Regulation of Glycolysis and Sugar Phosphotransferase Activities in Streptococcus lactis: Growth in the Presence of 2-Deoxy-D-Glucose

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Streptococcus lactis K1 has the capacity to grow on many sugars, including sucrose and lactose, in the presence of high levels (>500 mM) of 2-deoxy-D-glucose. Initially, growth of the organism was transiently halted by the addition of comparatively low concentrations (<0.5 mM) of the glucose analog to the culture. Inhibition was coincident with (i) rapid accumulation of 2-deoxy-D-glucose 6-phosphate (ca. 120 mM) and preferential utilization of phosphoenolpyruvate via the mannose:phosphotransferase system, (ii) depletion of phosphorylated glycolytic intermediates, and (iii) a 60% reduction in intracellular ATP concentration. During the 5- to 10-min period of bacteriostasis the intracellular concentration of 2-deoxy-D-glucose 6-phosphate rapidly declined, and the concentrations of glycolytic intermediates were restored to near-normal levels. When growth resumed, the cell doubling time (T₉₀) and the steady-state levels of 2-deoxy-D-glucose 6-phosphate maintained by the cells were dependent upon the medium concentration of 2-deoxy-D-glucose. Resistance of S. lactis K1 to the potentially toxic analog was a consequence of negative regulation of the mannose:phosphotransferase system by two independent mechanisms. The first, short-term response occurred immediately after the initial "overshoot" accumulation of 2-deoxy-D-glucose 6-phosphate, and this mechanism reduced the activity (fine control) of the mannose: phosphotransferase system. The second, long-term mechanism resulted in repression of synthesis (coarse control) of enzyme IImannose. The two regulatory mechanisms reduced the rate of 2-deoxy-D-glucose translocation via the mannose: phosphotransferase system and minimized the activity of the phosphoenolpyruvate-dependent futile cycle of the glucose analog (J. Thompson and B. M. Chassy, J. Bacteriol. 151:1454–1465, 1982). Phosphoenolpyruvate was thus conserved for transport of the growth sugar and for generation of ATP required for biosynthetic and work functions of the growing cell.

In a recent study (23), we showed that growth of Streptococcus lactis 133 on sucrose or lactose was inhibited by relatively low concentrations of 2-deoxy-D-glucose (2DG). In S. lactis all three sugars are translocated simultaneously with phosphorylation by the multi-component, phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) (for reviews, see references 2, 4, and 11). We suggested that bacteriostasis was a consequence of futile recycling of the glucose analog by the following sequence of vectorial reactions:

\[
2DG_{\text{out}} + \text{PEP}_{\text{in}} \xrightarrow{\text{mannose:PTS}} 2DG-6P_{\text{in}} + \text{Pyruvate}_{\text{in}} \tag{1}
\]

\[
2DG-6P_{\text{in}} + \text{H}_2\text{O} \xrightarrow{\text{hexose 6P:phosphatase}} 2DG_{\text{in}} + P_i \tag{2}
\]

\[
2DG_{\text{in}} \xrightarrow{\text{EIIman or permease}} 2DG_{\text{out}} \tag{3}
\]

Net: \[
\text{PEP}_{\text{in}} + \text{H}_2\text{O} \xrightarrow{} \text{Pyruvate}_{\text{in}} + P_i \tag{4}
\]

where 2DG-6P is 2-deoxy-D-glucose 6 phosphate and EIIman is the enzyme II component of the mannose:PTS.

The futile cycle caused the dissipation of PEP and the generation of ATP via pyruvate kinase (ATP:pyruvate 2-O-phosphotransferase, EC 2.7.1.40) was reduced to a rate incompatible with the energy requirements for normal growth. When a genetic lesion was introduced into the enzyme II component of the mannose:PTS, stage 1 of the cycle was eliminated, and the mutant strain (S. lactis 133 man A) became resistant to the glucose analog.
In a continuation of this work, we found that some wild-type strains were resistant to high levels of 2DG even though such cells contained a functional mannose:PTS. For example, growth of S. lactis K1 in medium containing 10 mM sucrose was not significantly inhibited by >500 mM 2DG. In this communication we describe the resistance phenomenon and suggest mechanism(s) for bacterial immunity to the potentially toxic analog. Our data provide additional insight into in vivo regulation of glycolysis and sugar:PTS activities in growing cells of S. lactis.

