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Generation of a Proton Motive Force by the Anaerobic Oxalate-Degrading Bacterium *Oxalobacter formigenes*

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The generation of transmembrane ion gradients by *Oxalobacter formigenes* cells metabolizing oxalate was studied. The magnitudes of both the transmembrane electrical potential ($\Delta\psi$) and the pH gradient (internal alkaline) decreased with increasing external pH; quantitatively, the $\Delta\psi$ was the most important component of the proton motive force. As the extracellular pH of metabolizing cells was increased, intracellular pH increased and remained alkaline relative to the external pH, indicating that *O. formigenes* possesses a limited capacity to regulate internal pH. The generation of a $\Delta\psi$ by concentrated suspensions of *O. formigenes* cells was inhibited by the K^+ ionophore valinomycin and the protonophore carbonyl cyanide-*m*-chlorophenylhydrazone, but not by the Na^+ ionophore monensin. The H^+ ATPase inhibitor *N,N′*-dicyclohexyl-carbodiimide inhibited oxalate catabolism but did not dissipate the $\Delta\psi$. The results support the concept that energy from oxalate metabolism by *O. formigenes* is conserved not as a sodium ion gradient but rather, at least partially, as a transmembrane hydrogen ion gradient produced during the electrogenic exchange of substrate (oxalate) and product (formate) and from internal proton consumption during oxalate decarboxylation.

Oxalic acid and its salts are widely distributed and are normal components of human and animal diets, sometimes at toxic levels (25). In 1980, Dawson et al. (19) isolated and described a ruminal anaerobe capable of utilizing energy from the decarboxylation of oxalate for growth. Similar strains of this organism, *Oxalobacter formigenes*, have since been isolated from the large bowels of various nonruminant herbivores (4, 18), from human feces (2), and from aquatic sediments (39). *O. formigenes* isolates are neutrophilic gram-negative, nonmotile, non-spore-forming rods which utilize oxalate as the sole energy-yielding substrate.

Oxalic acid is degraded by *O. formigenes* to approximately equimolar amounts of CO_2 and formate (19). Oxalate metabolism by concentrated cell suspensions of *O. formigenes* is accompanied by the consumption of 1 mol of H^+ per mol of oxalate degraded (4). The decarboxylation appears to occur inside the cell (8) after oxalate is translocated across the cell membrane via an oxalate-formate antiporter (5, 38) and after activation to oxalyl-coenzyme A (CoA) by an acyl-CoA transferase (6, 7).

The conservation of energy from oxalate degradation by most bacteria occurs via aerobic or anaerobic respiratory processes. *Pseudomonas oxalaticus* (35), *Methylobacterium extorquens* (10), and *Bacillus oxalophilus* (43) decarboxylate oxalate (via oxalyl- and formyl-CoA) to formate and CO_2. Formate oxidation is coupled to ATP generation by electron transport phosphorylation with O_2 as the terminal electron acceptor. *Desulfovibrio vulgaris* subsp. *oxamicus* conserves energy from oxalate degradation through dissimilatory sulfate reduction; this process is also thought to occur via the oxidation of formate (34). *Clostridium thermoaceticum* converts oxalate to acetate and CO_2. The energetics of this process are likely linked to the generation of reductant which is used to synthesize acetate via the acetyl-CoA (Wood) pathway (3, 17). In contrast to production in these organisms, formate is produced as an end product by *O. formigenes*, as well as by *Oxalobacter vibrioformis* (20) and *Oxalophagus oxalicus* (*Clostridium oxalicum*) (16, 20), and energy for growth from oxalate catabolism must therefore be conserved by some other means. The yield of ATP for oxalate-dependent growth by *O. formigenes* is expected to be low, in accordance with the free-energy change associated with oxalate hydrolysis to formate and CO_2 ($\Delta G^{\circ\prime} = -26.7$ kJ/mol [41]). The low growth yields found for *O. formigenes* (1.0 to 1.1 g of cells per mol of oxalate degraded [19, 39]) are consistent with this.

As a mechanism whereby *O. formigenes* generates ATP has not yet been determined, measurements of ion gradients were made in this study as a step toward gaining an understanding of the means by which this unique organism conserves energy for growth.

MATERIALS AND METHODS

Culture methods. *O. formigenes* OxB was grown by the anaerobic culture methods of Hungate, as modified by Bryant (13) and Holdeman et al. (26). *O. formigenes* cells were grown in a 14-liter fermentor under CO_2 at 39°C in medium B (initial pH, 6.8) as previously described (4). The cells were harvested in the late logarithmic phase of growth, washed twice, and resuspended either in anaerobic dilution solution (14) or in an anaerobic buffer. The anaerobic buffer [100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 10 mM Mg_2SO_4] was prepared by boiling and then cooling the solution under N_2. Dithiothreitol (5 mM) was added, and the pH was adjusted to 6.0 with 100 mM tris(hydroxymethyl)aminomethane (Tris). The gas phase was 100% CO_2 for the anaerobic dilution solution and 100% N_2 for the anaerobic buffer.

