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Uptake and Metabolism of Sucrose by *Streptococcus lactis*

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Transport and metabolism of sucrose in *Streptococcus lactis* K1 have been examined. Starved cells of *S. lactis* K1 grown previously on sucrose accumulated [^{14}C]sucrose by a phosphoenolpyruvate-dependent phosphotransferase system (PTS) (sucrose-PTS; K_m, 22 μM; V_{max}, 191 μmol transported min^{-1} g of dry weight of cells^{-1}). The product of group translocation was sucrose 6-phosphate (6-*O*-phosphoryl-D-glucopyranosyl-1-α-β-2-D-fructofuranoside). A specific sucrose 6-phosphate hydrolase was identified which cleaved the disaccharide phosphate (K_m, 0.10 mM) to glucose 6-phosphate and fructose. The enzyme did not cleave sucrose 6′-phosphate (D-glucopyranosyl-1-α-β-2-D-fructofuranoside-6′-phosphate). Extracts prepared from sucrose-grown cells also contained an ATP-dependent mannofructokinase which catalyzed the conversion of fructose to fructose 6-phosphate (K_m, 0.33 mM). The sucrose-PTS and sucrose 6-phosphate hydrolase activities were coordinately induced during growth on sucrose. Mannofructokinase appeared to be regulated independently of the sucrose-PTS and sucrose 6-phosphate hydrolase, since expression also occurred when *S. lactis* K1 was grown on non-PTS sugars. Expression of the mannofructokinase may be negatively regulated by a component (or a derivative) of the PTS.

The consequences of streptococcal growth on disaccharides can be detrimental or beneficial to human welfare. For example, metabolism of dietary sucrose and lactose by *Streptococcus mutans* has been implicated in plaque formation and the development of dental caries (13). By contrast, the fermentation of lactose in milk by group N species (*Streptococcus lactis*, *S. cremoris*, *S. diacetylactis*) is of major importance in the manufacture of cheese and other dairy products (18).

In *S. lactis* (21, 29, 30) and *S. mutans* (6, 7) transport and intracellular accumulation of lactose is mediated via the multicomponent phosphotransferase system (PTS; for reviews see references 15 and 24). The product of phosphoenolpyruvate (PEP)-dependent group translocation (lactose-6-phosphate [16, 30]) is subsequently cleaved by phospho-β-D-galactoside galactohydrolase (7, 17). The galactose 6-phosphate and glucose moieties are further metabolized by the D-tagatose 6-phosphate (2, 14) and glycolytic pathways, respectively, to yield lactic acid.

Recent studies with *S. mutans* suggest that sucrose may also be metabolized by a functionally analogous series of enzymes. St. Martin and Wittenberger (26) and Slee and Tanzer (25) have demonstrated sucrose-PTS activity and sucrose 6-phosphate (6-*O*-phosphoryl-D-glucopyranosyl-1-α-β-2-D-fructofuranoside) formation in *S. mutans*. Recently, Chassy and Porter identified sucrose 6-phosphate hydrolase (8) and fructokinase (23) activities in cell-free extracts of *S. mutans*, and suggested that these enzymes catalyze the entry of the phosphorylated disaccharide into the glycolytic pathway. In a preliminary report, LeBlanc and co-workers (19) described sucrose-PTS activity in permeabilized whole cells of *S. lactis* DR1251 cultured on sucrose, but neither the products of transport nor the route(s) of sucrose catabolism were reported.

The existence of a large PEP pool in starved cells of *S. lactis* was first described by Thompson and Thomas (32). From the results of in vivo experiments, Thompson and Thomas (32), Thompson (29), and Waggoner et al. (20) suggest that formation of this PEP reserve is due to inactivation of pyruvate kinase in response to (i) reduced levels of glycolytic activators (27) and (ii) high intracellular P_i concentration. Starved cells—being thus "primed" with a readily available energy source—permit PTS functions to be studied in vivo (29-32). In this report,

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we have shown that uptake of sucrose by starved cells of *S. lactis* K1 is mediated by a sucrose-specific PTS and have identified the product of group translocation. In addition to the sucrose-PTS, growth of *S. lactis* K1 on the disaccharide also elicits induction of sucrose 6-phosphate hydrolase and fructokinase. The activities of the three enzyme systems were assayed after growth of *S. lactis* K1 on a variety of PTS and non-PTS sugars. The data suggest that expression of the fructokinase gene may be regulated independently of the genes which code for sucrose 6-phosphate hydrolase and the components of the sucrose-PTS.

