JOURNAL OF BACTERIOLOGY, Nov. 1988, p. 5006–5011
0021-9193/88/115006-06$02.00/0

Vol. 170, No. 11

Dextransucrase Secretion in *Leuconostoc mesenteroides* Depends on the Presence of a Transmembrane Proton Gradient

DAVID R. OTTS[1]† AND DONAL F. DAY[2]*

Department of Microbiology, Louisiana State University,[1] and Audubon Sugar Institute, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center,[2] Baton Rouge, Louisiana 70803

Received 16 March 1988/Accepted 22 July 1988

The relationship between proton motive force and the secretion of dextransucrase in *Leuconostoc mesenteroides* was investigated. *L. mesenteroides* was able to maintain a constant proton motive force of −130 mV when grown in batch fermentors at pH values 5.8 to 7.0. The contribution of the membrane potential and the transmembrane pH gradient varied depending on the pH of the growth medium. The differential rate of dextransucrase secretion was relatively constant at 1,040 ΔmU/Δmg (dry weight) when cells were grown at pH 6.0 to 6.7. Over this pH range, the internal pH was alkaline with respect to the external pH. When cells were grown at alkaline pH values, dextransucrase secretion was severely inhibited. This inhibition was accompanied by an inversion of the pH gradient as the internal pH became more acidic than the external pH. Addition of nigericin to cells at alkaline pH partially dissipated the inverted pH gradient and produced a fourfold stimulation of dextransucrase secretion. Treatment of cells with the lipophilic cation methyltriphenylphosphonium had no effect on the rate of dextransucrase secretion at pH 5.5 but inhibited secretion by 95% at pH 7.0. The reduced rate of secretion correlated with the dissipation of the proton motive force by this compound. Values of proton motive force greater than −90 mV were required for maximal rates of dextransucrase secretion. The results of this study indicate that dextransucrase secretion in *L. mesenteroides* is dependent on the presence of a proton gradient across the cytoplasmic membrane that is directed into the cell.

Over the past 7 years, evidence has accumulated indicating a role for proton motive force (Δp) in the process of protein secretion in bacteria (2, 5–9, 32). This requirement has been demonstrated as obligatory for the export of several proteins (2, 25) but nonessential for others (4, 5). Most studies on the energetic requirements of protein secretion in bacteria have focused on *Escherichia coli*. This organism maintains a Δp of approximately −200 mV when grown under aerobic conditions (1, 2, 16). Secretion of β-lactamase by this organism was inhibited by 50% when the Δp was lowered to −150 mV (2). This level of Δp is typical for bacteria that derive their cellular energy through strictly fermentative pathways. Examples include Δp values of −120 mV for *Streptococcus cremoris* (29), −143 mV for *Streptococcus lactis* (16), and −120 mV for *Clostridium thermoaceticum* (3). The involvement of Δp in protein secretion in strictly fermentative bacteria has not been thoroughly examined. Do bacteria that generate relatively low levels of Δp require Δp for efficient protein secretion?

To answer this question, we studied the role of Δp in dextransucrase secretion in the strictly fermentative, gram-positive bacterium *Leuconostoc mesenteroides*. Earlier work demonstrated the inhibition of secretion of this enzyme upon treatment of cells with various ionophores (10, 26). In this study, we correlated the secretion of dextransucrase with the level of Δp generated by the cells. We report that dextransucrase secretion requires the presence of a proton gradient directed into the cell and that an electrical potential alone is not sufficient.

* Corresponding author.
† Present address: The Hormel Institute, University of Minnesota, Austin, MN 55912.

MATERIALS AND METHODS

Growth of organism. *L. mesenteroides* ATCC 10830 was maintained on All Purpose Twee agar and broth (Difco Laboratories, Detroit, Mich.). For all experiments, cells were grown overnight for 16 h in All Purpose Twee broth. A 5% (vol/vol) inoculum from this culture was transferred to a 500-ml fermentor (model C30 Bioflo; New Brunswick Scientific Co., Inc., Edison, N.J.) that contained LMK100 medium. This growth medium consisted of 1.0% tryptone, 0.79% yeast extract, 0.87% K_2HOP_4, 1.0% Tween 80, and 5% sucrose. Sucrose was prepared separately as a 50% (wt/vol) stock solution and added to the growth medium after sterilization. Constant pH was maintained throughout the fermentation by the automatic addition of 5 N KOH, controlled by a pH controller and pump module (model pH-40; New Brunswick Scientific). All fermentation experiments were carried out at 30°C with an agitation rate of 100 rpm. Cell growth was followed by measurement of the turbidity of the culture at 660 nm. Cellular dry weights were calculated from a standard curve.