Internal cell volume. Internal cell volumes were determined as described by Rottenberg (37), by using ^3H_2O as a measure of total volume and [^{14}C]sucrose as the marker for extracellular volume. Cell suspensions (5 or 10 ml) were incubated at 39°C in a jacketed polypropylene titration vessel (Radiometer, Copenhagen, Denmark). Samples were continuously stirred and bubbled with N_2 to maintain anaerobic conditions. Cells were separated from the incubation medium by centrifugation through a mixture of silicone oils (550 and 556; Dexter Hysol, Olean, N.Y.); the appropriate oil density was determined for each sample cell concentration. Samples (200 to 400 μl) were layered over 300 μl of silicone oil. After centrifugation (12,000 × *g*, 5 min), 50 to 200 μl of the supernatant was placed in a scintillation vial and the tip of the microcentrifuge tube containing

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the pellet was cut off into a separate scintillation vial. One milliliter of perchloric acid (1 M) was added to each vial, and the pellet was dissolved by using a vortex mixer. Biofluor (10 ml; New England Nuclear Corp., Boston, Mass.) was added to each vial, and the samples were counted for radioactivity. Nonenergized cells were cells which had been incubated for 10 min prior to the addition of oxalate. Oxalate [usually $(NH_4^+)_2$-oxalate, 20 to 100 mM] was added to energize the cells, and samples were removed at maximum transmembrane electrical potential ($\Delta\psi_{max}$) (as monitored with the tetraphenylphosphonium [TPP] electrode). Internal cell volume was calculated from the means for triplicate samples.

To study the effects of pH and oxalate addition on the internal cell volume, transmembrane electrical potential ($\Delta\psi$), and proton motive force (Δp), cells were added to 0.1 M anaerobic buffer containing 10 mM KCl, 5 mM dithiothreitol, 10 mM $MgSO_4$, and 50 μM chloramphenicol. The anaerobic buffers were adjusted to the appropriate pH by using combinations of 0.1 M MES, 0.1 M 3-*N*-morpholinopropanesulfonic acid (MOPS), 0.1 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 0.1 M Tris.

Δp measurements. Δp was determined according to the method of Rottenberg (37). The Δψ was calculated from estimates of intracellular concentrations of the lipophilic cation TPP. These estimates were made either by adding [^{3}H-phenyl] TPP to cells which were subsequently separated from the supernatant fluid by the silicone oil method described above or from measurements of changes in extracellular TPP concentration made with a TPP-specific electrode constructed as described by Kamo et al. (27). The internal reference solution for the electrode was 1 mM TPP; prior to use, each electrode was placed in 1 mM TPP for 12 to 16 h. New electrodes were used for each set of experiments. The reference electrode was a calomel electrode filled with saturated KCl, which also served as the reference for the pH electrode. TPP electrodes were calibrated prior to use to ensure that the electrode response was linear (millivolts versus log [TPP]) over the concentration range used. The TPP electrode was sensitive, and the response was proportional to the TPP concentration at levels of 0.8 to 10 μM TPP.

Estimates of TPP binding were made by incubating cells for 1.7 h in the presence of 1% toluene (37°C, pH 6.5). By using [^{3}H]TPP to calculate Δψ, the values for nonenergized, toluene-treated cells were similar to values calculated for nonenergized cells that had not been exposed to toluene; thus, values calculated for nonenergized cells were assumed to represent TPP binding. The Δψ values for energized cells were corrected by subtracting the counts associated with nonenergized cells.

Internal pH was determined from the distribution of [^{14}C]benzoic acid across the cell membrane (28). External pH was monitored with a pH electrode, and pH was held constant by the automatic addition of HCl or oxalic acid (Recording Titration System RTS822; Radiometer). Cells were separated from suspending fluids by centrifugation through silicone oil and processed as described for internal-volume measurements.

Measurements of internal cellular pH based on ^{31}P nuclear magnetic resonance (NMR) measurements at 121.5 MHz with a Brucker WM-300 spectrometer were conducted essentially as described by Ugurbil et al. (42). Freshly harvested cells were suspended in buffer {1 g [wet weight] in 3 ml of 0.1 M buffer [MES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), or MOPS at pH 6.0, 6.5, or 7.0, respectively} plus 10 mM NaCl and 10% D_2O. Cell suspensions with and without added KH_2PO_4 (0.25 to 1.3 μM) were maintained in 10-mm NMR tubes under argon gas during analysis at 23°C. Calculations of pH values were made from a standard curve prepared by using spectra obtained with the above-described buffers plus KH_2PO_4 and with 85% phosphoric acid in a capillary tube used as an external standard.

Δp was calculated as follows: $\Delta p = \Delta\psi - Z\Delta pH$, where $Z = 62$ at 39°C. The results obtained in this study indicate that internal cell volume changed with cellular metabolic state and experimental conditions; calculations of Δp were therefore made with estimates of internal cell volume that compensated for these volume changes.

The stoichiometry of proton consumption by cell extracts of *O. formigenes* in relation to oxalate metabolism was measured with pH held constant by the automatic addition of HCl (Recording Titration System). The cell extracts were prepared by using a French pressure cell (American Instruments Co., Silver Spring, Md.), and reaction mixtures were similar to those previously described (4, 6) with reactions under N_2 and with CoA supplied as the succinyl ester.

Stock solutions of ionophores and *N*,*N*′-dicyclohexyl-carbodiimide (DCCD) were prepared in absolute ethanol; no effects of solvent on Δψ generation were observed.

Protein was measured by the method of Lowry, as modified by Peterson (33). Bovine serum albumin was used as the standard.

All values presented represent the results of triplicate experiments.

Chemicals. [^{14}C]sucrose (40 mCi/mmol), ^3H_2O (225 mCi/g), [^{14}C]benzoic acid (10.7 mCi/mmol), and [^{3}H]TPP (35.5 Ci/mmol) were purchased from New England Nuclear. Valinomycin, nigericin, carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP), and DCCD were obtained from Sigma Chemical Company (St. Louis, Mo.). Monensin was obtained from Eli Lilly and Company (Indianapolis, Ind.).

