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Energy-Spilling Reactions of *Streptococcus bovis* and Resistance of Its Membrane to Proton Conductance

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Glucose-excess cultures of *Streptococcus bovis* consumed glucose faster than the amount that could be explained by growth or maintenance, and nongrowing chloramphenicol-treated cells had a rate of glucose consumption that was 10-fold greater than the maintenance rate. Because *N,N*-dicyclohexylcarbodiimide, an inhibitor of the membrane-bound F_1F_0 ATPase, eliminated the nongrowth energy dissipation (energy spilling) without a decrease in ATP and the rate of energy spilling could be increased by the protonophore 3,3′,4′,5-tetrachlorosalicylanilide, it appeared that a futile cycle of protons through the cell membrane was responsible for most of the energy spilling. When the rate of energy spilling was decreased gradually with iodoacetate, there was only a small decrease in the phosphorylation potential ($\Delta G'p$) and the theoretical estimate of H^+ per ATP decreased from 4.2 to 3.6. On the bases of this ratio of H^+ to ATP and the rate of ATP production, the flux of protons (amperage) across the cell membrane was directly proportional to the rate of energy spilling. Amperage values estimated from $\Delta G'p$ were, however, nearly twice as great as values which were estimated from the heat production (ΔH) of the cells [amperage = (0.38 × wattage)/Δp]. The last comparison indicated that only a fraction of the ΔG of ATP hydrolysis was harvested by the F_1F_0 ATPase to pump protons. Both estimates of amperage indicated that the resistance of the cell membrane to proton conductance was inversely proportional to the log of the energy-spilling rate.

Since Lipmann demonstrated the function of phosphate esters as key intermediates in the energy transformation of living cells, it has generally been assumed that "catabolism and anabolism are knit together into a largely reversible reaction continuum" (10). On the basis of the assumption that "cells are capable of regulating their metabolic reactions and the biosynthesis of their enzymes to achieve maximum efficiency and economy" (9), one would expect a strong correlation between the ATP production of catabolic pathways and the formation of bacterial biomass (2).

Theoretical calculations by Stouthamer (29) indicated that the Y_{ATP} of bacteria should be as high as 32 g of cells per mol of ATP, but most bacteria had growth yields which were significantly lower than this value (31). Some of this difference was explained by maintenance energy and variations in cell composition, but even liberal corrections still could not account for much of the missing ATP. $Y_{ATP/MAX}$ values (Y_{ATP} corrected for maintenance) were rarely greater than 16 g of cells per mol of ATP (30).

Because the ruminal bacterium *Streptococcus bovis* (i) requires carbohydrates as an energy source for growth (27), (ii) has clearly defined mechanisms of glucose transport (15, 24) (iii) does not accumulate polysaccharide, (iv) converts virtually all of its glucose to lactate or acetate, formate, and ethanol (25), and (v) derives all of its ATP from substrate-level phosphorylation (26), the ATP production of glucose fermentation can be estimated precisely. On the basis of these characteristics, *S. bovis* provided an ideal model for the study of bacterial bioenergetics.

Nongrowing *S. bovis* cells fermented glucose, and recent work indicated that this nongrowth energy dissipation (energy spilling) could be completely eliminated by *N,N*-dicyclohexylcarbodiimide (DCCD), an inhibitor of the F_1F_0 ATPase (27). On the basis of this result, it appeared that a futile cycle of protons through the cell membrane was causing a turnover of ATP (27). Because glucose-limited cells did not spill energy, it appeared that the mechanism of energy spilling might be regulated (25). The aims of the following experiments were to (i) estimate proton flux through the cell membrane (amperage), (ii) compare proton flux with the rate of ATP turnover by energy-spilling cells, and (iii) correlate membrane resistance changes with the rates of proton flux and energy spilling.

MATERIALS AND METHODS

Materials. All chemicals were analytical reagent grade. Myokinase, pyruvate kinase, and purified luciferin-luciferase extract were purchased from Sigma (St. Louis, Mo.). Tetra-[^{3}H]phenylphosphonium bromide (TPP^+) (0.5 μCi; 30 μCi/μmol), [7-^{14}C]benzoate (1.0 μCi; 21.8 μCi/μmol), [1,2-^{14}C]polyethylene glycol (1.0 μCi/mg), and ^3H_2O (4.00 μCi; 3.6 μCi/μmol) were from Amersham International (Amersham Laboratories, Rockford, Ill.).