Purification and assay of extracellular dextransucrase. Dextransucrase was purified from culture supernatants through the use of an aqueous two-phase partition technique as previously described (26). For each sample, 0.35 ml of culture supernatant was added to a 1.5-ml microfuge tube containing 0.35 ml of a 10% dextran T-500 solution. The tube contents were mixed, and then 0.7 ml of a 20% polyethylene glycol solution (molecular weight, 3,350) was added. The contents were again mixed and centrifuged in a microfuge for 3 min. The upper phase, which consisted primarily of polyethylene glycol, was removed with a Pasteur pipette and discarded; the lower, dextran-rich phase was brought to a volume of 0.5 ml with distilled water. Then 0.5 ml of the polyethylene glycol solution was added, and the contents were mixed and centrifuged. This procedure was repeated twice, and then the final dextran phase was diluted to 0.5 ml

with 0.1 M sodium acetate, pH 5.0. This procedure recovered dextransucrase in high yield (95%) with a specific activity of 30 U of protein per mg. This represented a 1,500-fold purification from the culture supernatant. Dextransucrase activity was determined by measuring reducing sugar formation from sucrose by the 3,5-dinitrosalicylic acid method (24). Fructose was used as the standard. The assay buffer consisted of sucrose, 150 mM; $CaCl_2$, 1 mM; sodium acetate, pH 5.0, 100 mM; and dextran T-40, 1 mg/ml. One unit is defined as the amount of enzyme that catalyzes the formation of 1 μmol of fructose per min at 30°C and pH 5.0.

The differential rate of dextransucrase secretion was determined from a graph of the slope of extracellular dextransucrase activity versus cellular dry weight. The unit was expressed as the amount of dextransucrase secreted per increase in cellular dry weight (ΔmU/Δmg [dry weight]).

Determination of pH stability of dextransucrase. To determine the pH stability of dextranase, the supernatant of a culture grown for 11 h in LMK100 medium was isolated and separated into 50-ml fractions. This culture, which had an initial pH of 6.7 and a final pH of 5.2, was then adjusted to various pH values between 5.0 and 8.0 with KOH or HCl. Immediately after adjustment of the pH, dextransucrase was purified and assayed as described above. To mimic the conditions in the fermentor, the 50-ml fractions were held at 30°C for 7 h. At this point, dextransucrase was again purified and assayed.

Determination of intracellular volume. Intracellular volume was determined by incubating cells in the presence of [^{14}C]inulin (0.49 μCi/ml) and ^3H_2O (0.98 μCi/ml) (16, 27). A 1-ml sample of growing cells was transferred to a capped culture tube (15 by 100 mm), the radiolabeled probes were added, and the tube was incubated in a 30°C water bath for 5 min. Then 0.4 ml of this mixture was centrifuged through 0.4 ml of silicone oil (79 to 82%) dimethyl–18 to 21% diphenylsiloxane copolymer) for 1 min at 10,000 rpm in an Eppendorf microfuge. The upper aqueous layer and the silicone oil were removed by siphoning with vacuum with a Pasteur pipette. The cell pellet was then counted for each radiolabel through the use of standard scintillation counting techniques. Intracellular volumes were calculated as described by Rottenberg (27). Volumes were determined for each experiment, but the intracellular volume did not vary significantly (1.62 ± 0.8 μl/mg [dry weight]; n = 40).

Determination of pH. Intracellular pH was determined from measurement of the distribution of [^{14}C]benzoate (6.4 μM, 43 Ci/mol) or [^{14}C]methylamine (2.1 μM, 49 Ci/mol) (16, 27). Cells were processed as described above for determination of intracellular volumes. The pH gradient was defined as the difference between the internal and external pHs. The pH gradient was converted to millivolts by multiplying by 2.3 RT/F, where R equals the gas constant, F is Faraday's constant, and T is the temperature in degrees Kelvin (27). This multiplication factor, Z, is equal to 60 to 30°C.