RESULTS

Internal cell volume. The values obtained for internal volumes of cells that were energized by the addition of oxalate were smaller than those calculated for nonenergized cells at all pH values tested. To study the effects of pH and oxalate addition on internal cell volume, cells (2.4 mg of protein per ml) were added to 0.1 M buffer containing 45 μCi of ^3H_2O and 4.8 μCi of [^{14}C]sucrose in a total volume of 10 ml (as described in Materials and Methods). Constant pH was maintained by the addition of 0.1 N HCl. For external pH values of 5.00, 5.85, and 6.90, the internal cell volumes calculated for nonenergized cells averaged 2.14 ± 0.05, 2.06 ± 0.06, and 1.92 ± 0.06 μl/mg of protein, respectively. At $\Delta\psi_{max}$ [as measured with the TPP electrode after the addition of 20 mM $(NH_4^+)_2$-oxalate], the corresponding volumes for the same cells averaged 1.9 ± 0.07, 1.86 ± 0.06, and 1.71 ± 0.02 μl/mg of protein.

Measurement of electrical potential. The values obtained for transmembrane electrical potential with [^{3}H]TPP differed somewhat from values determined with the TPP-specific electrode; however, relative changes in the magnitude of Δψ after the addition of oxalate to nonenergized cells were similar. Utilizing data obtained from three experiments in which external pH varied from 5 to 7 and either MES or phosphate buffers were used, the changes in Δψ measured with [^{3}H]TPP were 83, 70, and 22 mV, respectively. The corresponding changes in Δψ from TPP electrode measurements that were recorded simultaneously were 90, 73, and 24 mV, respectively.

Effect of extracellular pH on Δp generated by *O. formigenes* cells. The intracellular pH of *O. formigenes* cells was determined for cells maintained at pH values ranging from 5.0 to 8.0. The external pH was held constant by the automatic addition of 0.1 N HCl. With suspensions of washed nonenergized cells, the ΔpH (internal alkaline) remained relatively constant (0.42 ± 0.03) at pH 6.0 to 8.0. At pH 5.0, the ΔpH for nonenergized cells was 0.59. When these cells were energized by the addition of oxalate, the internal pH changed but was kept alkaline relative to the pH of the external medium (Fig. 1a). The magnitude of the ΔpH, however, generally decreased as the external pH was increased from 5.0 to 8.0. At external pH values of 5.0, 6.0, 7.0, and 8.0, the values for ΔpH were 0.64, 0.32, 0.35, and 0.16, respectively. Although it is difficult to accurately measure small differences in ΔpH by using ^{31}P NMR (28), measurements of pH gradients made by this method were similar to those found with the [^{14}C]benzoate probe. With nonenergized cells, measurements of intracellular pH (as determined with the ^{31}P NMR method) were 6.43 when cells were suspended at pH 6.02 in 0.1 M MES and 6.77 when cells were suspended at pH 6.44 in 0.1 M PIPES. The corresponding values calculated for internal pH by using [^{14}C]benzoate were 6.31 at a pH_{out} of 6.03 and 6.7 at a pH_{out} of 6.44. Reliable NMR measurements could be made only with nonenergized cells because with the concentrated cell suspensions needed for the NMR determinations, substrate levels required to maintain energized cells during observation periods in the NMR were high and rates of proton consumption under these conditions were such that a stable pH could not be maintained long enough to collect the needed NMR data.

In energized, nongrowing *O. formigenes* cells, the values for Δψ were greatest at pH 5.0 and decreased with increasing external pH (Fig. 1b). When oxalate was added to nonenergized cells, the Δψ increased by 80, 44, 31, and 8 mV at external pH values of 5.0, 6.0, 7.0, and 8.0, respectively. The magnitude of the Δp in energized cells generally decreased with increasing external pH.

