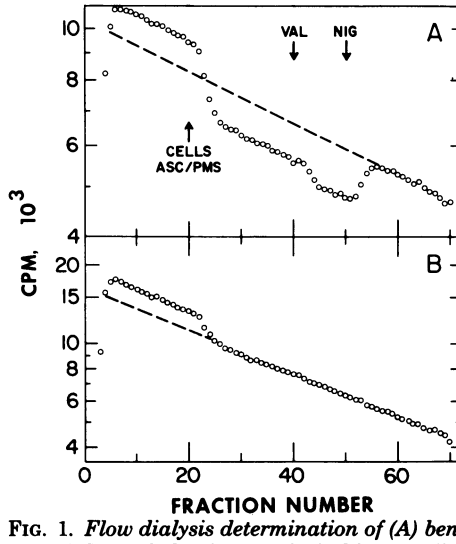

FIG. 1. *Flow dialysis determination of (A) benzoic acid and (B) methylamine uptake in M. lysodeikticus cells at pH 5.5 and 8.5, respectively. [7-^{14}C]benzoic acid (13.6 mCi/mmol) and [^{14}C]methylamine (49.6 mCi/mmol) were used at final concentrations of 55 and 20 μM, respectively. As shown by the arrows, cells, potassium ascorbate (ASC), PMS, valinomycin (VAL), and nigericin (NIG) were added to the upper chamber at concentrations given in the text. The dashed line indicates the calculated reduction in the concentration of benzoate and methylamine secondary to dilution of the radioactive solution in the upper chamber induced by the addition of the cell suspension at the inception of the experiment (first arrow). CPM, counts per minute.*

Using the equilibrium concentration of [^{14}C]benzoate in the dialysate after the addition of cells, ascorbate, and PMS (i.e., fractions 25 to 40), the amount of benzoate taken up by the cells could be determined. With this value, together with the external concentration of benzoate (broken line in Fig. 1A), its pK value (4.19), and the intracellular volume of 4.74 μl/mg of cell protein, it could be calculated that the intracellular pH was 7.10. Similarly, using the equilibrium concentration recovered in the dialysate after addition of valinomycin (i.e., fractions 45

to 50), it could be calculated that the intracellular pH was 7.31. Given the external pH values at those times, the ΔpH obtained before addition of valinomycin was 1.4 pH units (7.1 inside minus 5.7 outside), corresponding to −82 mV, and the ΔpH obtained after addition of valinomycin was about 1.56 pH units (7.31 inside minus 5.75 outside), corresponding to −92 mV. Similar results were obtained with [2-^{14}C]DMO and [1-^{14}C]acetate (data not shown).

Since it has been reported that the internal pH of intact *E. coli* becomes acid relative to the external medium at pH values of 8.0 and above (24), uptake of [^{14}C]methylamine by *M. lysodeikticus* was assayed at pH 8.5 (Fig. 1B). As shown, significant accumulation of the permeant amine was not observed in the presence of ascorbate and PMS. Similar results were also obtained at pH 8.0 (data not shown).

Although flow dialysis tracings will not be presented, internal pH and ΔpH in membrane vesicles from *M. lysodeikticus* were also determined from the equilibrium distribution of [7-^{14}C]benzoate, [2-^{14}C]DMO, [1-^{14}C]acetate, and [^{14}C]methylamine (see Fig. 3B). In these experiments, however, the reaction was initiated by addition of ascorbate and PMS (see Materials and Methods).

Effect of valinomycin on internal pH. As shown above (Fig. 1A), addition of valinomycin to *M. lysodeikticus* cells led to enhanced accumulation of benzoic acid, indicating that the ionophore increases the alkalinity of the intracellular space relative to the external medium. The results presented in Table 1 demonstrate that this effect was relatively constant from pH 5.5 to pH 7.0. Addition of valinomycin induced an increase in intracellular pH of about 0.2 pH units (i.e., −10 mV) over this range of external pH. The effect was somewhat less pronounced at pH 7.5 and was not observed at all at pH 8.0 and 8.5.