MATERIALS AND METHODS

Bacterial strain and cultivation conditions. *S. lactis* K1 was obtained from the culture collection of the New Zealand Dairy Research Institute. The organism was maintained and cultured in a complex medium (31) containing 0.5% (wt/vol) of the appropriate filter sterilized sugar.

Preparation of starved cells. Cells from 200 ml of culture were harvested during the mid-logarithmic phase of growth, and the pellet was washed twice by suspension in and centrifugation from 200 ml of 0.01 M $MgSO_4$ (28). The starved cells contained an intracellular PEP potential comprised of 2- and 3-phosphoglycerates and PEP (20, 29, 32).

Sugar uptake by starved cells. The accumulation of sugars by starved cells of *S. lactis* K1 was monitored in the presence of iodoacetate as described previously (29–31). In kinetic studies, the initial rates of sucrose uptake were determined after 5 s of incubation.

Preparation and breakage of cells. Flasks containing 800 ml of complex medium were inoculated with stationary-phase cultures of *S. lactis* K1 (2.5%, vol/vol) and incubated overnight at 30°C (16 h). The cells were collected by centrifugation and washed twice with 250 ml of 0.01 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, pH 7.5, containing 1 mM dithiothreitol (DTT). This and all subsequent operations (except enzyme assays) were carried out at 0 to 4°C. The cells were resuspended in the same buffer (approximately 140 ml), mixed with 200 ml of 0.10- to 0.17-mm glass beads, and subjected to two 3-min intervals in a Bead Beater (Biospec Products), separated by a 3-min cooling period. The resulting suspension was centrifuged at 27,000 × *g* for 1 h, and the supernatant fluid was reserved for enzyme assays and purification. For larger-scale preparation, 4 liters of cells was used. To determine specific activity in extracts of cells cultured on various energy sources, ultrasonic disruption was employed. Washed cells obtained from an 800-ml culture were suspended in 10 ml of the breakage buffer containing 2 g of 5-μm-diameter glass beads. The suspension was subjected to four 3-min periods of full power in a Branson W-185 sonifier. Three minutes of cooling was allowed between each exposure. The cell-free supernatants were collected as previously described.

Enzyme purification. Supernatants derived from sucrose-cultured cells were dialyzed against 0.01 M HEPES buffer, pH 7.5, containing 1 mM DTT (4 liters, one change and concentrated to 100 ml by pressure ultrafiltration) (Amicon PM-30 membrane). The retentate was clarified by ultracentrifugation at 140,000 × *g* for 2 h. The supernatant was then loaded onto a DEAE-Sephacel column (2.5 by 40 cm) previously equilibrated with the same buffer (flow rate, 1.6 ml/min). After the crude extract had been loaded on the column, a linear gradient of 0.1 M HEPES buffer (pH 7.5) containing 1 mM DTT and 0 to 0.5 M KCl was initiated. Fractions (10 ml) were collected and analyzed for fructokinase and sucrose 6-phosphate hydrolase activity. The two fractions containing sucrose 6-phosphate hydrolase and the fructokinase-containing fractions were pooled separately and concentrated to 3 to 5 ml by ultrafiltration as described above, followed by final concentration in dialysis tubes covered with Carbowax 20,000 (polyethylene glycol 20M). The concentrated fractions were each chromatographed by gel filtration on a Sephacryl S-200 column (2.5 by 90 cm; flow rate, 0.5 ml/min; 3.5-ml fractions), equilibrated, and developed with 0.1 M HEPES (pH 7.5) containing 1 mM DTT and 0.1 M NaCl. Activity-containing fractions were concentrated to 2 to 4 ml as described above and used for subsequent enzyme characterizations. Molecular weights were estimated from the Sephacryl column by comparison of peak fraction numbers with a plot of log molecular weight versus fraction number (1) constructed using ferritin (M_r 540,000) bovine serum albumin (M_r 68,000), ovalbumin (M_r, 45,000), chymotrypsinogen A (M_r 25,000), and RNase (M_r 13,800).

Enzymatic analysis. Free sugars and glycolytic intermediates present in trichloroacetic acid extracts of whole cells were assayed by enzymatic procedures (29, 32). The intracellular solute concentrations were calculated on the assumption that 1 g (dry weight) of *S. lactis* K1 cells contained 1.67 ml of intracellular (protoplast) fluid (28). Glucokinase (ATP:D-glucose-6-phosphotransferase, EC 2.7.1.2), fructokinase (ATP:D-fructose-6-phosphotransferase, EC 2.7.1.4), and sucrose 6-phosphate hydrolase (8) were assayed by published procedures. Protein was determined by the Coomassie brilliant blue dye-binding assay described by Bradford (4), using equine gamma globulins as the protein standard. Glucokinase and fructokinase activities in gels were localized by using an activity strain based on precipitation of nitroblue tetrazolium in its reduced form (12, 23). Proteins separated by gel electrophoresis were visualized by the method of Diezel et al. (11).