Cell growth. *S. bovis* JB1 was grown anaerobically at 39°C in basal medium containing the following (per liter): K_2HPO_4, 292 mg; KH_2PO_4, 292 mg; $(NH_4)_2SO_4$, 480 mg; NaCl, 480 mg; $MgSO_4 \cdot 7H_2O$, 100 mg; $CaCl_2 \cdot 2H_2O$, 64 mg; cysteine hydrochloride, 600 mg; Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 1g; yeast extract, 0.5 g; and glucose. The medium was adjusted to pH 6.7, and the final pH was never less than 6.5. The incubation temperature was 39°C. Glucose-limited (5.5 mM) continuous cultures were grown anaerobically (O_2-free CO_2) at a dilution rate of 0.1 h^{-1}. Growth was monitored by the increase in turbidity (1-cm cuvette, 600 nm). The relationship between optical density and cell protein was 160 mg of protein per liter per turbidity unit.

Heat production and glucose consumption. Bacterial heat production was measured with an LKB model 2277 bioactivity

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monitor that was equipped with semiconducting Peltier elements as thermopiles and gold flow cells. The machine was calibrated with an internal electric heat source and gave very stable digital and strip chart readouts. The flow cell was sterilized with 95% ethanol and 1 M HCl. Cells and medium were pumped through the flow cell at a rate of 40 ml/h. LKB indicated that the effective volume of the flow cell was 0.678 ml at this flow rate. The flow cell temperature was set at 39.00°C, and total transit time from the culture vessel through the flow cell and back to the culture vessel was approximately 4 min. The glucose consumption rate was estimated from the enthalpy (ΔH) of glucose conversion to lactate (21 cal [88.2J]/mmol of glucose) and the conversion factor, 1.16 mW/cal/h.

Measurement of electrochemical proton gradient (Δp). Exponentially growing cultures were anaerobically transferred with a hypodermic syringe (2.0 ml) to tubes (13 by 100 mm) which contained ^{3}H-TPP$^+$, [7-^{14}C]benzoate, [1,2-^{14}C]polyethylene glycol, or ^3H_2O. After incubation for 5 min at 39°C, the cultures were centrifuged through silicon oil (equal parts mixture of Dexter Hysol 550 and 560; Hysol Co., Olean, N.Y.) in microcentrifuge tubes (13,000 × *g*, 5 min, 22°C), and 20-μl samples of supernatant were removed. The tubes and contents were frozen (−15°C), and the bottoms (cell pellets) were removed with dog nail clippers. Supernatant and cell pellets were dissolved in scintillation fluid and counted.

The intracellular volume (4.30 μl/mg of protein) was estimated from the difference between the partitioning of ^3H_2O and [^{14}C]polyethylene glycol. The electrical potential across the cell membrane ($\Delta\Psi$) was calculated from the uptake of ^{3}H-TPP$^+$ according to the Nernst relationship. Nonspecific TPP$^+$ binding was estimated from cells which had been treated with valinomycin and nigericin (10 μM each). The ΔpH was determined from the distribution of [^{14}C]benzoate, using the Henderson-Hasselbalch equation (22), and ZΔpH was calculated as 62 mV × ΔpH.

Sampling and extraction of adenylates. Adenine nucleotides were extracted from the cells by perchloric acid-EDTA treatment after separation of the cells from the growth medium. At appropriate time intervals, 0.5-ml samples of culture were added to an Eppendorf microcentrifuge tube containing 0.15 ml of ice-cold 14% (wt/wt) perchloric acid which contained 9 mM EDTA on which was layered silicon oil (equal parts mixture of Dexter Hysol 550 and 560). Samples were layered on top of the silicon oil, and the tubes were centrifuged (13,000 × *g*, 5 min, 22°C) for 2 min. After centrifugation, the top layer was removed (200 μl) and mixed with 100 μl of ice-cold 14% perchloric acid containing 9 mM EDTA, and the residual top layer and oil layer were removed. The cell pellet was resuspended in the perchloric acid layer, and both layers were kept on ice for 20 min. The samples were then centrifuged (13,000 × *g*, 5 min, 22°C) for 5 min to remove cell debris, and the supernatants (100 μl) were neutralized with 50 μl of KOH-$KHCO_3$ (1 M each; 0°C) and frozen at −20°C. Prior to analysis, the samples were thawed and the potassium perchlorate was removed by centrifugation (13,000 × *g*, 5 min, 22°C).