The effect of pH on the rate of proton consumption during

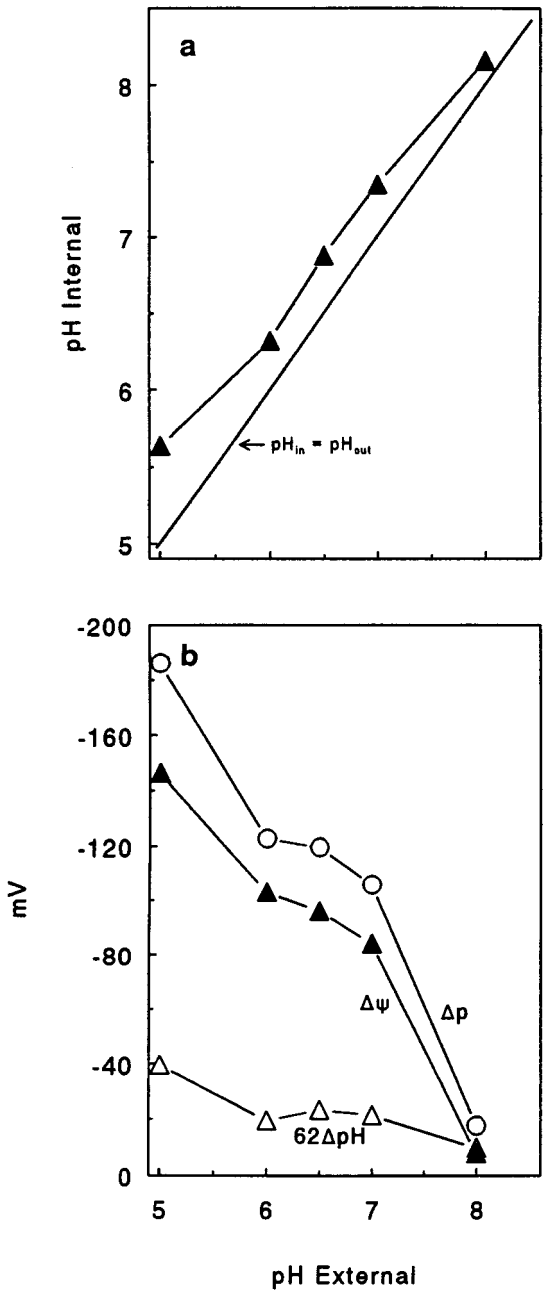

FIG. 1. (a) Internal pH versus external pH values of oxalate-energized cells. Cells (4.5 mg of protein per ml; total volume, 5 ml) were incubated in 0.1 M anaerobic buffer as described in Materials and Methods. [^{14}C]benzoic acid (0.75 μCi) was used to calculate internal pH (pH_{in}). Cells were energized by the addition of $(NH_4^+)_2$-oxalate (20 mM). pH_{out}, external pH. (b) Δp, Δψ, and ΔpH for energized cells at various external pH values. Cells were incubated as described for panel a. [^{14}C]benzoic acid (0.75 μCi) and [^{3}H]TPP (17 μCi) were used to calculate ΔpH and Δψ, respectively. Each datum point represents the mean of three measurements.

oxalate degradation was examined by using cell extracts. With proton consumption as the index of activity, the pH optimum for oxalate degradation by cell extracts from *O. formigenes* was approximately 6.4 (Fig. 2a). The 1:1 stoichiometry of proton consumption with oxalate degradation in the cell-free system (Fig. 2b) was the same as that found with suspensions of whole cells (4).

Effects of ionophores and DCCD on Δψ generation. Incubation of nonenergized cells with the ionophore valinomycin or CCCP inhibited the abilities of these cells to generate a Δψ and to metabolize oxalate (data not shown). When added to energized cells, both the potassium ionophore valinomycin (Fig. 3a) and the protonophore CCCP (Fig. 3b) caused a release of TPP from the cells. Alkalinization of the reaction medium stopped immediately after CCCP was added (Fig. 3b); proton consumption was not greatly affected by the addition of valinomycin at 1.0 or 2.5 μM (Fig. 3a). The addition of nigericin (final concentration, 50 μM) which effects the electroneutral exchange of K^+ and H^+, did not cause a release of TPP or decrease the rate of proton consumption by cells which had

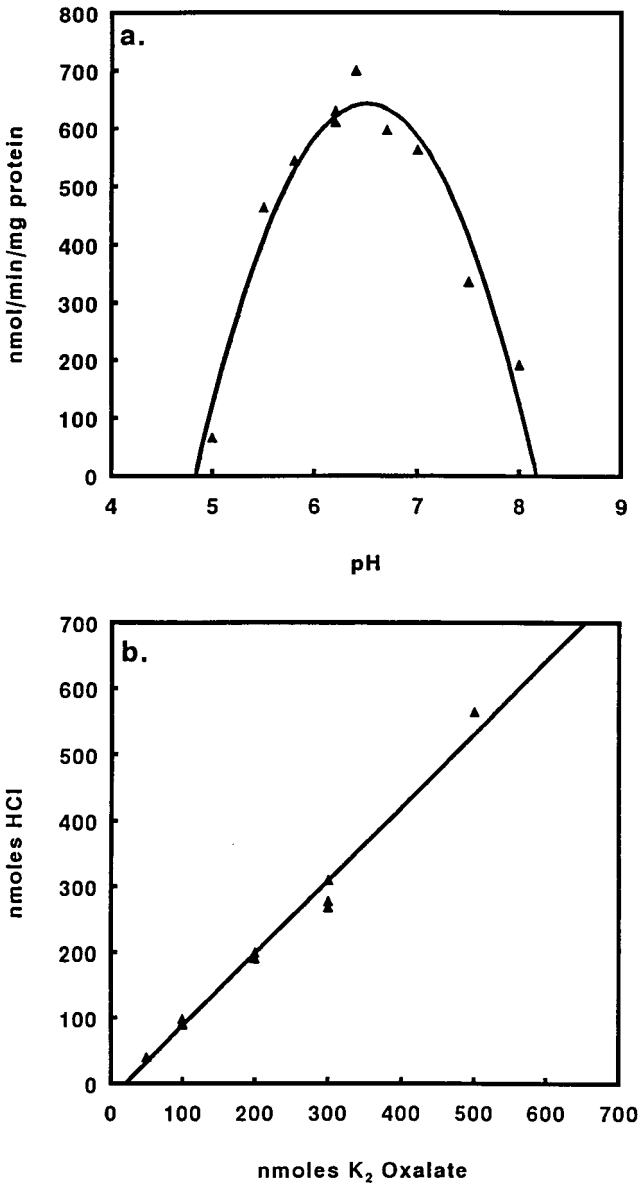

FIG. 2. (a) Rate of proton consumption during oxalate decarboxylation by cell extracts as affected by pH. The reaction mixture (at 37°C, 100% N_2 gas phase, 2 ml) contained thiamine PP_i (0.05 mM), succinyl CoA (0.2 mM), Mg^{2+} (5 mM), and cell extract (0.1 mg of protein), and the reaction was started by adding 100 nmol of K_2-oxalate. (b) Stoichiometry of proton consumption during oxalate decarboxylation by cell extracts. Reaction mixtures were as described for panel a, with the pH stat set at 6.0.