TABLE 1. *Effect of valinomycin on the internal pH*[a]

External pH	Internal pH	
	−VAL	+VAL
5.5	7.10	7.31
6.0	7.23	7.48
6.5	7.49	7.70
7.0	7.63	7.74
7.5	7.74	7.90

[a] Internal pH in *M. lysodeikticus* cells at external pH 5.5 to 7.5 was calculated from flow dialysis experiments (27, 30, 31) as described in Fig. 1 and 3 and in the text. Cells, potassium ascorbate, PMS, valinomycin (VAL), and nigericin were added as indicated in Fig. 1. The external pH values given represent the initial pH before addition of ascorbate-PMS. Although not shown, no ΔpH was detected at pH 8.0 and pH 8.5, and addition of valinomycin had no effect on the distribution of either benzoic acid or DMO at these pH values.

Determination of ΔΨ (interior negative). The data shown in Fig. 2 are representative results from experiments in which ascorbate/PMS-dependent TPP^+ accumulation by intact *M. lysodeikticus* cells was assayed by flow dialysis (Fig. 2A) and filtration (Fig. 2B). The steady-state level of TPP^+ accumulation increased significantly as external pH increased from pH 6.0 to 7.0 to 8.0, and essentially all of the accumulated lipophilic cation was released from the cells when valinomycin was added. The results are consistent with the conclusion that *M. lysodeikticus* cells generate a ΔΨ (interior negative) and that the magnitude of ΔΨ increases with external pH as reported recently for *E. coli* (40; Porter et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, p. 145). Moreover, comparison of ΔΨ values obtained using flow dialysis and filtration techniques (Table 2) demonstrates that both methods yield similar values with intact cells. In contrast, with isolated membrane vesicles, the ΔΨ values obtained using flow dialysis were approximately −10 to −20 mV higher than those obtained using the filtration assay.

Effect of external pH on internal pH, ΔpH, ΔΨ, and $\Delta\bar{\mu}_{H^+}$ in intact cells and membrane vesicles. Measurements of internal pH, ΔpH, ΔΨ, and $\Delta\bar{\mu}_{H^+}$ in cells and isolated membrane vesicles over a range of pH values from pH 5.5 to 8.5 are summarized in Fig. 3. In each instance, a given parameter was determined from the equilibrium distribution of permeant weak acids or [^{3}H]TPP^+ or both in the presence of ascorbate and PMS using flow dialysis. It is apparent that ΔpH varies markedly with external pH. At pH 5.5, a value of approximately −85 mV (i.e., 1.45 pH units) was obtained in both preparations; above pH 5.5, ΔpH decreased drastically and essentially linearly, and it was negligible at about pH 7.8 and above. Clearly, the variation in ΔpH with external pH is due to the maintenance of internal pH at a relatively constant value. Internal pH increased only slightly from pH 7.1 to pH 7.8 in intact cells (Fig. 3A) and from pH 7.1 to pH 7.5 in membrane vesicles (Fig. 3B) from an external pH of 5.5 to pH 7.5.

In intact cells (Fig. 3A), ΔΨ increased markedly with increasing external pH from −110 mV at pH 5.5 to −211 mV at pH 8.0 and above, and the increase was essentially linear from pH 5.5 to 7.0. In isolated membrane vesicles (Fig. 3B), the increase in ΔΨ with external pH was less