MATERIALS AND METHODS

Organism. S. lactis K1 was obtained from the culture collection of the New Zealand Dairy Research Institute, Palmerston North, New Zealand.

Culture maintenance and growth studies. Cultures were maintained, and the organism was grown in complex medium containing 28 mM hexose or 14 mM disaccharide as described previously (24). Growth experiments were performed with 3-ml volumes of culture in sterile, screw-top cuvettes, and growth of cells (absorbance at 600 nm) was followed by using a Gilford 300N microspectrophotometer adapted to accept the growth cuvettes (23). Accumulation of [14C]2DG by growing cells was monitored as described by Thompson and Chassy (23).

Determination of mannose:PTS activity in vivo. Cells of S. lactis K1 were grown, harvested, and washed as previously described (21). The starved cells, which contain an endogenous reserve of PEP (10, 25), were suspended at a concentration of 200 μg (dry weight) of cells per ml in 50 mM potassium phosphate buffer (pH 7) containing 1 mM MgCl2 (KPM buffer) and 5 mM sucrose. After 10 min of incubation at 30°C, 0.2 mM [14C]2DG (specific activity, 0.2 μCi/μmol) was added to the cell suspension. 1.0-ml samples were withdrawn at intervals of 5, 10, 15, 20, and 30 s, and cells were collected by membrane filtration. The initial rates of 2DG-6P accumulation were derived from tangents drawn to each reaction plot. The concentrations of intracellular 2DG-6P were calculated on the basis that 1 g (dry weight) of S. lactis K1 cells was equivalent to 1.67 ml of intracellular (protoplast) fluid (25).

Identification of [14C]-labeled glycolytic intermediates. The effects of 2DG accumulation upon the concentrations of intracellular glycolytic intermediates were determined by modification of a previously described procedure (23). Cells of S. lactis K1 were grown in complex medium containing 10 mM [U-14C]sucrose (specific activity, 0.2 μCi/μmol) and the appropriate concentrations of 2DG. In the exponential phase of growth (absorbance at 600 nm, 0.5 or 0.75; Gilford 300 N spectrophotometer), 30 ml of culture containing 4.7 or 7 mg total dry weight of cells was rapidly filtered onto a 47-mm-diameter membrane filter (Millipore Corp.; type HA, 0.22-μm pore size). The filter with the cells was immediately transferred to 5 ml of boiling water for 5 min, and intracellular metabolites were extracted. The cell extract was clarified by centrifugation at 13,000 × g for 30 min at 4°C. The supernatant fluids were removed, lyophilized, and reconstituted with 50 μl of distilled water. Radiolabeled glycolytic intermediates present in 5-μl volumes of the cell extracts (ca. 80,000 to 100,000 cpn) were separated and identified by polyethyleneimine-cellulose thin-layer chromatography (21, 23). Intracellular concentrations of glycolytic intermediates were calculated from the radioactivity of each metabolite as described previously (23).

Enzyme assays. Cells of S. lactis K1 were permeabilized with a mixture of toluene-acetone, and PTS activities were determined by the in vitro spectrophotometric method of St. Martin and Wittenberger (19). ATP was determined by using firefly luciferase-luciferin in according to the procedure described in the lucinescence reagent kit obtained from Packard Instrument Co. Determinations were made with an AminoChem Glow (model J4-7441) photometer and an integrator timer (model J4-7462-A). Samples of reconstituted cell extracts were diluted 100-fold, and 10 μl of diluted solution was used for ATP analysis. The final reaction volume was 400 μl, and calibration curves of 100 to 800 pmol of ATP were prepared from triplicate standards. Protein was determined by the Coomassie brilliant blue dye-binding assay of Bradford (1) with bovine gamma globulin as the protein standard.