Measurement of adenine nucleotides. Adenine nucleotides were measured by the luciferin-luciferase method (13). ATP was measured directly; ADP and AMP were measured after enzymatic conversion to ATP. For ATP, ADP, and AMP determinations, 20 μl of neutralized extract was mixed with 20 μl of the appropriate buffer (A, B, or C, respectively). Buffer mix A (for ATP) contained 40 mM Tris-SO_4 (pH 7.75), 10 mM $MgSO_4 \cdot 7H_2O$, 2 mM EDTA, and 0.3 mM phosphoenolpyruvate. Buffer mix B (for ADP) contained buffer mix A with 3 U of pyruvate kinase added per ml. Buffer mix C (for AMP) contained buffer mix B with 20 U of myokinase (resuspended in Tris-SO_4) added per ml.

The enzymatic conversion of ADP and AMP into ATP was carried out at 39°C for 30 min. After incubation, the reaction mixes (40 μl) were diluted with 760 μl of 40 mM Tris-SO_4 buffer (pH 7.75) containing 9 mM EDTA and 10 mM $MgSO_4 \cdot 7H_2O$. The luciferase reaction was initiated by adding 100 μl of a purified luciferin-luciferase mix to 100 μl of diluted extract according to the supplier's recommendations (Sigma). Light output was immediately measured with a luminometer (model 1250; LKB Instruments, Inc., Gaithersburg, Md.), using ATP as a standard. All samples were corrected for extracellular adenine nucleotides.

Phosphate determination. The determination of intracellular phosphate concentration was carried out in cell extracts which were prepared according to the method of Bulthuis et al. (4). The assay consisted of 40 μl of cell extract and 500 μl of ammonium heptamolybdate, malachite green Sterox color reagent (6). Standard curves were constructed with KH_2PO_4 solution (0 to 200 μM). All cell manipulations were carried out with acid-washed glassware, and determinations were carried out in triplicate.

Other analyses. Glucose was analyzed by an enzymatic method, using hexokinase and glucose-6-phosphate dehydrogenase (3). Protein from NaOH-hydrolyzed cells (0.2 M NaOH, 100°C, 15 min) was assayed by the method of Lowry et al. (12). Cellular polysaccharide was assayed by the anthrone method (7). Lactate, ethanol, and fermentation acids in cell-free supernatant samples were analyzed by high-pressure liquid chromatography with a Beckman 334 liquid chromatograph equipped with a model 156 refractive index detector and a Bio-Rad HPX-87H organic acid column. The sample size was 20 μl, the elutant was 0.0065 M H_2SO_4, the flow rate was 0.5 ml/min, and the column temperature was 50°C.

Statistics. All of the experiments were performed two or more times, and the measurements were highly reproducible. The coefficient of variation (standard deviation/mean) was always less than 10%.

RESULTS

Heat production and glucose fermentation of batch cultures. Exponentially growing cells (2.0 h^{-1}) consumed glucose at a rate of 80 μmol/mg of protein per h, and the heat production (1.95 mW/mg of protein) was directly proportional to the enthalpy (ΔH) of glucose conversion to lactate (21 cal [88 J]/mmol). More than 90% of the glucose was recovered as lactate, and the cells never accumulated polysaccharide (<0.2 mg of anthrone-reactive material per mg of protein). The heat production declined to zero as soon as the glucose was depleted. On the basis of these results, it appeared that heat production was an accurate indicator of glucose fermentation rate and ATP production. Under these conditions, maintenance energy (2.6 μmol of ATP per mg of protein per h; see reference 26) accounted for a very small fraction of total energy utilization.

Continuous cultures with pulse doses of glucose. When *S. bovis* was grown in glucose-limited (5 mM) continuous culture (0.1 h^{-1}; pH 6.7), acetate, formate, and ethanol were the end products of glucose fermentation, the glucose consumption was 3.9 μmol of glucose per mg of protein per h, and the heat production was 0.14 mW/mg of protein (Fig. 1a). When a pulse dose of glucose (35 mM) was added to the continuous-culture vessel at 0 h, the fermentation was homolactic. The rate of bacterial growth increased, but during this time the specific growth rate was always less than the maximum growth rate of