Chromatographic methods. Free and phosphorylated sugars were qualitatively separated by paper chromatography and identified by autoradiography (30).

Materials. Reagent grade chemicals were obtained from Fisher Chemical Co. and Baker Chemical Co. Media components were purchased from Difco Laboratories and BBL Microbiology Systems. Enzymes and biochemicals were obtained from Sigma Chemical Co. Sucrose 6′-phosphate (5) was the generous gift of J. G. Buchanan (Department of Chemistry, Heriot-Watt

University, Edinburgh, Scotland). Sucrose 6-phosphate was synthesized as previously described (8).

RESULTS

Induction of the sucrose-PTS. Starved cells of *S. lactis* K1 contained an endogenous PEP potential (29, 32), and in the presence of iodoacetate (an inhibitor of glyceraldehyde 3-phosphate dehydrogenase), the products of sugar transport could be assessed without extensive glycolysis of accumulated derivatives. Starved cells grown previously on sucrose, lactose, or fructose accumulated glucose and the non-metabolizable glucose analog 2-deoxyglucose via a constitutive glucose-PTS (Fig. 1). Maximum uptake of the sugars (as phosphorylated derivatives) approached 35 to 40 mM within 30 s of incubation, by which time the endogenous PEP potential had been consumed (29). By contrast, rapid accumulation of sucrose was observed only in cells which had been previously grown on the disaccharide (Fig. 1A). Cells grown previously on the separate hexose moieties fructose (Fig. 1C) or glucose (data not shown) were unable to accumulate significant concentrations of sucrose. Results (Fig. 1) confirmed the separate identities of the sucrose- and lactose-PTSs in *S. lactis* K1. Sucrose was not accumulated by lactose-grown cells (Fig. 1B), and lactose was not significantly accumulated by cells grown previously on sucrose (Fig. 1A).

Kinetics of the sucrose-PTS. Translocation of sucrose by intact cells of *S. lactis* K1 displayed simple saturation kinetics (Fig. 2), and the PEP-dependent PTS had a high affinity for the disaccharide (V_{max}, 191 μmol mg^{-1} [dry weight of cells] min^{-1}; K_m, 22 μM). The corresponding kinetic parameters reported for the sucrose-PTS in permeabilized cells of *S. mutans* (26) are: V_{max}, 20 to 50 μmol mg^{-1} (dry weight of cells) min^{-1} and K_m 70 μM.

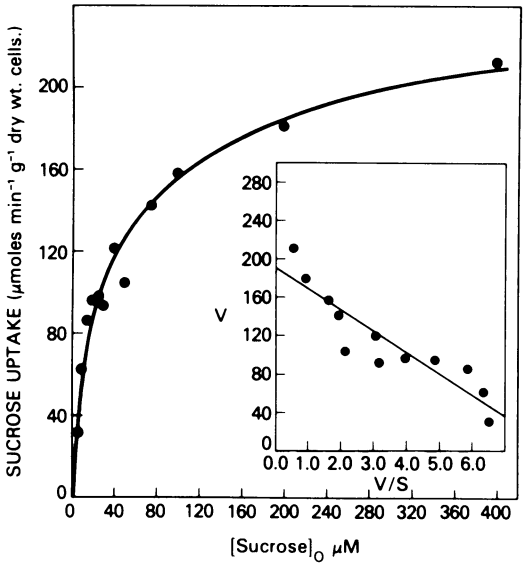

FIG. 2. *Kinetic analysis of PEP-dependent [^{14}C]sucrose accumulation by intact cells of S. lactis K1. Starved cells grown previously on sucrose were preincubated for 10 min in buffered medium containing 10 mM iodoacetate. Initial rates of sugar uptake (micromoles per minute per gram [dry weight] of cells) were determined after 5 s of incubation. Inset shows Hofstee transformation plot of initial rate data. The line of best fit was obtained by linear regression. "[Sucrose]₀" and "S" (inset) refer to medium concentrations (micromolar) of sucrose.*