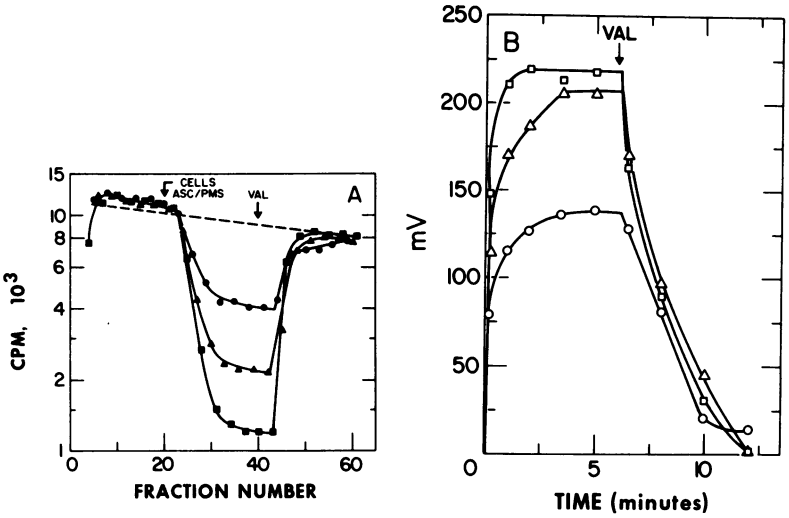

FIG. 2. *Determinations of [^{3}H]TPP$^+$ uptake in M. lysodeikticus cells by (A) flow dialysis and (B) filtration assays. The flow dialysis assays shown were carried out with cells suspended in 0.1 M KPO_4 buffer containing 2 mM $MgSO_4$, at pH 6.0 (●), 7.0 (▲), and 8.0 (■), as described previously (27, 30) and in the text. [^{3}H]TPP$^+$ (2,500 mCi/mmol) (11) was used at a final concentration of 10 μM. As shown by the arrows, cells, potassium ascorbate (ASC), PMS, and valinomycin (VAL) were added to the upper chamber of the flow dialysis cell at final concentrations of 0.36 mg of protein per ml, 20 mM, 0.1 mM, and 5 μM, respectively. The dashed line indicates the reduction in counts per minute (CPM) due to the dilution of [^{3}H]TPP$^+$ in the upper chamber by addition of cell suspension. Filtration assays were carried out with cells (0.29 mg of protein per ml; total volume, 1 ml) suspended in 0.1 KPO_4 buffer containing 2 mM $MgSO_4$, at pH 6.0 (○), 7.0 (△), and 8.0 (□). Potassium ascorbate and PMS were added to the cell suspension, and the reaction was initiated by addition of 10 μM [^{3}H]TPP$^+$. Samples were filtered and immediately washed with 2 ml of the same buffer. As indicated by the arrow, 5 μM valinomycin was added. The electrical potential (ΔΨ) was calculated as described in the text.*

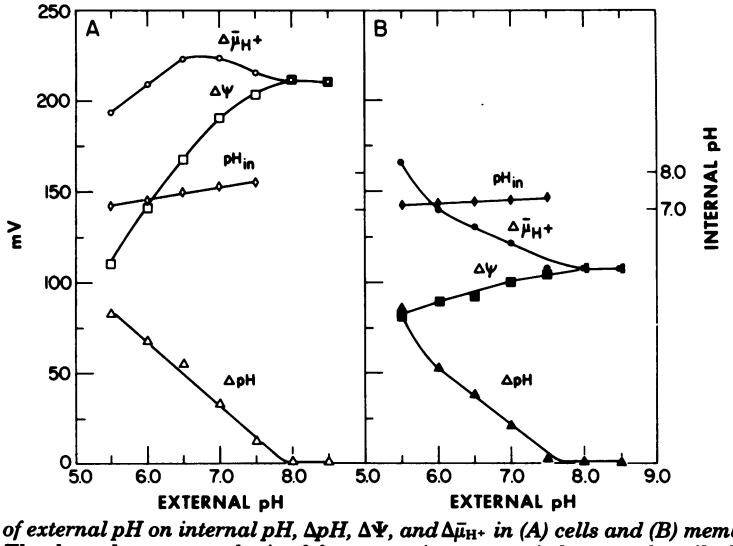

FIG. 3. *Effects of external pH on internal pH, ΔpH, ΔΨ, and $\Delta\bar{\mu}_{H^+}$ in (A) cells and (B) membrane vesicles of M. lysodeikticus. The data shown were obtained from experiments carried out as described in Fig. 1 and 2 and in the text. Internal pH values in cells (◇) and membrane vesicles (◆) were calculated from flow dialysis experiments with [7-^{14}C]benzoic acid (13.6 mCi/mmol), [2-^{14}C]DMO, or [1-^{14}C]acetate (in membrane vesicles only) at pH values of 5.5 to 7.5. ΔpH values in cells (△) and membrane vesicles (▲) were calculated in mV units as described in the text, after correcting the external pH for the changes induced by ascorbate-PMS oxidation (27). ΔΨ values in cells (□) were calculated using [^{3}H]TPP$^+$ from flow dialysis and filtration assays, and those for membrane vesicles (■) were calculated from flow dialysis assays, as described in Fig. 1 and 2 and in the text. $\Delta\bar{\mu}_{H^+}$ values in cells (○) and membrane vesicles (●) were calculated from ΔpH and ΔΨ values as described in the text.*