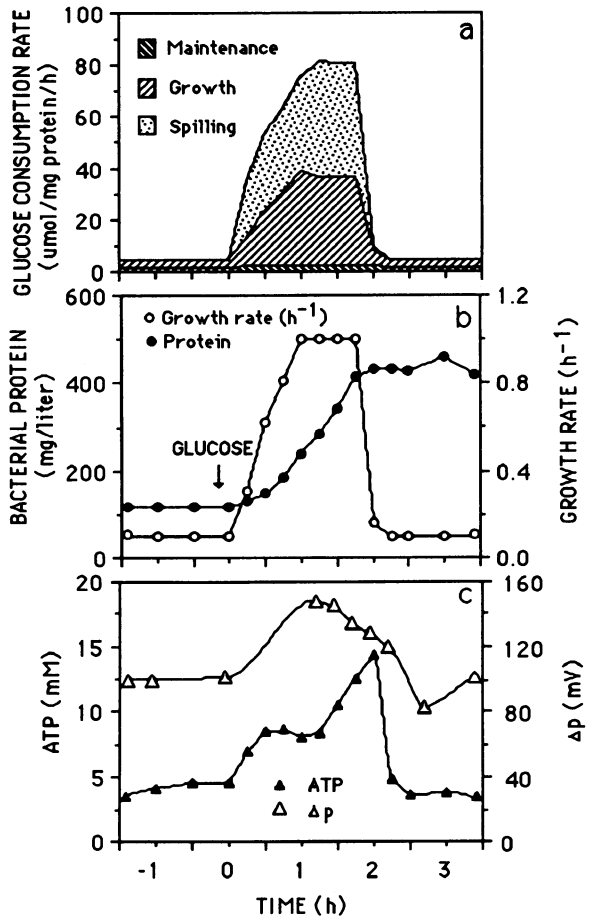

FIG. 1. Effect of a pulse dose of glucose at 0 h on glucose consumption rate (a), protein synthesis and growth rate (b), and ATP and Δp (c) of a continuous culture (0.1 h^{-1}; pH 6.7) of *S. bovis*. The maintenance rate was taken from Russell and Baldwin (26). Growth was calculated as 1/32 mg of cells/mmol of ATP × 1 mg of cells/0.5 mg of protein × 0.33 (glucose limited) or 0.5 mmol (glucose excess) of glucose/mmol of ATP × growth rate × 1,000. Spilling was the difference between the total rate of glucose consumption and that which was counted as maintenance and growth.

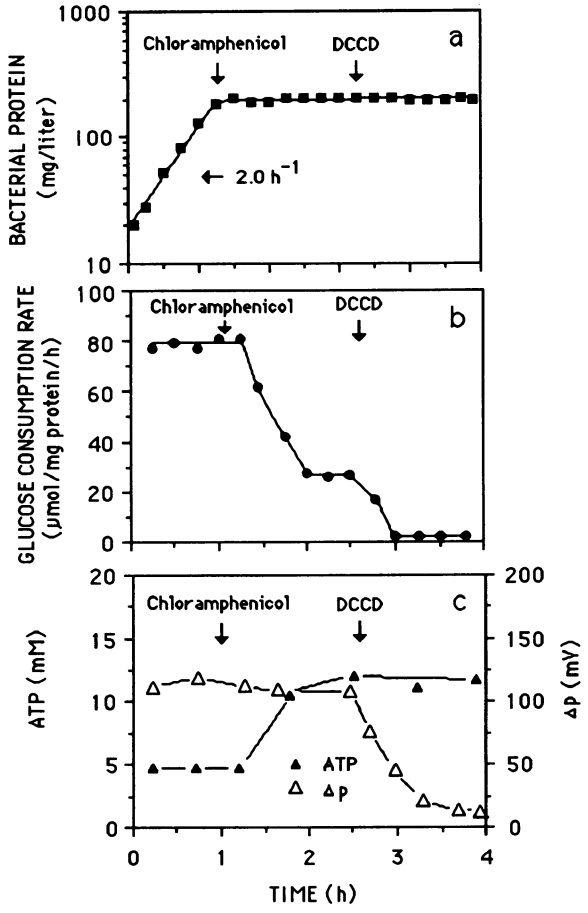

FIG. 2. Effect of chloramphenicol addition (200 μg/ml at 1.0 h) on growth (a), glucose consumption rate (b), and intracellular ATP and Δp (c) of *S. bovis*.

the bacterium (Fig. 1b). Glucose consumption increased at a faster rate than growth, but the cells never accumulated polysaccharide. The rate of glucose consumption was always proportional to the enthalpy change of the homolactic fermentation. The glucose-limited cells had an ATP content of 3 mM, but ATP increased fourfold after the glucose pulse (Fig. 1c). Glucose-limited cells had a Δp of 100 mV, and this value increased to 145 mV after the glucose pulse. When the excess glucose was depleted, ATP, Δp, and the rate of glucose consumption returned to their steady-state values.