In vitro PTS complementation assay. Cells of S. lactis K1 were grown in 3.2 liters of complex medium containing 14 mM sucrose, or 14 mM sucrose and 10 mM 2DG (22). The cells were harvested, washed, and disrupted in a Bead-Beater (Biospec Products, Bartlesville, Okla.) as described previously (22). Cell debris and residual whole cells were removed by centrifugation at 42,000 × g for 30 min, and the supernatant fluid was further centrifuged for 120 min at 220,000 × g. The high-speed supernatant fluid was concentrated to 20 ml in an Amicon pressure ultrafiltration cell (UM-05 membrane). The pellet obtained by high-speed centrifugation was suspended in 10 ml 0.1 M HEPES (N-2-hydroxyethylpiperazine-N-2'-ethanesulfonic acid) buffer (pH 7.5) containing 1 mM diithiothreitol. The formation of [14C]2DG-6P was followed by its binding to DEAE-cellulose (DE-81) filter circles by the method of Sherrington et al. (18). The PTS assay contained 100 mM HEPES buffer (pH 7.5), 1 mM diithiothreitol, 5 mM PEP, 10 mM MgCl2, 10 mM NaF, 5 mM [14C]2DG (specific activity, 0.2 μCi/μmol), 0 to 25 μl of high-speed centrifugation supernatant, and 0 to 50 μl of high-speed pellet membranes in a final reaction volume of 100 μl. Incubation was at 37°C for 20 min. Samples (30 to 50 μl) of the reaction mixture were transferred to DE-81 filter circles, washed free of unreacted [14C]2DG, and air dried. [14C]2DG-6P retained on the filter was determined by liquid scintillation counting.

Reagents. Chemicals and reagents were purchased from the Sigma Chemical Co., St. Louis, Mo. Radiolabeled compounds, [1-14C]2DG and [1-14C]2DG-6P (disodium salt), were obtained from New England Nuclear Corp., Boston, Mass. Luminescence reagents (Picozyme Kits, I, II, III, IV) were purchased from Packard Instrument Co., Inc., Downers Grove, Ill.

RESULTS

Effect of 2DG on growth of S. lactis K1. The doubling time (T_{d}) of S. lactis K1 in complex medium containing 14 mM sucrose was approximately 44 min (Fig. 1). When 2DG was added to a series of actively growing cultures at concen-
trations of >0.05 mM, an immediate, but transient, inhibition of growth occurred. After a short period of bacteriostasis (ca. 5 to 10 min), growth was resumed at a rate dependent upon the concentration of the analog in the medium (e.g., $T_d$ at 10 mM 2DG was approximately 65 min).

Accumulation of 2DG and identification of intracellular products. Previously we showed that inhibition of growth of $S.\ lactis$ 133 occurred simultaneously with the accumulation and maintenance of 2DG-6P to an intracellular concentration of >120 mM (23). It was important, therefore, to determine whether the analog was also accumulated by the 2DG-insensitive strain. The addition of 0.5 mM $[^{14}\text{C}]2\text{DG}$ to a culture of $S.\ lactis$ K1 caused an immediate inhibition of growth, coincident with accumulation of $[^{14}\text{C}]2\text{DG}-6\text{P}$ to ca. 130 mM (Fig. 1, inset). Approximately 95% of the radioactive material extracted from the cells cochromatographed with authentic 2DG-6P, and the remainder was $[^{14}\text{C}]2\text{DG}$. Within 2 to 3 min of attainment of maximum 2DG-6P uptake, the intracellular concentration of the derivative rapidly declined, and growth was resumed. After 25 min the 2DG-6P concentration had decreased by approximately 70% and throughout subsequent growth was maintained at ca. 25 mM. The major intracellular derivative was $[^{14}\text{C}]2\text{DG}-6\text{P}$, but additional products (~5% of the total radioactive material) were detected by paper chromatography. One derivative was tentatively identified as 6-phospho-2-deoxygluconate, and a second compound remained at the point of origin, but was not identified. The steady-state concentration of 2DG-6P (ca. 25 mM) maintained by $S.\ lactis$ K1 during growth (Fig. 1, inset) was four- to fivefold lower than that maintained by $S.\ lactis$ 133 during bacteriostasis (23).

2DG-6P accumulation and depletion of glycolytic intermediates. The experiment shown in Fig. 2 was conducted to study the effect of 2DG uptake upon the concentrations of intracellular glycolytic intermediates. Cells of $S.\ lactis$ K1 were grown (Fig. 1.) in medium containing [U-$^{14}$C]sucrose, and extracts were prepared from cells sampled before (Fig. 1, arrow 1) and at intervals of 3 and 30 min after the addition of 2DG to the culture (Fig. 1, arrows 2 and 3, respectively). Radiolabeled glycolytic intermediates in the three extracts were quantitatively