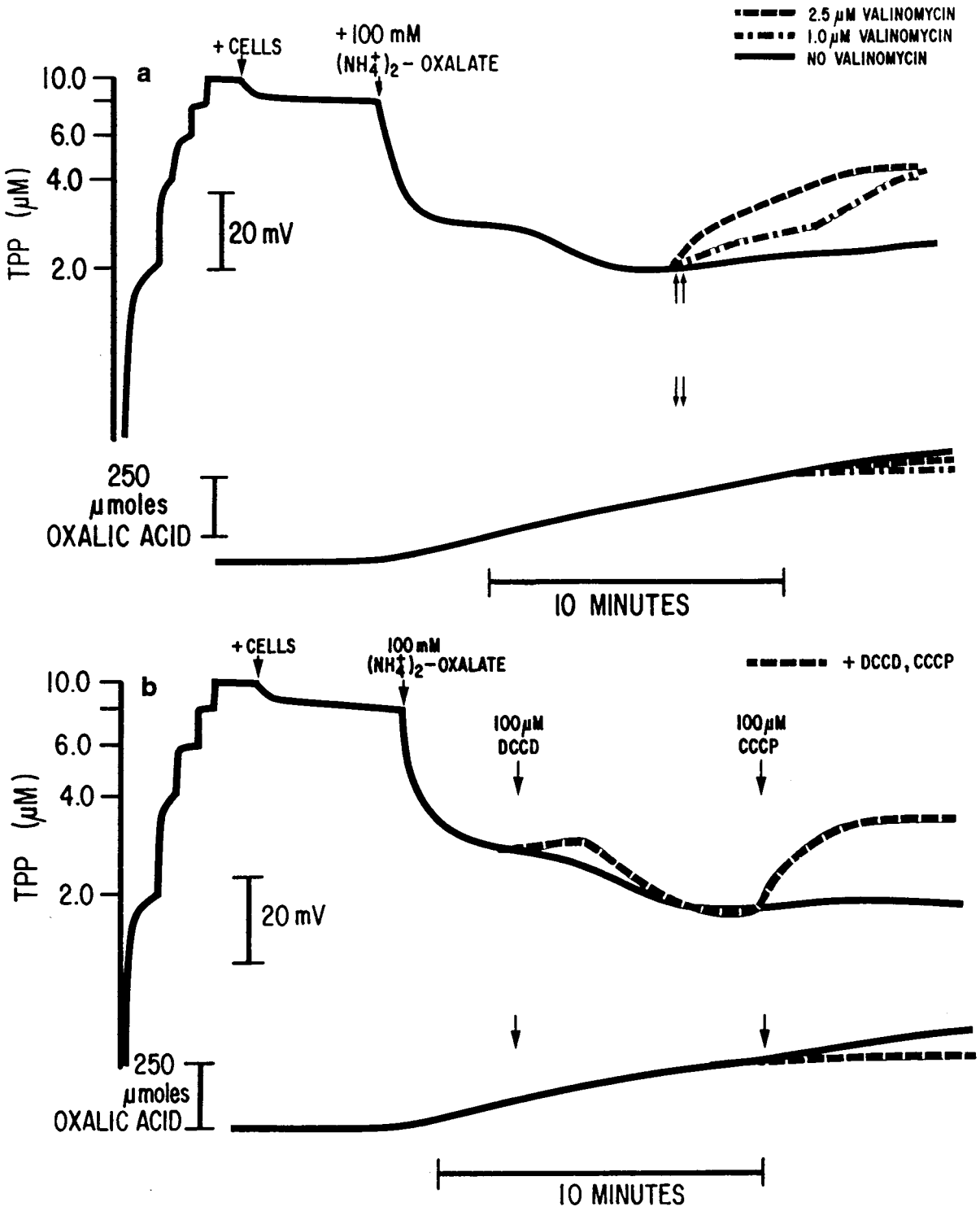

FIG. 3. Uptake of TPP by energized cells as monitored with the TPP electrode. The TPP electrode was calibrated by successive additions of TPP (to a final concentration of 10 μM). Cells (4.5 mg of protein per ml) were added to MES buffer (see Materials and Methods) containing 10 mM KCl, 2 mM EDTA, and 50 μM chloramphenicol. $(NH_4^+)_2$-oxalate (to 100 mM) was added to energize the cells. The bottom sets of curves represent oxalic acid (0.25 M) addition to maintain pH at 6.0. (a) Effect of valinomycin (arrows indicate valinomycin addition). (b) Effects of DCCD (100 μM) and CCCP (100 μM).

been energized with oxalate. However, the magnitude of the $\Delta\psi$ did not increase after addition of nigericin (Fig. 4a). The sodium ionophore, monensin, at concentrations up to 4.0 μM (Fig. 4b) did not decrease the magnitude of the transmembrane electrical potential generated by energized cells. The rate of proton utilization was also unaffected by monensin.