Determination of Δψ. The membrane potential (Δψ) was determined by measuring the uptake of [^{3}H]tetraphenylphosphonium (0.014 μM, 35.5 Ci/mmol) (16, 27). A 1-ml portion of cells was processed as described above. Nonspecific binding of the probe to cellular components was determined by treatment of a separate sample with 5% (vol/vol) n-butanol for 30 min before the addition of [^{3}H]tetraphenylphosphonium (17). The value obtained from liquid scintillation counting for the butanol-treated cells was subtracted from that of the untreated culture. The Δψ values were calculated according to the Nernst equation (27).

Calculation of proton motive force. The proton motive force was calculated according to the following equation: $\Delta p = \Delta\psi - Z\Delta pH$ (27), where Δψ is the membrane potential, pH is the transmembrane pH gradient, and Δp is the proton motive force.

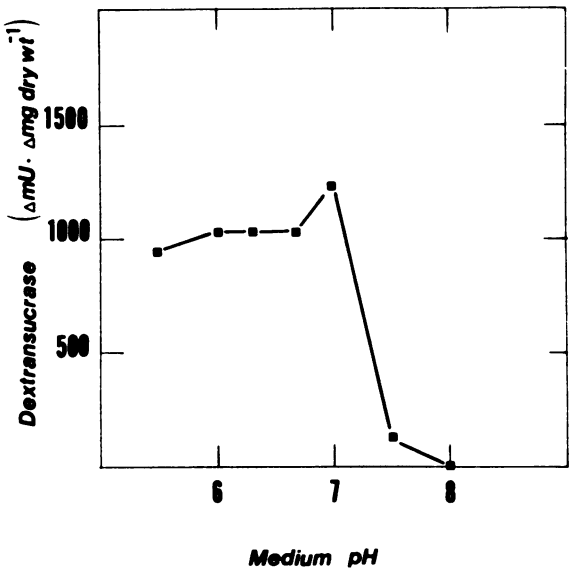

FIG. 1. Dextransucrase secretion by *L. mesenteroides* ATCC 10830 as a function of the culture pH. *L. mesenteroides* ATCC 10830 was grown in LMK100 medium in batch fermentors at various pH values. Extracellular dextransucrase was purified from culture supernatants at 15-min intervals for 1 h after 6 h of growth to establish the rate of enzyme secretion.

Preparation and use of ionophores. Methyltriphenylphosphonium bromide ($MTPP^+$) and nigericin were prepared as ethanolic solutions at concentrations of 200 mM and 5 mg/ml, respectively. For all experiments, the ionophores were added to the cultures 6 h after inoculation.

Chemicals. All radiolabeled compounds were obtained from Dupont, New Research Products, Boston, Mass. Nigericin and $MTPP^+$ were purchased from Sigma Chemical Co., St. Louis, Mo. Silicone oil was obtained from Petrarch Systems, Bristol, Pa. All other chemicals were of reagent grade and are available commercially.

RESULTS

Dextransucrase secretion by cells grown at constant pH. *L. mesenteroides* ATCC 10830 grew well in LMK100 medium over a wide range of external pH values. The optimum pH for growth was 6.0 to 7.0, where the mean doubling time was 31 ± 2 min (n = 4). The doubling time increased when the culture pH was outside this range. At pH 5.0, the mean doubling time increased to 60 min, and at pH 8.0 it increased to 67 min. *L. mesenteroides* was able to secrete dextransucrase over a wide range of external pH values (Fig. 1). A maximum secretion rate of 1,240 mU/mg (dry weight) was found at pH 7.0. A decrease in the external pH had little effect on the rate of dextransucrase secretion, as a rate of 1,000 mU/mg (dry weight) was observed at pH 5.5. Above pH 7.0, the rate of secretion decreased sharply. Dextransucrase secretion was reduced by 95% at pH 7.5, and no dextransucrase activity was detected in culture supernatants from cells grown at pH 8.0. To determine whether the lowered levels of dextransucrase at alkaline pH were the result of inactivation of the enzyme, the stability of dextran-