Chloramphenicol-treated batch cultures. When exponentially growing cells were treated with chloramphenicol, there was an almost immediate inhibition of protein synthesis (Fig. 2a). The rates of glucose consumption and heat production declined, but even nongrowing cells consumed glucose at a rate of 28 μmol/mg of protein per h (Fig. 2b). More than 90% of the glucose was still accounted for as lactate, the rate of heat production was proportional to the enthalpy change of glucose consumption of glucose conversion to lactate, and the cells did not accumulate polysaccharide. During exponential growth, the intracellular ATP concentration was 5 mM, and Δp was 110 mV (Fig. 2c). Chloramphenicol addition caused an increase in ATP (Fig. 2c), but it had little effect on Δp. When DCCD, an inhibitor of the F_1F_0 ATPase (18), was added to the chloramphenicol-inhibited cells, glucose consumption, heat production, and Δp declined, but there was no change in ATP concentration. When chloramphenicol-inhibited cells were treated with the protonophore 3,3′,4′,5-tetrachlorosalicylanilide (TCS), Δp was abolished, and the specific rate of glucose consumption increased twofold (data not shown). DCCD inhibited the glucose consumption of chloramphenicol- and TCS-treated cells. When chloramphenicol-inhibited cells were treated with the glycolytic inhibitor iodoacetate, the specific rate of glucose consumption decreased gradually (Fig. 3a). This decrease in the specific rate of glucose consumption was associated with a gradual decrease in ATP, but the Δp did not decline (Fig. 3b).

Proton flux (amperage). When chloramphenicol-inhibited cells were treated with iodoacetate to decrease the nongrowth glucose consumption (energy spilling), ATP declined (Fig. 3b), but on the basis of ATP, ADP, and P_i (Fig. 4a), there was little change in ΔG′p (Fig. 4b). The H^+/ATP ratio of the F_1F_0 ATPase was then estimated from H^+/ATP = ΔG′p/Δp (Fig. 4c). As the rate of DCCD-sensitive energy spilling declined, H^+/ATP decreased from 4.2 to 3.6.

On the bases of H^+/ATP, the rate of glucose consumption, and the assumption that the ATP/glucose ratio of a homolactic fermentation is 2, it was possible to estimate the proton flux

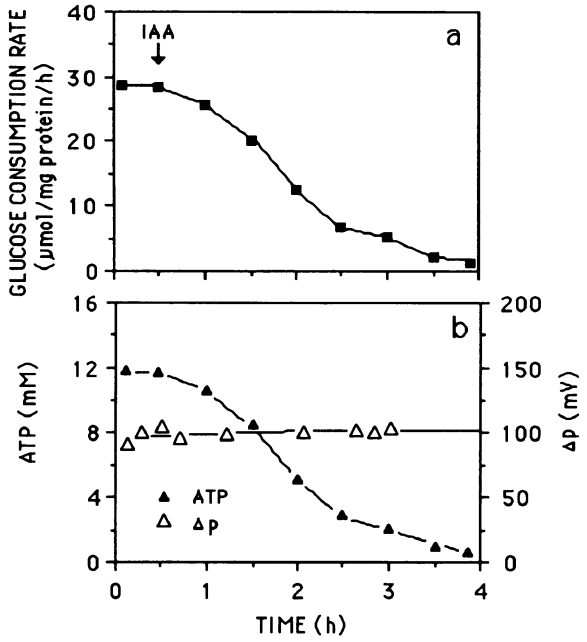

FIG. 3. Effect of iodoacetate (IAA; 50 μM) on glucose consumption rate (a) and intracellular ATP and Δp (b) of nongrowing chloramphenicol-treated *S. bovis* cultures.

(amperage) across the cell membrane: μmol of glucose/mg of protein/h × 6.03 × 10^{14} molecules/μmol × 2 μmol of ATP/μmol of glucose × *n* H^+/ATP ÷ 6.22 × 10^{18} H^+/C × C/s/amp ÷ 3,600 s/h. These calculations indicated that the addition of iodoacetate to the chloramphenicol-treated cells was causing a decrease in the proton flux (amperage) across the membrane (Fig. 5a).

Because the rate of heat production was proportional to the rate of glucose consumption, it was also possible to estimate amperage directly from the heat production. When glucose is converted to lactate, only 38% of the enthalpy is trapped as ATP {[(4 cal/mmol of ATP) × (2 mmol of ATP/mmol of glucose)] ÷ 21 cal/mmol of glucose, where 4 cal = ca. 17 J and 21 cal = ca. 88 J; see reference 28}. Because DCCD had no effect on ATP production but completely eliminated energy spilling (Fig. 2c), it appeared that all of the ATP was being used by the F_1F_0 ATPase. Thus, membrane amperage = Δp/(total wattage × 0.38). The last calculations also indicated that iodoacetate was decreasing proton flux, but amperage values derived from ΔH were 50% lower than those based exclusively on ΔG (Fig. 5a). In both cases, the relationship between Δp and the amperage was nonohmic (Fig. 5b).

