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Effects of Sodium Ions on the Electrical and pH Gradients Across the Membrane of Streptococcus lactis Cells

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Energized cells of Streptococcus lactis conserve and transduce energy at the plasma membrane in the form of an electrochemical gradient of hydrogen ions (Δp). An increase in energy-consuming processes, such as cation transport, would be expected to result in a change in the steady state Δp. We determined the electrical gradient ($\Delta\psi$) from the fluorescence of a membrane potential-sensitive cyanine dye, and the chemical H^+ gradient (ΔpH) from the distribution of a weak acid. In glycolyzing cells incubated at pH 5 the addition of NaCl to 200 mM partially dissipated the Δp by decreasing $\Delta\psi$, while the ΔpH was constant. The Δp was also determined independently from the accumulation levels of thiomethyl-β-galactoside. The Δp values decreased in cells fermenting glucose at pH 5 or pH 7 when NaCl was added, while the ΔpH values were unaffected; cells fermenting arginine at pH 7 showed similar effects. Thus, these nongrowing cells cannot fully compensate for the energy demand of cation transport.

Key words: protonmotive force, sodium ions, Streptococcus lactis

The work entailed in the transport of solutes against electrochemical gradients requires the input of metabolic energy. However, what portion of the cells' energy budget is expended for this work is not known. To approach this problem, we have begun our studies with resting-cell suspensions of the homolactic fermenter Streptococcus lactis. The rationale was based on the findings, that, in general, bacterial catabolism does not appear to be finely regulated. A number of workers has concluded that bacteria lack control mechanisms for the coupling of energy production to processes involving energy utilization (1–6). For example, Thomas and Batt (7) showed that nongrowing S. lactis cells ferment glucose at rates governed by the rate of sugar supply. Thus, it would not be surprising to encounter situations where the rate of energy supply, e.g., by glycolysis, has not been increased to meet an increased demand by energy-consuming processes. Thus, solutes which are massively transported, such as K^+ and Na^+, could be expected to utilize sufficient metabolic energy to cause measurable changes, even when the supply of energy-yielding substrates is not limiting.

The index of metabolic energy chosen for study is the protonmotive force (Δp), since bacteria transduce and conserve energy at the plasma membrane in for the form of an electrochemical gradient of hydrogen ions, according to the chemiosmotic theory of Mitchell (8, 9). The gradient (Δp) is generated by the proton-translocating membrane-bound ATPase complex which uses the ATP formed only by fermentative pathways in S. lactis cells. The Δp consists of a membrane potential ($\Delta\psi$) and a pH gradient (ΔpH) across the bacterial plasma membrane. These parameters bear the relationship

$$\Delta p = \Delta\psi - 59\ \Delta pH$$

where ΔpH equals the pH_{out} (pH of the bulk medium) minus the pH_{in} (pH of the cytosol). The value 59 is a combination of constants for expression of ΔpH in mV at 25°C.

In a previous communication we reported that addition of K^+ to fermenting cells of S. lactis increased the ΔpH and decreased the $\Delta\psi$ (10). Thus, when the increase in ΔpH could not compensate for the decrease in $\Delta\psi$, the Δp was partially dissipated. In the present communication we report that the addition of Na^+ causes no change in ΔpH, but decreases the $\Delta\psi$, again leading to partial dissipation of Δp. More Na^+ than K^+ is required to effect a measurable change in Δp in these fermenting cells. A preliminary report has appeared (11).

METHODS

Streptococcus lactis ATCC 7962 cells were grown and prepared as described previously (10). Stock suspensions of cells (1.4 mg dry weight/ml) plus (5×10^{-5} M) 1, 1′-dipropyl-2,2′-thiodicarbocyanine dye were prepared as before (10).

The transmembrane electric potential ($\Delta\psi$) was calculated from the fluorescence quenching of the cyanine dye. The fluorescence intensity was related to the K^+ diffusion potential obtained in valinomycin-treated cells suspended in media of various K^+ concentrations (10). Under the conditions of the present experiments, addition of NaCl to 100 mM or 200 mM had no effect on the fluorescence intensity, with K^+ diffusion potentials ranging from 0 to 190 mV.