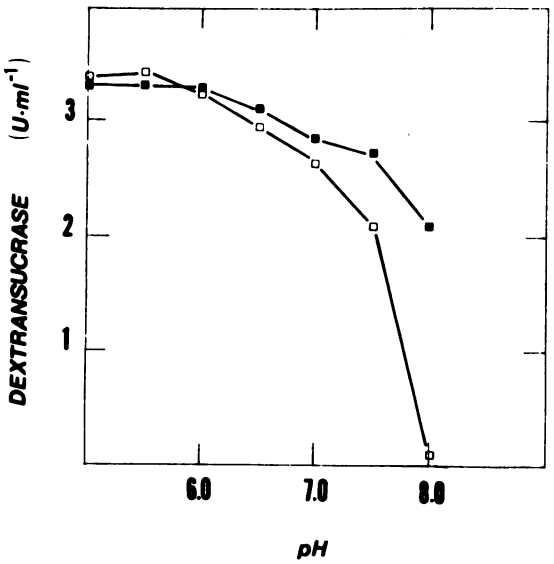

FIG. 2. Stability of dextransucrase as a function of pH. Dextransucrase was prepared and assayed as described in Materials and Methods. Symbols: ■, t = 0; □, t = 7 h after adjustment of pH.

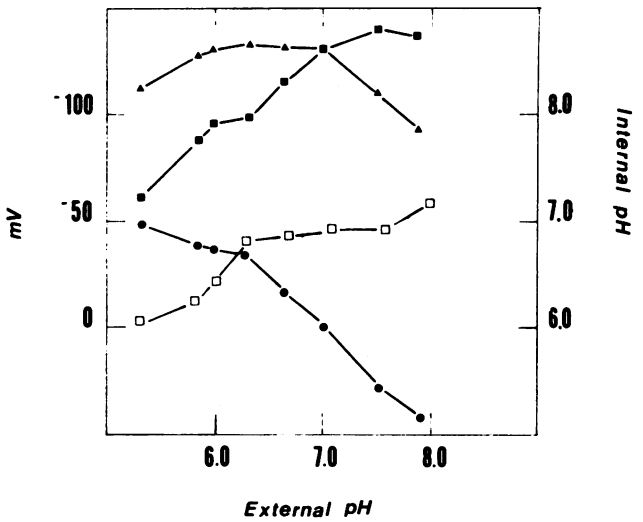

FIG. 3. Generation of Δp by *L. mesenteroides* as a function of the culture pH. Cells were grown at different pH values in LMK100 medium for 6 h. At that point, ΔpH, Δψ, and Δp were determined as described in Materials and Methods. Symbols: ▲, Δp; ■, Δψ; ●, $-Z\Delta pH$; □, internal pH.

sucrase at various pH values was determined (Fig. 2). Dextransucrase was completely inactivated when held at pH 8.0 for 7 h, whereas its activity decreased by only 40% at pH 7.5. Thus, the low level of dextransucrase observed in culture supernatant at pH 7.5, could not be attributed to inactivation of the enzyme. To confirm this, a culture grown at pH 6.7 for 6 h was raised to pH 7.5, and extracellular dextransucrase was determined at 10-min intervals for 1 h. The pH shift decreased the rate of dextransucrase secretion from 1,040 to 107 mU/mg (dry weight) but had no effect on cell growth. After 1 h at pH 7.5, the pH of the culture was lowered to 6.7, and extracellular dextransucrase was again determined. The rate of secretion increased to 1,130 mU/mg (dry weight) within 10 min after the pH shift. These results suggested that the low levels of extracellular dextransucrase found in cultures at pH 7.5 were due to inhibition of secretion rather than to pH inactivation of the enzyme.

Determination of Δp in cells grown at constant pH. The two components of the proton motive force were determined at various pH values (Fig. 3). Cells maintained a constant proton motive force of −130 mV when grown within a pH range of 5.8 to 7.0. Below pH 5.8, Δp decreased until it reached a value of −112 mV at pH 5.3. The contribution of $-Z\Delta pH$ to Δp increased from 0 mV at pH 7.0 to −50 mV at pH 5.3. The membrane potential decreased from −130 to −62 mV over this pH range. The organism was able to maintain a relatively constant internal pH of 7.0 when grown within a pH range of 6.6 to 7.5. Thus, at external, pH values above 7.0, the pH gradient was inverted (i.e., the interior was acidic with respect to the medium). The inverted pH gradient steepened with increasing external pH such that an internal pH of 7.18 was observed at an external pH of 7.9. The inversion resulted in a decrease in Δp even though Δψ increased to −140 mV. Below an external pH of 6.6, the organism was unable to maintain a constant internal pH.