Although the $\Delta\psi$ generated by energized cells was not dissipated by the ATPase inhibitor DCCD at a concentration of

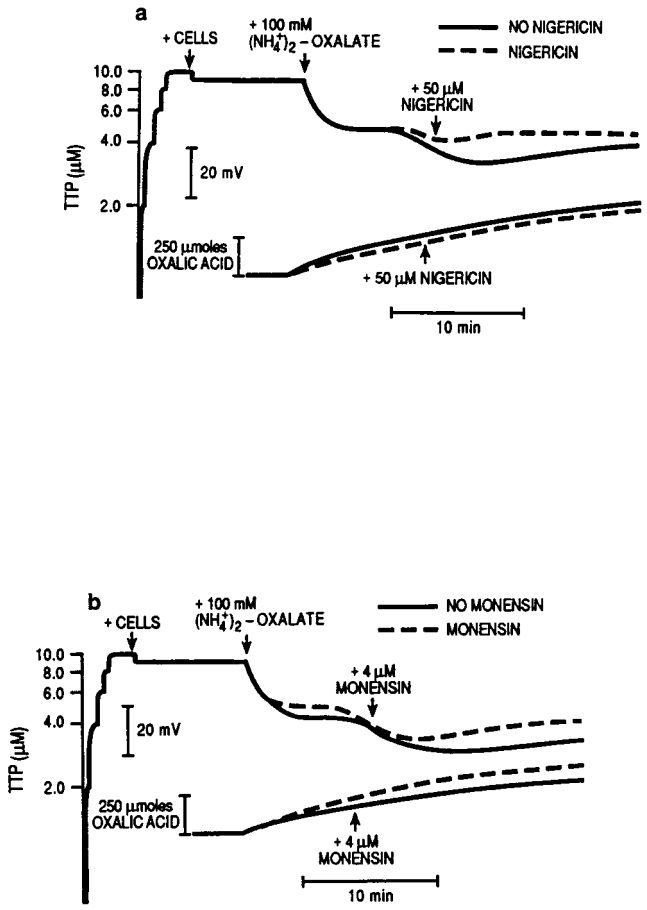

FIG. 4. Uptake of TPP by energized cells as monitored with the TPP electrode. The experiment was performed as described in the legend to Fig. 3. (a) Effect of nigericin (50 μM). (b) Effect of monensin (4.0 μM).

100 μM (Fig. 3b), this amount of DCCD (about 22 nmol/mg of protein) did temporarily stop the uptake of TPP by cells without having a detectable effect on the rate of proton consumption. Proton consumption was, however, inhibited when the DCCD was added to give a final concentration of 500 μM (data not shown).

DISCUSSION

Although a number of species of oxalate-degrading bacteria have been described (3, 25), information about the biochemistry of microbial oxalate degradation is mainly that for *P. oxalaticus* and *O. formigenes*. With *P. oxalaticus*, reactions that are coupled to formate oxidation are considered to provide the energy that is needed for growth (35). As formate is not oxidized by *O. formigenes*, some other mechanism for energy conservation must operate. We have thus examined the generation of transmembrane ion gradients when oxalate is degraded.

Our first hypothesis was that oxalate decarboxylation was coupled to the generation of a sodium gradient. This was based on results obtained with several species of bacteria (15, 21, 23) in which membrane-bound decarboxylase reactions are coupled to an active transport of Na^+ through the cell membrane. The energy of the decarboxylation reaction is thus converted to an Na^+ ion gradient, which then can drive an Na^+-activated ATPase. It was proposed that all the energy needed for life by *Propionigenium modestum* is obtained by decarboxylating succinate to produce propionate and CO_2 (24).

Our results do not support the hypothesis of sodium gradient as an energy conservation mechanism for *O. formigenes*. Growth of *O. formigenes* was not impaired when all sodium was deleted from a chemically defined culture medium or in the presence of monensin at concentrations up to 100 μM (unpublished observations). Furthermore, we also found that oxalate metabolism with production of an electrochemical gradient across cell membranes was not appreciably inhibited by monensin. Addition of the proton conductor CCCP to energized cells, however, caused a rapid dissipation of the electrochemical gradient and inhibited further proton consumption (Fig. 3b). This is in contrast to results obtained with *P. modestum* (24), in which decarboxylation of methylmalonyl-CoA was coupled to the generation of a sodium gradient and formation of that gradient was strongly inhibited by monensin but not by the uncoupler carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP). Furthermore, the above-mentioned sodium-translocating decarboxylases are membrane-bound biotin enzymes, whereas the oxalyl-CoA decarboxylase of *O. formigenes* was readily separated from membrane fractions of the cell and was not inhibited by avidin (6).

The internal volume of cells of *O. formigenes* that were supplied with oxalate (i.e., energized) was about 89% of the volume measured with cells that were not supplied with oxalate (i.e., nonenergized), and there was a trend toward increasing cell volumes as extracellular pH was decreased. Most estimates of Δp have used a constant value for internal cell volume for a given organism, but the decrease in cellular volume when substrate was supplied to *O. formigenes* was a consistent finding in our experiments. A loss of cell volume was also observed when substrate (glucose) was supplied to *Mycoplasma gallisepticum* (36), while with *Escherichia coli* incubation with energy-yielding substrates led to increases in cell volume (1, 30). With *Leuconostoc lactis*, intracellular volume decreased with a decrease in extracellular pH (22); thus, the changes observed with *O. formigenes* were in the opposite direction. We do not have an explanation for the observed changes in cell volume.

Results obtained earlier (4) showed that when whole cells metabolize oxalate, the culture or cell suspension medium becomes alkalinized and that the ratio of protons consumed to oxalate metabolized is approximately 1:1. Results obtained in the present study with cell extracts gave the same stoichiometry as was obtained with whole cells. This is an indication that the amount of protons consumed by intact cells was essentially independent of membrane function and was due to the proton-consuming reaction inside the cells ($^-$OOC-COO-CoA + H^+ $\rightarrow$ CO_2 + HCOO-CoA). The pH optimum in cell extracts, with proton consumption as the index of activity, agreed with the optimum of pH 6.7 obtained for purified oxalyl-CoA decarboxylase (determined by using rates of product formation) (6).