FIG. 1. Effect of increasing concentrations of 2DG upon growth of $S.\ lactis$ K1 in complex medium containing 14 mM sucrose as the energy source. At the midlog phase of growth (absorbance at 600 nm, 0.5), 2DG was added to the cultures to obtain the following (millimolar) concentrations: (●) control (no 2DG); (○) 0.05; (▲) 0.5; (▲) 1; (□) 10. Inset. Accumulation and regulation of intracellular $[^{14}\text{C}]2\text{DG}-6\text{P}$ by cells of $S.\ lactis$ K1. $[^{14}\text{C}]2\text{DG}$ (0.5 mM; specific activity, 0.2 µCi/µmol) was added to the culture, and uptake of $[^{14}\text{C}]2\text{DG}-6\text{P}$ during the initial overshoot, transient inhibition, and resumption of growth was followed as described in the text. Samples from a duplicate system containing $[^{14}\text{C}]$sucrose were removed at times indicated by arrows 1, 2, and 3, and extracts were analyzed for $^{14}$C-labeled glycolytic intermediates as described in the legend to Fig. 2.
identified by thin-layer fluorography (Fig. 2). The control cells contained ca. 20 mM fructose 1,6-diphosphate, 10 mM of a mixture of PEP and 2- and 3-phosphoglyceric acids, and lower concentrations of hexose 6-phosphate and sucrose 6-phosphate (Fig. 2, lane 1). Within 3 min after 2DG addition (i.e., at the point of growth inhibition and maximum uptake of 2DG-6P) the cells were essentially depleted of glycolytic intermediates (Fig. 2, lane 2). When growth resumed (Fig. 1, arrow 3) the concentrations of the metabolites were restored and began to approach those present in control cells (Fig. 2, lane 3). At 3 min after the addition of 2DG to the culture, the intracellular ATP concentration had fallen from 4.4 to 1.8 mM (a decrease of approximately 60%), and when growth resumed the ATP concentration (1.4 mM) was still considerably lower than the concentration in control cells.

**Accumulation of 2DG by resting and chloramphenicol-treated cells.** Cells of \( S. \) lactis \( K1 \) were suspended in buffered medium containing sucrose, and after 10 min, when steady-state glycolysis had been attained, \([^{14}C]2DG \) was added to the suspension. Within 15 min the resting cells accumulated \([^{14}C]2DG-6P \) to ca. 100 mM (Fig. 3), but in contrast to growing organisms this high steady-state level of sugar phosphate was maintained for the duration of the experiment. When this experiment was conducted with resting cells grown previously in the presence of 2DG, the concentration of 2DG-6P maintained at the steady state was only 30% of that maintained by cells grown in the absence of the analog (Fig. 3). In neither case did the addition of the complex medium components elicit a reduction in intracellular 2DG-6P concentration (data not shown). However, accumulation and rapid expulsion of 2DG-6P was found in cells whose growth had been terminated by chloramphenicol (Fig. 4). This result showed that the short-term capacity of \( S. \) lactis \( K1 \) to regulate the intracellular concentration of the glucose analog was independent of de novo protein synthesis.

**Short-term regulation of intracellular 2DG-6P by growing cells.** The resumption of growth after exposure of the cells to 2DG (Fig. 1) resulted from a regulatory mechanism which reduced the steady-state concentration of 2DG-6P. This short-term response could have been achieved by either (A) an increased rate of dephosphorylation and expulsion of 2DG or (B) a reduction in the rate of 2DG-6P accumulation by the mannose:PTS. Possibility (A) seemed unlikely from...
FIG. 4. Regulation of 2DG-6P accumulation by chloramphenicol-treated cells of S. lactis K1. Growth conditions were as described in Fig. 1. Chloramphenicol (○) was added to the culture (arrow 1) to a final concentration of 25 μg ml⁻¹. When growth had ceased (absorbance at 600 nm, 0.7), 10 mM sucrose was added (arrow 2) to ensure continued glycolysis, and 15 min later 0.5 mM [¹⁴C]2DG (specific activity, 0.2 μCi/μmol) was added to the cuvette (arrow 3). Intracellular concentrations of [¹⁴C]2DG-6P (inset) were determined by standard procedures.

An energetic standpoint, because this would not reduce the rate of PEP dissipation by the futile cycle. Possible mechanisms for (B) could include: (i) inactivation or reduced synthesis of the mannose:PTS, (ii) changes in kinetic parameters of this PTS toward 2DG, (iii) reduced levels of intracellular PEP or the phosphorylated histidine-containing phosphocarrier protein of the PTS (HPr ~ P), or (iv) product inhibition of mannose:PTS activity by intracellular 2DG-6P. The four possibilities were considered in the following experiments.