Effect of $MTPP^+$ on Δp and dextransucrase secretion. To assess the role of Δp in dextransucrase secretion, the lipophilic cation $MTPP^+$ was used to manipulate the membrane potential, while the transmembrane pH gradient was manipulated by varying the pH of the growth medium. The results of a titration of Δp with $MTPP^+$ are shown in Table 1. The effects of $MTPP^+$ were not specific for Δψ, as ΔpH was slightly dissipated. The addition of $MTPP^+$ to the cells inhibited dextransucrase in a pH-dependent manner (Fig. 4). At a culture pH of 5.5, $MTPP^+$ had no effect on the rate of dextransucrase secretion. However, $MTPP^+$ decreased dextransucrase secretion, a function of increasing culture pH, until at pH 7.0 95% less enzyme was secreted than by cells not treated with the lipophilic cation. This inhibition was not the result of a generalized effect on the health of the cells. When added to cells at pH 5.5, $MTPP^+$ increased the doubling time from 96 to 143 min; at pH 7.0, $MTPP^+$ increased the doubling time from 113 to 134 min. The rate of dextransucrase secretion observed after treatment of cells with $MTPP^+$ was dependent on the level of Δp present (Fig. 4). Dextransucrase secretion was severely inhibited at Δp values of less than −80 mV. Between −80 and −90 mV, there was a rapid rise in the rate of dextransucrase secretion. Above −90 mV, there was no significant change in the rate of secretion, as extracellular enzyme levels reached those found with untreated cells. The ability of *L. mesenteroides* to secrete dextransucrase after treatment with $MTPP^+$ was directly related to the presence of a pH gradient (interior alkaline) across the cell membrane (Fig. 4). These results indicated that a proton motive force directed into the cells was required for efficient dextransucrase secretion.

Effect of nigericin on Δp and dextransucrase secretion. At pH 7.5, extracellular dextransucrase production was severely inhibited. The proton motive force of the organism at

TABLE 1. Effect of $MTPP^+$ on the generation of Δp at pH 6.0

$MTPP^+$ concn (mM)	Value obtained (mV)		
	$-Z\Delta pH$	Δψ	Δp
0	−35	−95	−130
0.1	−31	−85	−116
0.5	−31	−61	−92
1.0	−27	−54	−81

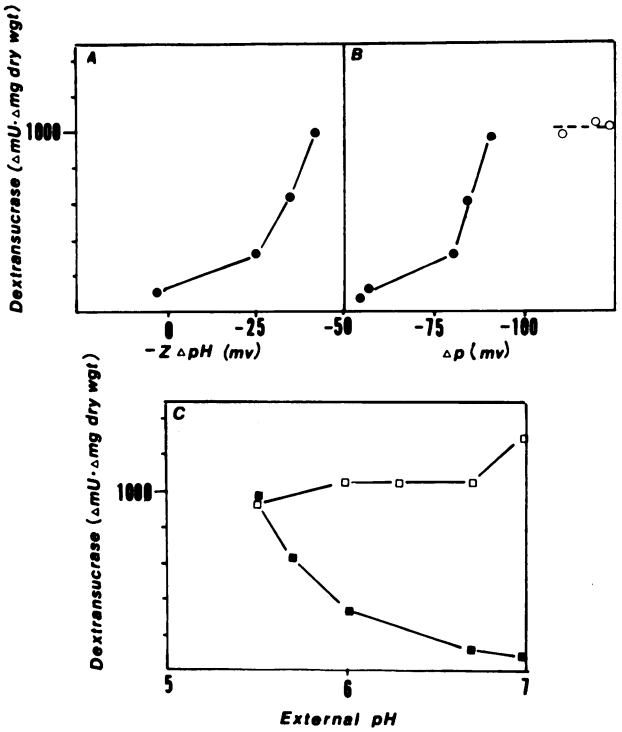

FIG. 4. Inhibition of dextransucrase secretion by $MTPP^+$. Cells were grown in batch fermentors at various pH values between 5.5 and 7.0. After 6 h of growth at the given pH, $MTPP^+$ was added to give a final concentration of 1 mM. (A) Dependence of dextransucrase secretion on the transmembrane pH gradient after treatment with 1 mM $MTPP^+$. (B) Dependence of dextransucrase secretion on Δp. Symbols: Δp of cells treated with 1 mM $MTPP^+$ at different values of external pH (●); Δp of control treated cells (○). (C) Dependence of $MTPP^+$-mediated inhibition of dextransucrase secretion on the external pH. Symbols: ■, 1 mM $MTPP^+$; □, control.