Maintenance of internal pH of *O. formigenes* cells within a narrow range did not appear to be essential for oxalate metabolism (as measured by alkalinization of the buffer) or for the generation of a Δp (Fig. 1b); thus, the ability of this organism to regulate internal pH appears to be limited compared with that of other neutrophiles (i.e., *E. coli* [12]). The internal pH of acid-tolerant anaerobes, such as *Streptococcus* (40), *Clostridium* (9), and *Lactobacillus* (29) spp., decreases during growth concomitantly with decreasing external pH. In contrast to the situation with these organisms, the ΔpH of *O. formigenes* cells began to increase below an external pH of 6, suggesting that internal pH is more highly regulated than it is with the acid-tolerant anaerobes.

Our results provide strong evidence that the metabolism of

oxalate by whole cells of *O. formigenes* leads to the generation of a Δp. Further, the data indicate that the $\Delta\psi$ was quantitatively the most important component of the Δp (at pH values below 8). Measurements showing that the addition of CCCP to energized cells resulted in a rapid dissipation of the $\Delta\psi$ and further inhibited proton consumption (Fig. 3b) also suggest that the proton gradient is an important component of the ion electrochemical gradient.

The exchange of oxalate and formate across membranes of *O. formigenes* has been shown to be an electrogenic reaction (5) mediated by a membrane protein, OxlT (32, 38). This antiport of divalent substrate and univalent product results in the development of an internally negative membrane potential. The internal consumption of protons during the reductive decarboxylation of oxalate (Fig. 2b) supports a model (31) in which oxalate degradation is coupled to energy conservation through an indirect proton pump consisting of OxlT and oxalate decarboxylase. The inhibition of $\Delta\psi$ generation by CCCP in whole cells and the failure to show this inhibition (Fig. 3b) with DCCD (except at very high concentrations) are consistent with the generation of a Δp through the action of an indirect proton pump. It is proposed that the resulting Δp supports ATP synthesis through the action of an F_0F_1 ATPase.

This role for ATPase is thus distinct from that usually accepted for fermentative anaerobes, where it functions as a pump that extrudes protons at the expense of ATP. There are, however, anaerobes in addition to *O. formigenes* that appear to use the membrane ATPase primarily for ATP synthesis. An example of this is the conversion to ATP of energy stored as a proton gradient when methanol is used for methanogenesis by *Methanosarcina barkeri* (11). The possible relationship of the proposed model to reactions in certain other anaerobes where anions are translocated across the membrane and where proton consumption occurs internally has been discussed (38).