Membrane resistance. By Ohm's law (membrane resistance = Δp/membrane amperage), it was then possible to calculate the resistance of the cell membrane to proton conductance (Fig. 5c). Both estimates of amperage (ΔH and ΔG) showed an increase in the membrane resistance as the rate of energy spilling decreased, but the effect was somewhat more dramatic for the ΔH estimate.

DISCUSSION

S. bovis cells treated with chloramphenicol did not grow but still consumed glucose one-third as fast as exponentially growing cells (Fig. 2). Because the nongrowth glucose consumption (energy spilling) was 10 times greater than the maintenance

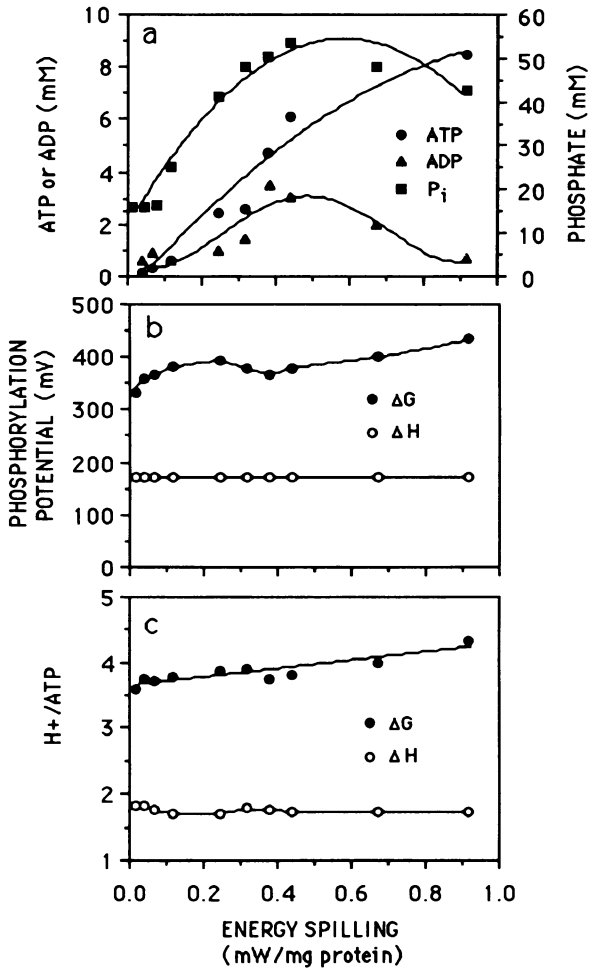

FIG. 4. Rate of energy spilling by chloramphenicol (200 μg/ml)- and iodoacetate (50 μM)-treated *S. bovis* cells and its effect on intracellular ATP, ADP, and inorganic phosphate (a), the phosphorylation potential (b), and the stoichiometry of H^+/ATP (c). The phosphorylation potential (b) (ΔG'p) was calculated from the equation ΔG'p = ΔG°p/F + Zlog [ATP]/[ADP] ×.[P_i] mV. The standard Gibbs energy charge (ΔG°p) was corrected for magnesium, using the data of Rosing and Slater (23). Because the intracellular pH (6.7) did not vary significantly under the experimental conditions used (<0.1 U), ΔG°p/F was 281 mV (6.50 kcal/mol) and Z had a value of 62 mV. The H^+/ATP was calculated as ΔG'p/Δp. The enthalpy-derived (ΔH) estimates for H^+/ATP (c) and phosphorylation potential (b) were based on (0.38 × wattage)/Δp/glucose consumption rate/2ATP/glucose and H^+/ATP × Δp, respectively.

rate of growing cells (26), it appeared that this phenomenon could not be categorized as "maintenance" per se. On the basis of the observation that the nongrowth energy transduction was enhanced by TCS and inhibited by DCCD, it appeared that the ATP turnover was due to the membrane-bound F_1F_0 ATPase and a cycle of protons through the cell membrane (Fig. 6). Since *S. bovis* uses glucose transport mechanisms which are not dependent on Δp (15, 24) and ATP content did not decrease, the effect of DCCD could not be explained by an inhibition of glucose uptake or catabolism per se.