this pH (−112 mV) was higher than the level required for optimal dextransucrase secretion as determined by $MTPP^+$ treatment. At a culture pH of 7.5, ΔpH was equal to −0.48 mV (interior acidic). Cellular processes dependent on proton influx would not be expected to function well at pH 7.5 because of the large amount of energy required to bring protons into the cell against the concentration gradient. If dextransucrase secretion was inhibited due to the inverted pH gradient, then treatment of cells with a dissipator of ΔpH, such as nigericin (1), should have stimulated dextransucrase secretion. Addition of nigericin to cells at pH 7.5 stimulated dextransucrase secretion fourfold (Table 2). The pH gradient partially collapsed (from 29 to 24 mV), and Δp increased from −110 to −119 mV. Thus, partial dissipation of the inverted pH gradient resulted in a stimulation of the secretion of dextransucrase. When nigericin was added to cells at pH 6.7, the generation of pH was severely, but not totally, inhibited, with a corresponding increase in Δψ. The increase in Δψ kept Δp relatively constant. The doubling time of the cells increased from 116 to 254 min, but there was no effect on the specific production of extracellular dextransucrase. This result provides evidence that secretion is independent of general growth effects caused by this ionophore.

TABLE 2. Effect of nigericin on Δp and dextransucrase secretion

Treatment	pH	Value obtained (mV)			Growth[a] (min)	Dextransucrase production (mU/mg [dry wt])
		−ZΔpH	Δψ	Δp		
Control	6.7	−26	−115	−141	116	1,038
Nigericin		−6	−135	−141	254	1,078
Control	7.5	29	−140	−111	107	124
Nigericin		24	−143	−119	105	494

[a] Doubling time between t = 6 to 7 h.

DISCUSSION

The dependence of dextransucrase secretion on the proton motive force was demonstrated by manipulation of the components of Δp with ionophores. Optimal rates of dextransucrase secretion require Δp to be greater than −90 mV. Above this level, there is no significant increase in the rate of secretion. This "saturation" of the dextransucrase secretion system by Δp is similar to the findings of Δp involvement in flagellar motility in *Bacillus subtilis* (19, 28). The maximal rate of flagellar rotation was achieved at values of Δp greater than −100 mV (28).

When grown at alkaline pH values, *L. mesenteroides* did not efficiently secrete dextransucrase, even though the proton motive force was above the saturation level of −90 mV. The inhibition occurred despite high levels of membrane potential at alkaline pH values (i.e., −140 mV at pH 7.5). Thus, enzyme secretion was not solely dependent on the electrical potential across the membrane. We attribute the low rate of dextransucrase secretion to the inverted pH gradient (interior acidic) at alkaline external pH values. The stimulation of secretion after nigericin treatment correlated with a partial dissipation of this inverted gradient. The membrane potential must be generated by the distribution of ions other than protons at alkaline pH values. Alkalophilic bacteria use Na^+ instead of protons to drive flagellar motility (13). Apparently, *L. mesenteroides* does not use this type of ion transport to support dextransucrase secretion.

The energy requirements of protein export in bacteria have been examined predominantly in *E. coli* (2, 4–9, 32). This organism uses both ATP (4, 5, 32) and Δp (4, 32) to drive protein export. The proton motive force in *E. coli* is typically −175 to −200 mV under aerobic conditions (1, 16). A decrease in Δp to −150 mV decreases the rate of β-lactamase export by 50% (2). This value of Δp is larger than the maximum observed in *L. mesenteroides* under optimal growth conditions. (The level of Δp where the rate of dextransucrase secretion was inhibited by 50% was calculated to be approximately −85 mV. This represents a 35% decrease in Δp, compared with approximately 25% for *E. coli* [2].)

Protein export in several gram-positive bacteria has been suggested to be dependent on Δp. The export of α-amylase in *Bacillus amyloliquefaciens* is inhibited by carbonyl cyanide *m*-chlorophenylhydrozone or valinomycin plus K^+ (25), and gramicidin inhibits the export of a glycosyltransferase in *Streptococcus sanguis* (18, 31). Neither of these studies addressed the question of whether protein export was driven solely by Δψ, ΔpH, or Δp. This study, therefore, represents the first examination of the actual relationship between Δp and protein secretion in gram-positive bacteria.