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REFERENCES

1. **Ahmed, S., and I. R. Booth.** 1981. Quantitative measurements of the proton-motive force and its relation to steady state lactose accumulation in *Escherichia coli*. Biochem. J. **200:**573–581.
2. **Allison, M. J., H. M. Cook, D. B. Milne, S. Gallagher, and R. V. Clayman.** 1986. Oxalate degradation by gastrointestinal bacteria from humans. J. Nutr. **116:**455–460.
3. **Allison, M. J., S. L. Daniel, and N. Cornick.** 1995. Oxalate-degrading bacteria, p. 131–167. *In* S. R. Khan (ed.), Calcium oxalate in biological systems. CRC Press, Inc., Boca Raton, Fla.
4. **Allison, M. J., K. A. Dawson, W. R. Mayberry, and J. G. Foss.** 1985. *Oxalobacter formigenes* gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. Arch. Microbiol. **141:**1–7.
5. **Anantharam, V., M. J. Allison, and P. C. Maloney.** 1989. Oxalate:formate exchange. The basis for energy coupling in *Oxalobacter*. J. Biol. Chem. **264:** 7244–7250.
6. **Baetz, A. L., and M. J. Allison.** 1989. Purification and characterization of oxalyl-coenzyme A-decarboxylase from *Oxalobacter formigenes*. J. Bacteriol. **171:**2605–2608.
7. **Baetz, A. L., and M. J. Allison.** 1990. Purification and characterization of formyl-coenzyme A transferase from *Oxalobacter formigenes*. J. Bacteriol. **172:**3537–3540.
8. **Baetz, A. L., and M. J. Allison.** 1992. Localization of oxalyl-coenzyme A decarboxylase, and formyl-coenzyme A transferase in *Oxalobacter formigenes* cells. Syst. Appl. Microbiol. **15:**167–171.
9. **Baronofsky, J. J., W. J. A. Schreuers, and E. R. Kashket.** 1985. Uncoupling by acetic acid limits growth of and acetogenesis of *Clostridium thermoaceticum*. Appl. Environ. Microbiol. **48:**1134–1139.
10. **Blackmore, M. A., and J. R. Quayle.** 1970. Microbial growth on oxalate by a route not involving glyoxylate carboligase. Biochem. J. **118:**53–59.
11. **Blaut, M., and G. Gottschalk.** 1985. Evidence for a chemiosmotic mechanism of ATP synthesis in methanogenic bacteria. Trends Biochem. Sci. **10:**486–489.
12. **Booth, I. R.** 1985. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. **49:**359–378.
13. **Bryant, M. P.** 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. **25:**1324–1328.
14. **Bryant, M. P., and L. A. Burkey.** 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. **36:**205–217.
15. **Buckel, W., and R. Semmler.** 1982. A biotin-dependent sodium pump: glutaconyl-CoA decarboxylase from *Acidaminococcus fermentans*. FEBS Lett. **148:**35–38.
16. **Collins, M. D., P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow.** 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int. J. Syst. Bacteriol. **44:**812–826.
17. **Daniel, S. L., and H. L. Drake.** 1993. Oxalate- and glyoxylate-dependent growth and acetogenesis by *Clostridium thermoaceticum*. Appl. Environ. Microbiol. **59:**3062–3069.
18. **Daniel, S. L., P. A. Hartman, and M. J. Allison.** 1987. Microbial degradation of oxalate in the gastrointestinal tracts of rats. Appl. Environ. Microbiol. **53:**1793–1797.
19. **Dawson, K. A., M. J. Allison, and P. A. Hartman.** 1980. Isolation and some characteristics of anaerobic oxalate-degrading bacteria from the rumen. Appl. Environ. Microbiol. **40:**833–839.
20. **Dehning, I., and B. Schink.** 1989. Two new species of anaerobic oxalate-fermenting bacteria, *Oxalobacter vibrioformis* sp. nov. and *Clostridium oxalicum* sp. nov., from sediment samples. Arch. Microbiol. **153:**79–84.
21. **Dimroth, P.** 1982. The generation of an electrochemical gradient of sodium ions upon decarboxylation of oxaloacetate by the membrane-bound and Na-activated oxaloacetate decarboxylase from *Klebsiella aerogenes*. Eur. J. Biochem. **121:**443–449.
22. **Fitzgerald, R. J., S. Doonan, L. L. McKay, and T. M. Cogan.** 1992. Intracellular pH and the role of *D*-lactate dehydrogenase in the production of metabolic end products by *Leuconostoc lactis*. J. Dairy Res. **59:**359–367.
23. **Hilpert, W., and P. Dimroth.** 1982. Conversion of the chemical energy of methylmalonyl-CoA decarboxylation into a Na^+ gradient. Nature (London) **296:**584–585.
24. **Hilpert, W., B. Schink, and P. Dimroth.** 1984. Life by a new decarboxylation-dependent energy conservation mechanism with Na^+ as coupling ion. EMBO J. **3:**1665–1670.
25. **Hodgkinson, A.** 1977. Oxalic acid in biology and medicine. Academic Press, Inc., New York.
26. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic and State University, Blacksburg.
27. **Kamo, N., M. Muratsugu, R. Hongoh, and Y. Kobatake.** 1979. Membrane potential of mitochondria measured with an electrode sensitive to tetraphenyl phosphonium and relationship between electrochemical potential and phosphorylation potential in steady state. J. Membr. Biol. **49:**105–121.
28. **Kashket, E. R.** 1985. The proton motive force in bacteria: a critical assessment of methods. Annu. Rev. Microbiol. **39:**219–242.
29. **Kashket, E. R.** 1987. Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. FEMS Microbiol. Rev. **46:**233–244.
30. **Kroll, R. G., and I. R. Booth.** 1981. The role of potassium transport in the generation of a pH gradient in *Escherichia coli*. Biochem. J. **198:**691–698.
31. **Maloney, P. C.** 1994. Bacterial transporters. Curr. Opin. Cell Biol. **6:**571–582.
32. **Maloney, P. C., V. Anantharam, and M. J. Allison.** 1992. Measurement of the substrate dissociation constant of a solubilized membrane carrier: substrate stabilization of OxlT, the anion exchange protein of *Oxalobacter formigenes*. J. Biol. Chem. **267:**10531–10536.
33. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. **83:**346–356.
34. **Postgate, J. R.** 1963. A strain of *Desulfovibrio* able to use oxamate. Arch. Mikrobiol. **46:**287–295.
35. **Quayle, J. R., D. B. Keech, and G. A. Taylor.** 1961. Carbon assimilation by *Pseudomonas oxalaticus* (OX1). Part 4. Metabolism of oxalate in cell-free extracts of the organism grown on oxalate. Biochem. J. **78:**225–236.
36. **Rottem, S., C. Linker, and T. H. Wilson.** 1981. Proton motive force across the membrane of *Mycoplasma gallisepticum* and its possible role in cell volume regulation. J. Bacteriol. **145:**1299–1304.
37. **Rottenberg, H.** 1977. The measurement of membrane potential and ΔpH in cells, organelles, and vesicles. Methods Enzymol. **55:**547–569.
38. **Ruan, Z.-S., V. Anantharam, I. T. Crawford, S. V. Ambudkar, S. Y. Rhee, M. J. Allison, and P. C. Maloney.** 1992. Identification, purification, and reconstitution of OxlT, the oxalate:formate antiport protein of *Oxalobacter formigenes*. J. Biol. Chem. **267:**10537–10543.
39. **Smith, R. L., F. E. Strohmaier, and R. S. Oremland.** 1985. Isolation of

anaerobic, oxalate-degrading bacteria from freshwater lake sediments. Arch. Microbiol. **141:**8–13.
40. **Ten Brink, B., and W. N. Konings.** 1982. Electrochemical proton gradient and lactate concentration gradient in *Streptococcus cremoris* cells grown in batch culture. J. Bacteriol. **152:**682–686.
41. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41:**100–180.
42. **Ugurbil, K., H. Rottenberg, P. Glynn, and R. G. Shulman.** 1982. Phosphorus-31 nuclear magnetic resonance studies of bioenergetics in wild-type and adenosinetriphosphatase(1-) *Escherichia coli* cells. Biochemistry **21:**1068–1075.
43. **Zaitsev, G. M., N. I. Govorukhina, O. V. Laskovneva, and Y. A. Trotsenko.** 1993. Properties of the new obligately oxalotrophic bacterium *Bacillus oxalophilus*. Microbiology **62:**378–382.