(i) Activity of the mannose:PTS during growth in the presence of 2DG. The data presented in Table 1 show that mannose:PTS activity remained constant (throughout at least four doublings of cell mass) after addition of 2DG to the medium. These results discounted the first possibility.

(ii) Kinetic parameters of the mannose:PTS. The effect of 2DG upon the kinetic characteristics of the mannose:PTS in vivo was studied during growth of the organism in medium containing increasing concentrations of [¹⁴C]2DG. The data (Fig. 5A) showed that after transient growth inhibition, the intracellular [¹⁴C]2DG-6P concentrations and the cell doubling times were dependent upon the concentration of the analog in the medium and, therefore, upon the activity

<table>
<thead>
<tr>
<th>Absorbance (600 nm)</th>
<th>Mannose:PTS activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>of growing cultures</td>
<td>Control (no 2DG) +2DG</td>
</tr>
</tbody>
</table>
| 0.1                 | 45.3                  | 36.8*
| 0.2                 | 42.2                  | 44.2
| 0.4                 | 50.7                  | 43.5
| 0.8                 | 52.2                  | 43.5
| 1.6                 | 54.8                  | 38.0

*Expressed as micromoles of 2DG-6P accumulated per gram (dry weight) of cells per minute. Cells were removed from the culture at the designated absorbance, harvested, and washed, and the initial rate of [¹⁴C]2DG uptake was determined.

a Cells were grown in complex medium containing 14 mM sucrose.

b 2DG (1 mM) was added to the culture immediately after removal of this first sample (at an absorbance at 600 nm of 0.1).
FIG. 5. (A) Effect of extracellular (medium) concentration of 2DG upon cell doubling time (●) and intracellular steady-state concentration of 2DG-6P (○) maintained by cells of S. lactis K1. (B) Double-reciprocal plot of intracellular steady-state 2DG-6P concentration maintained by growing cells versus concentration of 2DG in the medium.

of the mannose:PTS and rate of PEP utilization. In the present experiments the half-time ($T_{1/2}$) of 2DG efflux from growing cells was ca. 5 s, and the rate constant $k$ for efflux was 8.3 min$^{-1}$ (Thompson and Chassy, unpublished data).

The intracellular 2DG-6P concentrations maintained by S. lactis K1 during exponential growth represented a steady-state condition (Fig. 5A) in which the rate of accumulation of 2DG-6P via the mannose:PTS was equal to the rate of de-
phosphorylation and efflux of 2DG. Therefore \( \frac{d[2DG-6P]_{in}}{dt} = 0 \), and in the steady state (5):

\[
\frac{V_{max}^{en} [2DG]_{ex}}{[2DG]_{ex} + K_m^{en}} = k [2DG-6P]_{in}
\]

entry = efflux

\( K_m^{en} \) is the apparent "affinity" constant of the mannose:PTS for 2DG, \( V_{max}^{en} \) is the maximum rate of uptake of the analog, and \( k \) is the first-order rate constant for dephosphorylation and 2DG exit. \([2DG]_{ex}\) and \([2DG-6P]_{in}\) represent the extracellular and intracellular concentrations of the free and phosphorylated analog, respectively. Rearrangement and transposition of equation (5) yields the linear equation

\[
\frac{1}{[2DG-6P]_{in}} = \frac{kK_m^{en}}{V_{max}^{en} [2DG]_{ex} + k} + \frac{1}{V_{max}^{en}}
\]

The reciprocal plot of the data from Fig. 5A, according to this equation, and the slope \( (kK_m^{en}/V_{max}^{en}) \) and intercept \( (k/V_{max}^{en}) \) values are shown in Fig. 5B. \( V_{max}^{en} \) (600 \( \mu \)mol of 2DG-6P accumulated per g [dry weight] of cells per min) was determined by substitution for \( k = 8.3 \) min\(^{-1} \) in the intercept formula. A second substitution for \( V_{max}^{en} \) into the slope formula yields a value for \( K_m^{en} \) of 0.13 mM. These kinetic parameters determined from steady-state data in growing cells are in reasonable agreement with previous data \( (K_m^{en}, 0.08 \) mM; and \( V_{max}^{en}, 360 \) \( \mu \)mol of 2DG-6P accumulated per g [dry weight] of cells per min) determined from initial rate studies of 2DG uptake by PEP-preloaded, starved cells (21). The kinetic parameters of the mannose:PTS toward 2DG were not changed significantly during growth of \( S. lactis \) K1 in the presence of 2DG.