TABLE 2. *Measurements of ΔΨ (interior negative) in cells and membrane vesicles of M. lysodeikticus*[a]

External pH	Cells (mV)		Membrane vesicles (mV)	
	Filtration	Flow dialysis	Filtration	Flow dialysis
5.5	111	109	60	82
6.0	138	143	64	89
6.5	172	162	70	92
7.0	194	185	79	100
7.5	203	203	95	104
8.0	205	213	100	107
8.5	209	212	97	107

[a] ΔΨ (interior alkaline) in intact cells and membrane vesicles of *M. lysodeikticus* at given external pH values was calculated from flow dialysis (27, 30, 31) and filtration (4, 35) assays with 10 μM [^{3}H]TPP$^+$ as described in Fig. 2 and 3 and in the text.

pronounced, increasing from about −82 mV at pH 5.5 to about −107 mV at pH 8.0 and above. Although not shown, it is important that treatment of the vesicles with 70 μM dicyclohexylcarbodiimide for 30 min at 25°C (25) did not effect an increase in the magnitude of ΔΨ in the vesicles at pH 7.5. Thus, it seems unlikely that passive membrane permeability to protons is limiting for ΔΨ at relatively high pH in the vesicles.

In cells, $\Delta\bar{\mu}_{H^+}$ remained relatively constant over the pH range tested, increasing slightly from −193 mV at pH 5.5 to −223 mV at pH 6.5 to 7.5, and then decreasing to −210 mV at pH 8.0 and above (Fig. 3A). In membrane vesicles, on the other hand, $\Delta\bar{\mu}_{H^+}$ decreased from −166 mV at pH 5.5 to −107 mV at pH 8.0 and above (Fig. 3B).

DISCUSSION

In the experiments presented in this paper, ΔpH (interior alkaline) and ΔΨ (interior negative) were determined in *M. lysodeikticus* and membrane vesicles prepared from this organism by measuring the equilibrium distribution of weak acids and the permeant lipophilic cation [^{3}H]TPP$^+$. It is apparent from the data that respiring cells and vesicles generate a ΔΨ and a ΔpH and that external pH dramatically affects the partitioning of $\Delta\bar{\mu}_{H^+}$ into ΔpH and ΔΨ in both systems. These results are similar to those reported previously with *E. coli* cells (24, 40) and membrane vesicles from both *E. coli* (27, 30) and *S. typhimurium* (38).

In intact *E. coli*, apparent ΔΨ values of −73.5 and −88 mV were reported at external pH values of 6.0 and 8.0, respectively, when $^{86}Rb^+$ distribution (in the presence of valinomycin) was determined by centrifugation (24), whereas ΔΨ values of about −95 and −150 mV, respectively, were reported using TPP$^+$ distribution and flow dialysis (40) as well as intracellular microelectrodes (Porter et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K_2, p. 145). As determined from DMO distribution, ΔpH (interior alkaline) values of similar magnitude were found with intact *E. coli* using either centrifugation (24) or flow dialysis (40), whereas in *E. coli* membrane vesicles, ΔpH can be determined only by flow dialysis because both filtration and centrifugation lead to essentially complete loss of accumulated permeant weak acids (30). Taken as a whole, these studies emphasize the importance of the technique used to measure the parameters of $\Delta\bar{\mu}_{H^+}$ and highlight the virtues of flow dialysis in this respect.