ATP-dependent proton flux should be proportional to the rate of glucose fermentation, the ratio of ATP to glucose, and the H^+/ATP stoichiometry of the F_1F_0 ATPase. Mitchell (17)

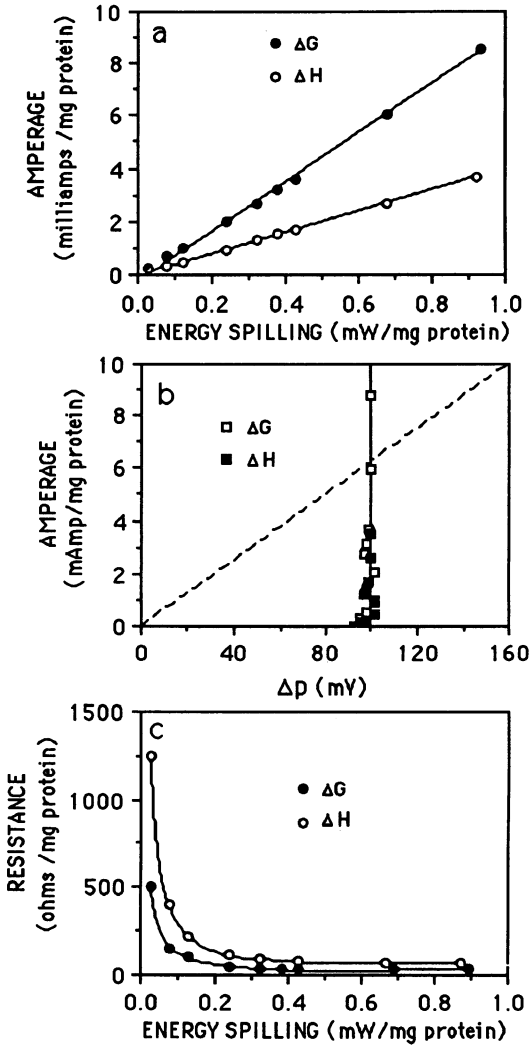

FIG. 5. Relationship between rate of energy spilling and flux of protons (amperage) across the cell membrane (a), between Δp and amperage (b), and between energy spilling and membrane resistance (c). The enthalpy-derived (ΔH) estimates for amperage were based on (0.38 × wattage)/Δp. The ΔG estimates for amperage were based on glucose consumption rate ÷ 2ATP/glucose, H^+/ATP, and Δp (see text).

originally indicated that the F_1F_0 ATPase should have an H^+/ATP stoichiometry of 2, but later work indicated that this value could be as high as 4.3 (14, 20). These estimates were based on the premise that the ΔG of ATP hydrolysis was in equilibrium with Δp or, conversely, that Δp should be a function of ΔG.

When the nongrowth glucose fermentation of *S. bovis* was decreased with iodoacetate, the rate of glucose consumption decreased more than 10-fold. Because there was little change in the ΔG of ATP hydrolysis, it appeared that iodoacetate was decreasing the flux of protons through the cell membrane (Fig. 5a). The failure of iodoacetate to affect Δp, however, indicated that the relationship between proton flux and Δp was nonohmic (Fig. 5b). On the basis of Ohm's law, the amperage of an electrical circuit should be proportional to the voltage.

Biochemists have used ΔG to describe energy changes in

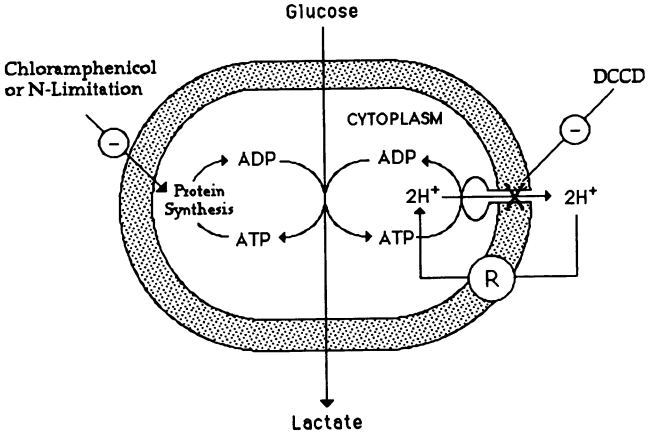

FIG. 6. Hypothetical schematic showing homolactic fermentation of glucose, protein synthesis, membrane ATPase activity, and the resistance of the membrane to proton conductance. The inhibition of membrane ATPase by DCCD and the inhibition of protein synthesis by chloramphenicol or nitrogen limitation are also shown.

biological systems because ΔG accounts for entropy (TΔS), which has the potential to drive thermodynamic reactions. ΔH is, however, a measure of the total energy change, and it represents the true inefficiency of biological reactions: ΔG = ΔH − TΔS. The ΔH of glucose conversion to lactate is 21 cal (88.2 J)/mmol, but this glycolytic scheme of catabolism is only able to produce 2 ATP per glucose. On the basis of the ΔH of ATP (4 cal [16.8 J]/mmol), only 38% of the ΔH is conserved as ATP (16).