The pH gradient across the membrane (ΔpH) was estimated from the distribution of [^{14}C]benzoic acid (12, 10). The Δp was also determined from the accumulation levels of [^{14}C]thiomethyl-β-galactoside (TMG) (13, 14). The sources of the chemicals used are listed in Ref. 10.

RESULTS

The experiments consisted of incubating S. lactis cells in media of varying NaCl concentrations and measuring the Δp. The energy was supplied by the fermentation of either glucose or arginine. The Δp was measured by 2 methods: a) from the sum of the values for $\Delta\psi$ (determined from the fluorescence quenching of the cyanine dye) plus those for ΔpH, and b) from the accumulation levels of TMG.

Effects of NaCl on $\Delta\psi$

The fluorescence intensity of suspensions of cells plus dye incubated at pH 5 decreased on addition of glucose, indicating an increase in $\Delta\psi$ (Fig. 1). The fluorescence intensity level reached and maintained for at least 3 min was higher as the concentration of

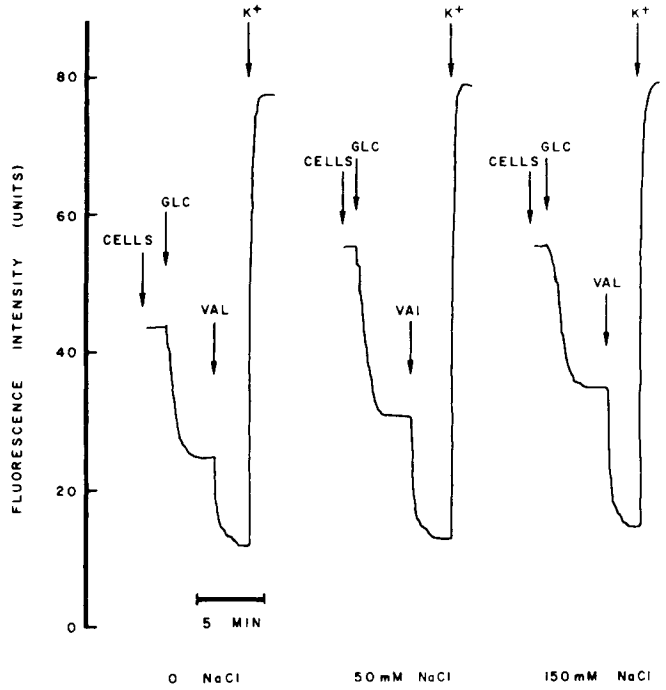

Fig. 1. Effect of NaCl on fluorescence intensity of fermenting cells: 3 representative tracings of chart recordings. Stock cells (1.4 mg dry weight/ml) and dye (5×10^{-5}M) (10) were diluted 1:10 with 0.1 M citrate-Tris buffer, pH 5.0, and NaCl was added to the concentrations indicated under each tracing. The reaction mixtures were added to the cuvettes at the time indicated "CELLS"; glucose was added at time "GLC." The values corresponding to the fluorescence intensity at 2 min after glucose addition were read off a calibration curve as described previously (10). At time "VAL," 2 μl of 1×10^{-2} M valinomycin was added; at "K^+," KCl was added to 230 mM final concentration.

NaCl in the medium was increased, indicating a progressively lower $\Delta\psi$. With these cells the maximum $\Delta\psi$ had not been reached (Fig. 1). When valinomycin was added, the fluorescence intensity decreased further, indicating an increase in $\Delta\psi$. This $\Delta\psi$ was attributed to a diffusion potential for K^+ ions, since the ionophore had rendered the membrane specifically permeable to that cation, and since the concentration ratio in/out of potassium was very large ($> 1{,}000$). Indeed, when K^+ was added to concentrations greater than 200 mM, the fluorescence rose, as expected from a decrease in the $\Delta\psi$ due to a decrease in the potassium diffusion potential. Cells incubated with NaCl were capable of showing a potassium diffusion potential almost as great as that of cells incubated without NaCl, since valinomycin addition lowered the fluorescence almost to the same value.