ATP cannot be ruled out as a possible energy source, as no measurements of intracellar ATP levels were made in this

study. It is possible that $MTPP^+$ exerts its effect by stimulating membrane hydrogenases to secrete protons from cells, thus lowering the internal ATP level. However, the effects of $MTPP^+$ on the growth of *L. mesenteroides*, at the extremes of the pH range examined, were quite similar, although dextransucrase secretion was inhibited only at the alkaline end. Additionally, $MTPP^+$ has been demonstrated to increase the internal ATP concentration in *B. subtilis* (15).

It is not yet clear what the physical role of Δp is in the process of protein secretion in bacteria. The most simplistic model would be the direct coupling of proton movement to protein translocation through the membrane. This type of model has been ascribed to Δp involvement in flagellar motility (19, 21, 23). Proton influx in motile streptococci was directly coupled to rotation of the flagellar filament (19). An alternative is the possibility discussed by Bakker and Randall (2), that Δp is required to maintain an export-competent conformation of a membrane complex. This type of model has also been ascribed to the role of Δp in peptidoglycan and teichoic acid synthesis in *B. subtilis* (12).

Membrane lipid composition has been postulated to play an important role in glucosyltransferase secretion in *Streptococcus salivarius* (22). An increase in the K^+ concentration of the growth medium stimulated glucosyltransferase secretion and an increase in the amount of unsaturated membrane lipid fatty acids (22). Hope and Cullis (14) demonstrated that the presence of a pH gradient across large unilamellar vesicle membranes affected the transbilayer distribution of the membrane lipids. It is conceivable that the direction of the pH gradient affects the distribution of membrane lipids in *L. mesenteroides* and thus alters dextransucrase secretion by disruption of export complexes within the membrane.

Apparently, the production and secretion of dextransucrase are not requirements for sucrose metabolism by *L. mesenteroides*. Although dextransucrase secretion was totally inhibited at pH 8.0, growth of the organism in a sucrose medium was not severely affected. *L. mesenteroides* is known to also produce a sucrose phosphorylase when grown in media containing sucrose (30). It is possible that at alkaline pH the organism relies on this enzyme for sucrose utilization.

It is interesting to note that although proton motive force is crucial for efficient protein secretion in bacteria, it is not a requirement for cell growth (11, 20). Further investigations are required to elucidate the actual mechanism of the involvement of proton motive force in protein secretion in bacteria.