(iii) Possibility of regulation of 2DG-6P uptake by PEP limitation. The possibility of regulation of 2DG-6P uptake by PEP limitation was investigated in the experiment described in Fig. 6. Cells

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FIG. 6. Identification and intracellular (millimolar) concentrations of \(^{14}\)C-labeled glycolytic intermediates present in growing cells of \( S. lactis \) K1. Cells were grown in complex medium containing \(^{14}\)C-sucrose and increasing (micromolar) concentrations of 2DG: 0, control lane 1; 25, lane 2; 75, lane 3; 150, lane 4; and 500, lane 5.
of *S. lactis* K1 were grown (Fig. 5A) in media containing [14C]sucrose and increasing concentrations of 2DG. Glycolytic intermediates present in the growing cells were determined after extraction and thin-layer fluorography (Fig. 6). The intracellular sucrose 6-phosphate and hexose 6-phosphate levels remained generally constant, but the concentrations of fructose 1,6-diphosphate, 3-phosphoglycerate, 2-phosphoglycerate, and PEP decreased with increasing 2DG level in the medium. Since the glycolytic reactions from fructose 1,6-diphosphate to PEP are reversible and close to equilibrium, a reduction in PEP concentration would be readily transmitted to the first metabolite in the sequence (fructose 1,6-diphosphate) so that: (i) intracellular fructose 1,6-diphosphate, 3-phosphoglycerate, 2-phosphoglycerate, and PEP concentrations would decrease simultaneously, and (ii) the ratio of the concentrations of these metabolites would remain constant (Fig. 7). The data in Fig. 6 and 7 support both predictions. The intracellular ATP levels in control cells and in cells growing in the presence of 25, 75, 150, and 500 μM 2DG were 4.7, 3.3, 4.4, 4.6, and 4.8 mM, respectively. The cells metabolized sucrose at near-normal rates and maintained an intracellular PEP level 60 to 65% of that found in control cells. It is unlikely that decreased uptake of 2DG-6P (after the initial accumulation; Fig. 1) can be attributed to limitation of intracellular PEP (Fig. 2, lane 3). When cells were grown in the presence of 2DG, fermentation of sucrose became increasingly heterolactic and, besides lactic acid, ethanol and acetate were detected in the medium (Thompson and Chassy, unpublished data).

(iv) Effect of 2DG-6P on mannose:PTS activity. In vitro complementation studies with cell extracts (Table 2) showed that 25 mM 2DG-6P had little inhibitory effect upon mannose:PTS activity. Furthermore, extracts prepared from cells grown in the presence of 2DG did not contain any factor which alone or in combination with 2DG-6P was able to inhibit the mannose:PTS in vitro.

Long-term repression of mannose:PTS synthesis. In the short term, the addition of 2DG to a growing culture of *S. lactis* K1 produced no significant repression of the mannose:PTS (Table 1). However, growth for several generations in medium containing 10 mM 2DG did result in repression of mannose:PTS synthesis, and both the initial rate and the maximum level of 2DG-6P accumulated by the cells were approximately 70% lower than those observed in control cells. (Fig. 3). This result was confirmed by in vitro assay of PTS activities in permeabilized cells (Table 3), where it was found that PEP-dependent phosphorylation of mannose:PTS substrates (glucose, mannose, 2DG, glucosamine, and N-acetylglucosamine) was reduced by 80%. In vitro complementation studies showed that the reduced mannose:PTS activity was due to
TABLE 2. Effect of 2DG-6P\(^{a}\) upon the activity of the mannose:PTS in vitro

<table>
<thead>
<tr>
<th>HSS(^{b})</th>
<th>HSS sample vol (µl)</th>
<th>Source of HSP(^{d})</th>
<th>HSP sample vol (µl)</th>
<th>Sp act(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. lactis K1</td>
<td>40</td>
<td>S. lactis K1</td>
<td>10</td>
<td>1.43</td>
</tr>
<tr>
<td>S. lactis K1</td>
<td>40</td>
<td>S. lactis K1 (2DG)(^{e})</td>
<td>10</td>
<td>0.75</td>
</tr>
<tr>
<td>S. lactis K1</td>
<td>20</td>
<td>S. lactis K1</td>
<td>10</td>
<td>1.60</td>
</tr>
<tr>
<td>S. lactis K1 (2DG)(^{e})</td>
<td>20</td>
<td>S. lactis K1</td>
<td>10</td>
<td>1.28</td>
</tr>
</tbody>
</table>

\(^{a}\) 2DG-6P was present in the assay at a final concentration of 25 mM.