The chemical gradients of H^+ (ΔpH) were determined in parallel reaction mixtures. To calculate Δp, the values obtained for $\Delta\psi$ and ΔpH were added. As shown on Fig. 2, increasing the NaCl concentration from 0 to 200 mM resulted in a decrease in the poise of Δp from about 150 to 90 mV. The ΔpH was found to remain constant at about 1 pH unit (inside alkaline), while the $\Delta\psi$ decreased with increasing NaCl in the medium. It should be noted, however, that the standard errors of the means were large, ranging from 5 to 22% of the mean values, suggesting that quantitative results obtained by this technique should be considered with reservation.

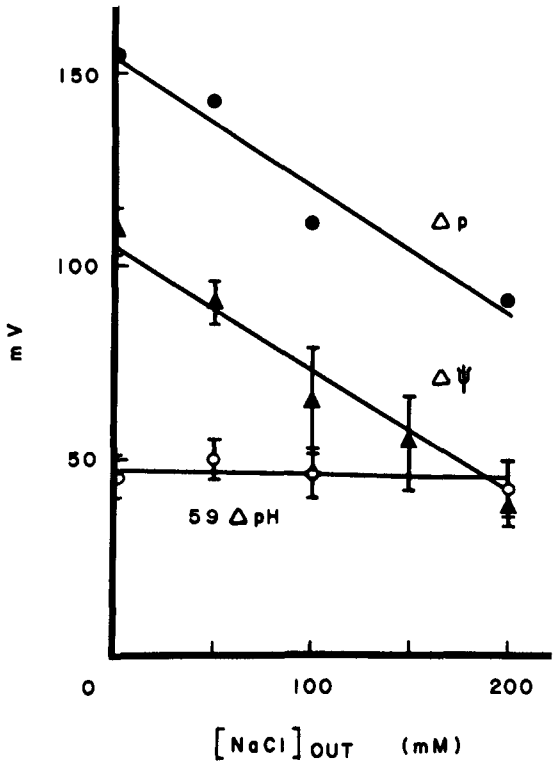

Fig. 2. Effect of NaCl on the electrochemical gradient of hydrogen ions. The $\Delta\psi$ was calculated from the fluorescence intensity and the ΔpH was measured from the distribution of [^{14}C] benzoic acid (see Methods). The Δp was calculated by adding $\Delta\psi$ and 59 ΔpH (see Introduction). Each point represents the average of 5 experiments ± the standard error of the mean. The lines were derived by the method of least squares.

Effects of NaCl on the Accumulation of TMG

The accumulation levels of TMG, which have been shown to correlate directly with Δp (13, 14) were also used to measure the Δp at various NaCl concentrations. This method, in addition to confirming in general the results obtained by the cyanine dye technique, also enabled extension of the experiments to incubation conditions (pH > 5) where the fluorescence intensity reaches levels beyond those in the linear portion of the calibration curve (10). We also established, by performing counterflow experiments (15, 16), that Na^+ (or K^+) had no effect on the TMG carrier per se.

When the effects of NaCl on Δp were tested, as calculated from TMG levels, there was a decrease in Δp with increasing amounts of NaCl, up to 200 mM NaCl (data not shown). At pH 5, with glucose as energy source, the addition of 200 mM NaCl decreased the Δp from 145.4 to 125.9 mV (Table I). This decrease is less than that calculated from the cyanine dye data, where the Δp decreased to 91.4 mV. However, the latter method has been noted to be inaccurate. At pH 7, the Δp decreased from 140.7 to 119.4 mV, with glucose as energy source.

When arginine was used as energy source, there was no significant decrease in Δp at pH 5. At pH 7, however, the Δp was lowered from 131.0 to 106.1 mV.