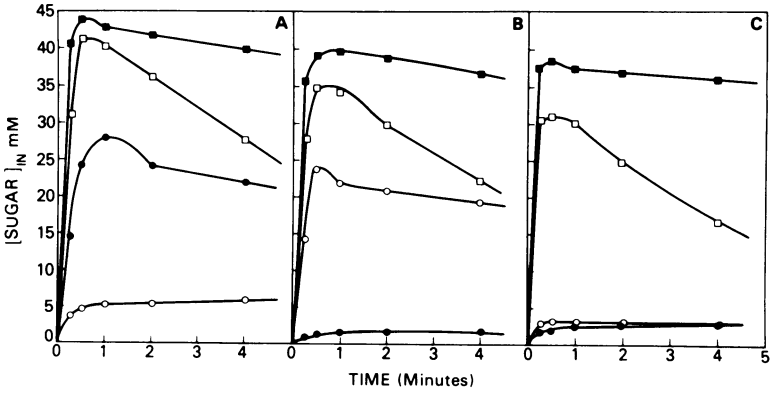

FIG. 1. *Accumulation of sugars by starved cells of S. lactis K1. Cells grown previously on sucrose (A), lactose (B), or fructose (C) were suspended at a density of 200 μg (dry weight) per ml in 0.1 M Tris-maleate buffer (pH 7.0) containing 10 mM iodoacetate. After 10 min of preincubation, the appropriate [^{14}C]-labeled sugar (0.2 mM; 0.2 μCi/μmol) was added to the medium: lactose (○), sucrose (●), 2-deoxy-D-glucose (□), and glucose (■). [Sugar]IN, Intracellular concentration (millimolar) of (phosphorylated) sugars.*

Identification of the sucrose-PTS product. Iodoacetate-starved cells of *S. lactis* K1 were incubated for about 1 s with [^{14}C]sucrose, and uptake was stopped by the addition of trichloroacetic acid. Trichloroacetic acid was removed by ether extraction, and the radioactive intracellular products were qualitatively identified by paper chromatography. A rapidly migrating material with an R_f corresponding to that found for authentic sucrose and a slower-moving, possibly ionic (phosphorylated?) derivative were found. This derivative migrated more slowly than either fructose 1-phosphate or fructose 6-phosphate, but overlapped the region occupied by sucrose 6′-phosphate and glucose 6-phosphate. The derivative was isolated by preparative chromatography, and when treated with (i) alkaline phosphatase, it yielded [^{14}C]-sucrose; (ii) with alkaline phosphatase and invertase, it produced only [^{14}C]glucose and [^{14}C]fructose; and (iii) after mild acid hydrolysis, it yielded [^{14}C]glucose 6-phosphate and [^{14}C]fructose. These results demonstrated that the derivative was sucrose 6-phosphate.

Enzymatic analysis of intracellular derivatives. To identify the intracellular products formed as a consequence of sucrose transport by the sucrose-PTS, iodoacetate-inhibited starved cells of *S. lactis* K1 (cultured on sucrose) were incubated with the sugar for 60 s. At this time, maximum uptake and accumulation had occurred (see Fig. 1A). The cells were collected by rapid membrane filtration, and the trichloroacetic acid-soluble material was extracted. Whereas cells pulsed for 1 s were shown to contain significant quantities of sucrose 6-phosphate, the enzymatic analysis of the extracts prepared from cells after 60 s of sucrose uptake (Table 1) showed that the cells contained high levels of glucose 6-phosphate, fructose, fructose 6-phosphate, fructose 1,6-diphosphate, and triose phosphates. These results indicate that sucrose 6-phosphate was rapidly hydrolyzed and further transformed by intact cells. Neither 2-phosphoglyceric acid, 3-phosphoglyceric acid or phosphoenolpyruvic acid were detected in the extracts, indicating that transport of sucrose had occurred at the expense of PEP potential and that glyceraldehyde 3-phosphate dehydrogenase was inhibited in the intact cells. The same analysis was performed on extracts prepared from cells which had been allowed to accumulate maximum levels of glucose. High levels of glucose 6-phosphate and fructose 1,6-diphosphate were observed (Table 1), but, in contrast to the results obtained after transport of sucrose, free fructose was not detected.