\(^{b}\) HSS, High-speed supernatant fluid.

\(^{c}\) Expressed as nanomoles of \([^{14}C]2DG-6P\) formed per milligram of high-speed supernatant fluid protein per minute. The assays contained ca. 28 µg of high-speed pellet protein, and other conditions were as described in the text.

\(^{d}\) HSP, High-speed (membrane) pellet.

\(^{e}\) Designates preparations derived from S. lactis K1 grown in the presence of 2DG.

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decreased levels of the membrane-associated EII\(^{\text{mann}}\) (data not shown).

DISCUSSION

When sucrose is the sole PTS sugar present during growth of S. lactis K1, intracellular PEP is consumed by the sucrose:PTS and via ATP:pyruvate 2-0-phosphotransferase (pyruvate kinase) for ATP generation. Throughout exponential growth the rate of sucrose transport must be closely linked to and controlled by glycolytic activity (7, 8, 13). In addition, this metabolic cycle (Fig. 8, route 1) must be regulated so that sucrose is translocated only as fast as it can be metabolized by the growing cell. One might expect that the addition to the medium of a second sugar (2DG)—which is both non-metabolizable and a substrate of a high-affinity PTS—would disturb the energetic balance of the growing cell. We attribute the initial inhibitory effect of the glucose analog to competition between the sucrose-EII\(^{\text{auc}}\) and 2DG-EII\(^{\text{mann}}\) complexes for the common high-energy phosphorlyl donor (HPr-P) (2, 4, 17). When 2DG was added to the culture, the analog was rapidly accumulated (as 2DG-6P) via the mannose:PTS with concomitant depletion of PEP and other glycolytic intermediates. At the same time growth was transiently halted. Previously we suggested that growth stasis in S. lactis 133 resulted from the dissipation of intracellular PEP after the establishment of an energetically wasteful 2DG futile cycle (23) (Fig. 8). It is clear from the data in Fig. 1 and Fig. 2 (lane 2) that, upon first exposure of S. lactis K1 to 2DG, intracellular PEP and HPr-P were utilized preferentially for translocation of 2DG via the mannose:PTS (Fig. 8, route 2). S. lactis K1 had the potential to establish, but did not sustain, a high level of futile cycle activity. Within minutes of attainment of maximum intracellular 2DG-6P (ca. 120 mM), a regulatory response occurred which reduced the activity of the futile cycle. This response resulted in (i) a rapid decrease in intracellular 2DG-6P, (ii) reestablishment of normal concentrations of glycolytic intermediates, and (iii) resumption of growth at a rate dependent upon the concentration of 2DG in the growth medium (Fig. 5A) and upon the degree of saturation of the mannose:PTS. The most significant feature of this short-term response was that PEP and HPr-P were no longer consumed preferentially by the mannose:PTS (Fig. 8, route 2), but were instead utilized primarily for translocation of the growth sugar (Fig. 8, route 1).

We have examined several possible mechanisms for this short-term regulatory response,

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TABLE 3. PTS activities\(^{a}\) in permeabilized cells in S. lactis K1 after prolonged growth\(^{b}\) in the presence (or absence) of 2DG

<table>
<thead>
<tr>
<th>Sugar tested</th>
<th>Control cells</th>
<th>Cells grown with 2DG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>161.7</td>
<td>&lt;11.5</td>
</tr>
<tr>
<td>Mannose</td>
<td>160.6</td>
<td>13.7</td>
</tr>
<tr>
<td>2DG</td>
<td>98.7</td>
<td>22.3</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>61.9</td>
<td>&lt;11.5</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>52.8</td>
<td>&lt;11.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>94.0</td>
<td>86.8</td>
</tr>
<tr>
<td>Galactose(^{d})</td>
<td>&lt;11.5</td>
<td>&lt;11.5</td>
</tr>
</tbody>
</table>

\(^{a}\) Determined by in vitro spectrophotometric assay (19) with permeabilized cells.

\(^{b}\) Cells were serially transferred and finally grown overnight in complex medium containing 14 mM sucrose and, when required, 10 mM 2DG.

\(^{c}\) Expressed as micromoles of NADH oxidized (i.e., sugar phosphate formed) per gram (dry weight) of cells per minute. Glucose, mannose, 2DG, glucosamine, and N-acetylglucosamine are transported by the mannose:PTS in S. lactis (24).