LITERATURE CITED

1. **Ahmed, S., and I. R. Booth.** 1983. The use of valinomycin, nigericin, and trichlorocarbanilide in control of the protonmotive force in *Escherhichia coli* cells. Biochem. J. **212**:105–112.
2. **Bakker, E. P., and L. L. Randall.** 1984. The requirement for energy during export of β-lactamase in *Escherichia coli* is fulfilled by the total protonmotive force. EMBO J. **4**:895–900.
3. **Baronofsky, J. J., W. J. A. Schreurs, and E. R. Kashket.** 1984. Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum*. Appl. Environ. Microbiol. **48**: 1134–1139.
4. **Chen, L., and P. C. Tai.** 1985. ATP is essential for protein translocation into *Escherichia coli* membrane vesicles. Proc. Natl. Acad. Sci. USA **82**:4384–4388.
5. **Chen, L., and P. C. Tai.** 1986. Roles of H^+-ATPase and proton motive force in ATP-dependent translocation in vitro. J. Bacteriol. **167**:389–392.
6. **Daniels, C. J., D. G. Bole, S. C. Quay, and D. L. Oxender.** 1981. Role of membrane potential in the secretion of protein into the periplasm of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**: 5396–5400.
7. **Date, T., J. M. Goodman, and W. T. Wickner.** 1980. Procoat, the precursor of M13 coat protein, requires an electrochemical potential for membrane insertion. Proc. Natl. Acad. Sci. USA **77**:4669–4673.
8. **Enequist, H. G., T. R. Hirst, S. Harayama, S. J. S. Hardy, and L. L. Randall.** 1981. Energy is required for maturation of exported proteins in *Escherichia coli*. Eur. J. Biochem. **116**: 227–233.
9. **Geller, B. L., N. R. Movva, and W. Wickner.** 1986. Both ATP and the electrochemical potential are required for optimal assembly of pro-OmpA into *Escherichia coli* inner membrane vesicles. Proc. Natl. Acad. Sci. USA **83**:4219–4222.
10. **Gomez-Puyou, A., and C. Gomez-Lojero.** 1977. The use of ionophores and channel formers in the study of the function of biological membranes. Curr. Top. Bioenerg. **6**:221–257.
11. **Harold, F. M., and J. Van Brunt.** 1977. Circulation of H^+ and K^+ across the plasma membrane is not obligatory for bacterial growth. Science **197**:372–373.
12. **Harrington, C. R., and J. Baddiley.** 1984. Synthesis of peptidoglycan and teichoic acid in *Bacillus subtilis*: role of the electrochemical proton gradient. J. Bacteriol. **159**:925–933.
13. **Hirota, N., and Y. Imae.** 1983. Na^+-driven flagellar motors of an alkalophilic *Bacillus* strain YN-1. J. Biol. Chem. **258**:10577–10581.
14. **Hope, M. J., and P. R. Cullis.** 1987. Lipid asymmetry induced by transmembrane pH gradients in large unilamellar vesicles. J. Biol. Chem. **262**:4360–4366.
15. **Joliffe, L. K., R. J. Doyle, and U. N. Streips.** 1981. The energized membrane and cellular autolysis in *Bacillus subtilis*. Cell **25**: 753–763.
16. **Kashket, E. R.** 1985. The proton motive force in bacteria: a critical assessment of methods. Annu. Rev. Microbiol. **39**:219–242.
17. **Kashket, E. R., A. G. Blanchard, and W. C. Metzger.** 1980. Proton motive force during growth of *Streptococcus lactis* cells. J. Bacteriol. **143**:128–134.
18. **Keevil, C. W., A. A. West, N. Bourne, and P. D. Marsh.** 1984. Inhibition of the synthesis and secretion of extracellular glucosyl- and fructosyltransferase in *Streptococcus sanguis* by sodium ions. J. Gen. Microbiol. **130**:77–82.
19. **Khan, S., and R. M. Macnab.** 1980. Proton chemical potential, proton electrical potential and bacterial motility. J. Mol. Biol. **138**:599–614.
20. **Kinoshita, N., T. Unemoto, and H. Kobayashi.** 1984. Proton motive force is not obligatory for growth of *Escherichia coli*. J. Bacteriol. **160**:1074–1077.
21. **Lancaster, M. J., C. W. Keevil, D. C. Ellwood, and R. C. W. Berkeley.** 1987. Environmental control of protein export of *Bacillus licheniformis* NCIB 6346 reveals two types of extracellular proteins. Arch. Microbiol. **147**:158–162.
22. **Markevics, L. J., and N. A. Jacques.** 1985. Enhanced secretion of glucosyltransferase by changes in potassium ion concentrations is accompanied by an altered pattern of membrane fatty acids in *Streptococcus salivarius*. J. Bacteriol. **161**:989–994.
23. **Meister, M., G. Lowe, and H. C. Berg.** 1987. The proton flux through the bacterial flagellar motor. Cell **49**:643–650.
24. **Monsan, P., and A. Lopez.** 1981. On the production of dextran by free and immobilized dextransucrase. Biotech. Bioeng. **23**: 2027–2037.
25. **Muren, E. M., and L. L. Randall.** 1985. Export of α-amylase by *Bacillus amyloliguefaciens* requires proton motive force. J. Bacteriol. **164**:712–716.
26. **Otts, D. R., and D. F. Day.** 1987. The effect of ionophores on the production of extracellular dextransucrase by *Leuconostoc mesenteroides*. FEMS Microbiol. Lett. **42**:179–183.
27. **Rottenberg, H.** 1979. The measurement of membrane potential and pH in cells, organelles, and vesicles. Methods Enzymol. **55**: 547–569.
28. **Shioi, J. I., S. Matsuura, and Y. Imae.** 1980. Quantitative measurements of proton motive force and motility in *Bacillus*

subtilis. J. Bacteriol. **144**:891–897.
29. **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.
30. **Vandamme, E. J., Van Loo, and A. Delaporte.** 1987. Dynamics of sucrose phosphorylase formation in *Leuconostoc mesenteroides* fermentations. Biotechnol. Bioeng. **29**:8–15.
31. **West, A. A., C. W. Keevil, P. D. Marsh, and D. C. Ellwood.** 1984. The effect of ionophores on growth and glycosyltransferase production of *Streptococcus sanguis*. FEMS Microbiol. Lett. **25**:133–137.
32. **Yamane, K., S. Ichihara, and S. Mizushima.** 1987. In vitro translocation of protein across *Escherichia coli* membrane vesicles requires both the proton motive force and ATP. J. Biol. Chem. **262**:2358–2362.