In intact *M. lysodeikticus*, ΔpH (interior alkaline) was maximal and ΔΨ (interior negative) was minimal at pH 5.5, and when external pH was increased, ΔpH decreased and ΔΨ increased (Fig. 3A). Thus, the cells maintain a relatively constant $\Delta\bar{\mu}_{H^+}$ over a wide range of external pH values, a situation that is physiologically advantageous. Moreover, this phenomenon appears to be common among a variety of bacterial species, since similar effects have been reported for the extreme halophile *Halobacterium halobium* (1), and the facultative aerobe *E. coli* (40). Despite the similarity in the effect of external pH on the partitioning of $\Delta\bar{\mu}_{H^+}$ into its components, there are significant differences in the magnitude of ΔpH and ΔΨ in different bacteria. In *M. lysodeikticus*, ΔpH is relatively low (see Fig. 3A, where the maximum value of ΔpH observed at pH 5.5 is about −85 mV), whereas in *E. coli* (24, 40) and *H. halobium* (1), maximum ΔpH values of about −120 mV are observed. On the other hand, ΔΨ in *M. lysodeikticus* is relatively high (see Fig. 3A, where the maximum value of ΔΨ observed at pH 8.0 and above is −211 mV) in comparison to *E. coli* (−150 mV at pH 8.0 and above) (40) and *H. halobium* (maximum values range from −115 to −140 mV) (1). Furthermore, in *M. lysodeikticus*, ΔΨ is the major component of $\Delta\bar{\mu}_{H^+}$ at all external pH values tested, and $\Delta\bar{\mu}_{H^+}$ is relatively constant as a function of pH (Fig. 3A), whereas in *E. coli* (24, 40) and *H. halobium* (1) ΔpH is the major component of $\Delta\bar{\mu}_{H^+}$ at acidic pH and $\Delta\bar{\mu}_{H^+}$ is maximal at acidic pH. Clearly, these differences may be important when studying the energetics of various $\Delta\bar{\mu}_{H^+}$-dependent processes in different organisms.

The $\Delta\bar{\mu}_{H^+}$ generated in respiring *M. lysodeikticus* is sufficient thermodynamically to drive the accumulation of solutes against a large concentration gradient. Specifically, $\Delta\bar{\mu}_{H^+}$ values in the range of −200 mV (Fig. 3A) can account for gradients of about 2,000-fold, and distribution

ratios of this magnitude have been reported for phosphate and arsenate in *M. lysodeikticus* under certain conditions (5). Moreover, the effects of valinomycin and nigericin on the steady-state levels of phosphate and arsenate accumulation at acid and alkaline pH (5) and the effects of these ionophores on ΔpH and ΔΨ at the same extremes of pH are consistent with the argument that phosphate and arsenate transport are coupled primarily to ΔpH at pH 5.5 and to ΔΨ at pH 8.0 and above, as suggested initially for the transport of certain organic anions in *E. coli* membrane vesicles (28, 29). Thus, phosphate and arsenate transport in *M. lysodeikticus* is inhibited by nigericin and stimulated by valinomycin at pH 5.5, whereas at pH 8.0, nigericin has essentially no effect and valinomycin becomes a potent inhibitor (5). Clearly, these findings are consistent with the following observations: (i) nigericin collapses ΔpH at pH 5.5 (Fig. 1A and data not shown); (ii) a significant ΔpH is not detected at pH 7.8 and above (Fig. 3A); (iii) valinomycin collapses ΔΨ at pH 5.5 and at pH 7.5 (Fig. 2); and (iv) valinomycin causes an increase in ΔpH at relatively acid external pH values (Fig. 1A and Table 1).

The maximum value for ΔpH at pH 5.5 in the vesicles was similar to that observed in intact cells at the same pH, and the response of ΔpH to external pH was similar in both systems. Moreover, the ΔΨ measured in cells and vesicles was comparable at pH 5.5. However, as external pH was increased, the ΔΨ measured in the vesicles did not increase to anywhere near the extent observed in the intact cell. As a result, there was a significant decrease in $\Delta\bar{\mu}_{H^+}$ with increasing external pH in the vesicles. This apparent difference has also been observed in *E. coli* cells and vesicles (compare reference 40 with references 27 and 30), and it is critical to the putative increase in H^+-solute stoichiometry with certain transport substrates at relatively alkaline pH in the vesicles (cf. references 29 and 40). In considering this problem, the following points are noteworthy: (i) intracellular recording and TPP^+ distribution measurements in *E. coli* giant cells yield similar values for ΔΨ (Porter et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K2, p. 145), and it is clear that ΔΨ varies with pH in much the same manner as observed here with *M. lysodeikticus*; (ii) the response of ΔΨ to increasing pH in membrane vesicles from *E. coli* (27, 30) and *S. typhimurium* (38) is similar to that observed with *M. lysodeikticus* vesicles, and essentially the same results are obtained in *E. coli* vesicles using quenching of 3,3'-dipropylthiodicarbocyanine fluorescence (37, 39) to measure ΔΨ (interior negative) (Ramos et al., manuscript in preparation). Given these observations, it is apparent that there may be a real difference in the magnitude of ΔΨ in cells and vesicles at high pH that is not due to a technical artifact.

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