Since the ΔpH values were not affected by increasing NaCl in the medium, the decreases in Δp are assigned to decreases in $\Delta\psi$.

TABLE I. Effects of NaCl on TMG Accumulation and Transmembrane pH Gradient

Energy source	pH_{out}	$\Delta p(59 \log [TMG]_{in}/[TMG]_{out})$		59 ΔpH (mV)	
		None	200 mM NaCl	None	200 mM NaCl
Glucose	5	145.4 ± 0.8	125.9 ± 1.4	88.8 ± 5.4[a]	66.2 ± 7.6[a]
Glucose	7	140.7 ± 1.7	119.4 ± 1.5	25.5 ± 4.4	23.5 ± 2.5
Arginine	5	99.3 ± 4.1	93.3 ± 5.6	58.9 ± 2.4	59.4 ± 1.9
Arginine	7	131.0 ± 2.7	106.1 ± 2.5	0.0	0.0

The accumulation of TMG and the ΔpH were measured as described previously (10). The values are expressed in equivalent units ± the standard error of the mean. Each value is the average of 6 to 32 determinations, using 2 to 5 batches of cells. The Δp values obtained with NaCl were significantly different from those without NaCl ($p < 0.02$ by Student's t test), except those for arginine-energized cells at pH 5.

[a] The ΔpH values with or without NaCl were not significantly different.

DISCUSSION

Experiments with other membrane systems have suggested that the rate of H^+ extrusion may be rate limiting. For example, Mitchell and Moyle (17) showed that Δp in mammalian mitochondria was about 230 mV when respiration was limited by insufficient inorganic phosphate acceptor (state 4 respiration). When ADP was added to the system (state 3 respiration) the Δp decreased to about 200 mV. Similarly, Pick et al. (18) showed a ΔpH of 3.7 units in illuminated chloroplast preparations deficient in phosphate acceptor; when ADP was added to the ΔpH decreased by about 0.3 units(there is no $\Delta\psi$ component in chloroplast preparations). In intact illuminated cells of Halobacterium halobium Michel and Oesterhelt (19) have measured $\Delta\psi$ from the distribution of the permeant cation triphenylmethyl phosphonium and the ΔpH with the dimethyloxazolidine method. Treatment with dicyclohexylcarbodiimide, an inhibitor of the membrane ATPase, resulted in an increase in Δp, the result of increases of both $\Delta\psi$ and ΔpH. Presumably this increase occured because the synthesis of ATP was abolished in the presence of the inhibitor, and thus influx of H^+ was no longer effected by this system. Thus the utilization of Δp for ATP synthesis appeared to lower the poise of Δp in these 3 systems.

The results reported here can be interpreted as follows: Na^+ ions enter the cell down their electrochemical gradient, thus depressing the $\Delta\psi$. As result, the membrane ATPase can pump more H^+ out of the cell, which would give rise to an increase in ΔpH, were it not for the Na^+/H^+ antiporter. Such a carrier is presumed to be analogous to that described for S. faecalis (20, 21) or Escherichia coli cells (22). The activity of this carrier results in the electroneutral exchange of Na^+ for H^+, thus preventing the expected increase in ΔpH and, at the same time, extruding Na^+.

The effects of Na^+ addition on Δp and its components are different from those seen with K^+ addition. Concentrations of 10 mM KCl or less resulted in decrease of $\Delta\psi$ and increase of ΔpH (10). In contrast, NaCl up to 200 mM effected no change in ΔpH, but decreased the $\Delta\psi$. Addition of 50 mM NaCl did not alter the effects seen with 50 mM KCl (data not shown), thus supporting the view that one cation is transported independently of the other.

In these nongrowing, fermenting cells of S. lactis, therefore, the poise of the proton electrochemical gradient can be altered by the addition of cations that the cells actively transport. While these experiments yield no information on cation flux rates, they suggest that these cells cannot fully compensate for the energy demand of cation transport.

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