\(^{d}\) Galactose (C-3, C-4 epimer of glucose) is not transported by the mannose:PTS, and this assay provided a measure of the endogenous rate of NADH oxidation.
but a satisfactory explanation is not yet at hand. Product (2DG-6P) inhibition of the mannose:PTS at the inner surface of the cytoplasmic membrane was a likely possibility, because inhibition of PTS activities by sugar phosphates has been reported in intact cells (4, 11, 14, 15) and in membrane vesicles (6). However, three observations militate against this attractive proposal. First, 2DG-6P at a concentration of 25 mM did not inhibit the mannose:PTS in vitro (Table 2). Second, from this mode of inhibition one would expect the same pattern of rapid accumulation and efflux of 2DG from resting cells, but this was not observed (Fig 3). Finally, 2DG-6P inhibition of mannose:PTS activity in vivo would preclude the establishment of the PEP-dependent futile cycle in sensitive strains, e.g., S. lactis 133 (23).

In the short term neither the synthesis nor the kinetic parameters of the mannose:PTS were significantly reduced by addition of 2DG to the medium. Furthermore, intracellular PEP does not appear to be rate limiting for 2DG translocation, because after a period of transient inhibition, cells growing in the presence of 2DG contained at least 60 to 65% of the PEP levels present in control cells (Fig. 6) and readily transported the growth sugar via the sucrose:PTS.

It should be noted that the level of 2DG-6P accumulated by S. lactis K1 is governed by the availability of HPr~, and if the short-term regulatory mechanism limits the supply of this high-energy phosphoryl donor to the mannose:PTS, then 2DG-6P accumulation will be reduced accordingly. Evidence for such a regulatory mechanism has recently been obtained by Scholte and Postma (17) in Salmonella typhimurium. These workers studied competition between the two PTS systems (EII-A/EII-B and EII~mannose/EII~mannose) which mediate glucose translocation in S. typhimurium. They concluded that the flow of phosphoryl groups via EI and HPr,
(rather than PEP concentration per se) imposed the rate limiting step for in vivo transport of glucose via the preferred EII^bc/EIIII^bc system. We believe that the regulatory response observed after the initial "overshoot" of 2DG-6P accumulation by S. lactis K1 may result from either (i) limitation of the availability of HPr-P for the mannose:PTS or (ii) reduction of the affinity of the mannose:PTS for HPr-P. Such limitations would reduce the accumulation of 2DG-6P, and this could occur without significant change in the kinetic parameters (V_max, K_m) of the 2DG transport system.

Short-term resistance to 2DG is a consequence of fine control of activity of the mannose:PTS. However, growth of S. lactis K1 for several generations in medium containing 2DG resulted in repression of synthesis (coarse control) of EII^man (Table 3); this also reduced the level of 2DG-6P uptake by the cell. The molecular basis for inhibition of EII^man synthesis is not known, but intracellular 2DG-6P may serve as an inhibitor of gene expression. London and Hausman (9) have described a similar repression of EII^vtilol synthesis by intracellular (and non-metabolizable) xylitol 5-phosphate in Lactobacillus casei. The net effect of short- and long-term regulation of the mannose:PTS is that both mechanisms prevent excessive dissipation of PEP by the 2DG futile cycle (Fig. 8). Energy is therefore conserved for biosynthetic and work functions of the growing cell.

In 2DG-sensitive strains, e.g., S. lactis 133, the short-term regulatory response does not occur (23). However, 2DG-resistant mutants of these strains arise with high frequency and are defective in the EII^man. By contrast, resistance in S. lactis K1 is achieved by modulation of extant regulatory mechanisms rather than by mutation. Our studies with S. lactis, and previous investigations with oral streptococci (e.g., Streptococcus salivarius and S. mutans [3, 12, 16, 20, 26]) have demonstrated that genetically similar organisms may exhibit wide variation in sensitivity to 2DG. The recent discovery of the PEP-dependent futile cycle in S. lactis (23) and the results of the present study may provide a biochemical basis for 2DG sensitivity and resistance in the streptococci.

ADDENDUM IN PROOF

After submission of this manuscript, Stock et al. (J. Biol. Chem. 257:14543-14552, 1982) reported repression of EII^man synthesis by 2DG in S. typhimurium.

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LITERATURE CITED


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