Because 38% of the total ΔH would be available to the F_1F_0 ATPase as ATP, it was possible to estimate the amperage of nongrowth energy transduction from the standard power equation, amperage = (0.38 × wattage)/Δp. On the basis of this calculation, however, the amperage was only half as great as the value which was derived solely from ΔG (Fig. 5a). In order for amperage to be as large as the one predicted by ΔG; (i) Δp would have to be very low (approximately 40 mV) or (ii) the heat production would have to be twofold higher than the amount we estimated with our calorimeter. Because the Δp values we measured (approximately 100 mV) are typical of growing or glucose-energized streptococci (14), and the heat measurements were always in close agreement with the enthalpy of glucose fermentation, neither of these possibilities was likely.

The difference in amperage estimates (ΔG versus ΔH) is probably due to the fact that the H^+/ATP stoichiometry is in reality much lower than the one predicted by ΔG. Such disequilibriums are, however, not an uncommon feature of biological systems. Most mechanisms of substrate phosphorylation conserve less than half of the ΔG as ATP (9). Why would chemiosmotic mechanisms be expected to operate at a 100% efficiency of energy transduction?

The nonohmic nature (amperage not proportional to Δp) of the nongrowth energy dissipation indicated that the resistance of the cell membrane to proton conductance was changing (Fig. 6). If DCCD were simply dissociating F_1 from the F_1F_0 so that it could act as a soluble ATPase, the heat production would not have been eliminated by DCCD, an F_0 channel blocker (18). If the DCCD-sensitive step was simply a soluble ATPase rather than the F_1F_0 ATPase, the membrane wattage estimate would have been even lower than the one predicted by ΔH (<0.38 × total wattage), the true amperage (proton

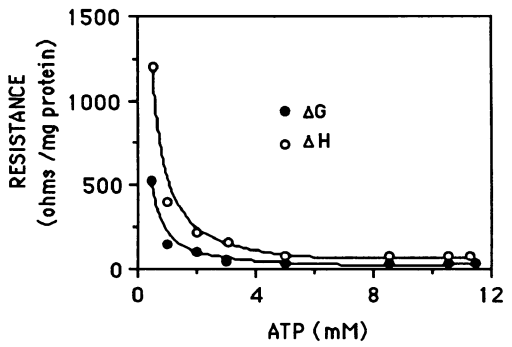

FIG. 7. Relationship between intracellular ATP and membrane resistance of *S. bovis*.

flux) would have been even lower, and the relationship between amperage and Δp would still have been nonohmic.

Krishnamoorthy and Hinkle (8) indicated that the nonohmic behavior of mitochondrial respiration was due to passive proton conductance, but Zoratti et al. (32) indicated that less than 30% of the energy dissipation of nonohmic mitochondrial respiration could be explained by "passive" leaks. Only a large change in membrane resistance could explain the very large change in nonohmic proton flux of *S. bovis* (Fig. 5c).

Konings and his colleagues proposed that electrogenic efflux of lactate via a carrier with a variable lactate/proton stoichiometry could create a proton motive force across the cell membrane (21), but the energetics of this mechanism have been questioned. As Anantharam et al. (1) recently noted, "a variable proton stoichiometry during efflux. . .carries with it the threat of an uncontrolled proton leak unless there is regulation of a kind not yet described." Recent work with *Lactobacillus plantarum* (19) and *Leuconostoc oenos* (11) indicated that lactate translocation was an electroneutral process. Since the yield of *S. bovis* in batch culture was unaffected by the addition of as much as 100 mM sodium lactate, it is unlikely that this bacterium has an electrogenic lactate translocation system (data not shown).

Further work is needed to define the pathway and regulation of proton conductance in bacteria. The brown adipose tissue of mammals has a membrane protein which can translocate protons and increase heat production (5), but such channels have not been demonstrated in bacteria. Because the membrane resistance of *S. bovis* was a function of the intracellular ATP concentration, ATP might be either directly or indirectly regulating membrane resistance. The membrane resistance was low until the intracellular ATP concentration was less than 3 mM (Fig. 7).

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