Induction patterns of sucrose 6-phosphate hydrolase and fructokinase. Since analysis of intracellular derivatives indicated a rapid turnover of sucrose 6-phosphate, an attempt was made to characterize enzymes capable of producing these metabolites. Cell-free extracts of *S. lactis* K1 were prepared from cells cultured on several carbohydrates and assayed for glucokinase, fructokinase, and sucrose 6-phosphate hydrolase activity (Table 2). High, and relatively constant, levels of a constitutive glucokinase activity was measured in all cell extracts, but sucrose 6-phosphate hydrolase activity was detected only in sucrose-cultured cells. By contrast, measurements of fructokinase activity on these same extracts revealed a pattern of regulation of expression different from that found for sucrose 6-phosphate hydrolase (Table 2). Fructokinase was induced during growth on sucrose; however, the enzyme was also induced after growth on maltose, galactose, ribose, and arginine (supplemented with 0.1% glucose). This gratuitous expression of fructokinase activity

TABLE 1. *Intracellular metabolites formed during uptake of glucose or sucrose by iodoacetate-inhibited cells of S. lactis K1*[a]

Sugar transported	Intracellular concn of metabolite (mM)[b]					
	Glucose	Glucose 6-phosphate	Fructose	Fructose 6-phosphate	Fructose-1,6-diphosphate	Triosephosphate[c]
Control (no sugar)	ND[d]	ND	ND	ND	0.94 ± 0.49	1.76 ± 0.55
Glucose	1.27 ± 0.90	13.65 ± 0.70	ND	2.94 ± 0.24	9.49 ± 0.86	2.52 ± 0.52
Sucrose	2.64 ± 0.29	13.21 ± 0.88	11.84 ± 0.85	2.84 ± 0.45	8.69 ± 0.52	2.31 ± 0.27

[a] Starved cells of *S. lactis* K1 (grown previously on sucrose) were suspended to a density of 1 mg (dry weight) per ml in 10 ml of 0.1 M Tris-maleate buffer (pH 7.0) containing 10 mM iodoacetate and preincubated for 10 min at 30°C. Sucrose or glucose was added to the appropriate system to a final concentration of 0.5 mM. After 60 s, the cells were collected by membrane filtration and immediately extracted with 10% trichloroacetic acid and ether as described previously (30, 32).

[b] Metabolites present in cell extracts were assayed by enzymatic analysis (30, 32), and concentrations were calculated on the basis that 1 g (dry weight) of cells contained the equivalent of 1.67 ml intracellular (protoplast) fluid volume (28).

[c] Combined concentrations of dihydroxyacetone phosphate plus glyceraldehyde 3-phosphate.

[d] ND, Not detectable.

was a reproducible phenomenon; however, no attempt was made to show that the same protein was responsible for fructose phosphorylation in each case.

Separation and partial purification of sucrose 6-phosphate hydrolase and fructokinase. To further characterize these two enzymes associated with sucrose metabolism, the crude extract resulting from the breakage of 4 liters of cells (sucrose grown, 15.86 g [wet weight]) was chromatographed on DEAE-Sephacel as described in the text. The fructokinase activity emerged in fractions 66 to 74; fractions 68 to 73 were concentrated for further purification (Fig. 3). Whereas the fructokinase emerged in a single nearly symetrical peak, sucrose 6-phosphate hydrolase activity emerged in at least three places (Fig. 3). The fractions comprising the early peak (I) and a second double peak (II) (fractions 14 to 23 and 44 to 67, respectively) were pooled separately and concentrated for further purification. Fructokinase, and both sucrose 6-phosphate hydrolase fractions, were chromatographed on a Sephacryl S-200 column which allowed an estimation of molecular weight (data not shown). Sucrose 6-phosphate hydrolase activity in peaks I and II eluted in the same fraction on gel filtration. The results of the partial purifications are summarized in Table 3. As judged by disc gel electrophoresis (9, 23), the two sucrose 6-phosphate hydrolase preparations were nearly homogeneous, having one major (activity-containing) and one minor band; the fructokinase preparation was less pure and con-

TABLE 2. *Specific activities of sucrose 6-phosphate hydrolase, fructokinase, and glucokinase in cell extracts of S. lactis K1 cultured on various growth substrates*

Growth substrate[a]	Enzyme sp act[b]		
	Sucrose 6-phosphate hydrolase	Fructokinase	Glucokinase
Glucose	0.0	1.6	302.4
Fructose	0.6	1.8	331.7
Sucrose	19.5	12.6	312.6
Maltose	1.9	14.2	394.2
Lactose	1.0	3.8	394.2
Ribose	1.4	18.2	291.7
Galactose	1.5	20.6	412.4
Arginine/glucose	0.3	16.3	219.7

[a] Cells were grown in complex medium containing 0.5% (wt/vol) of the appropriate carbohydrate, except the arginine/glucose system, which contained 1% (wt/vol) arginine plus 0.1% (wt/vol) glucose.

[b] Cell-free extracts were assayed for each of the three enzymes as described in the text. Activities are expressed as nanomoles of substrate utilized per minute per milligram of protein.

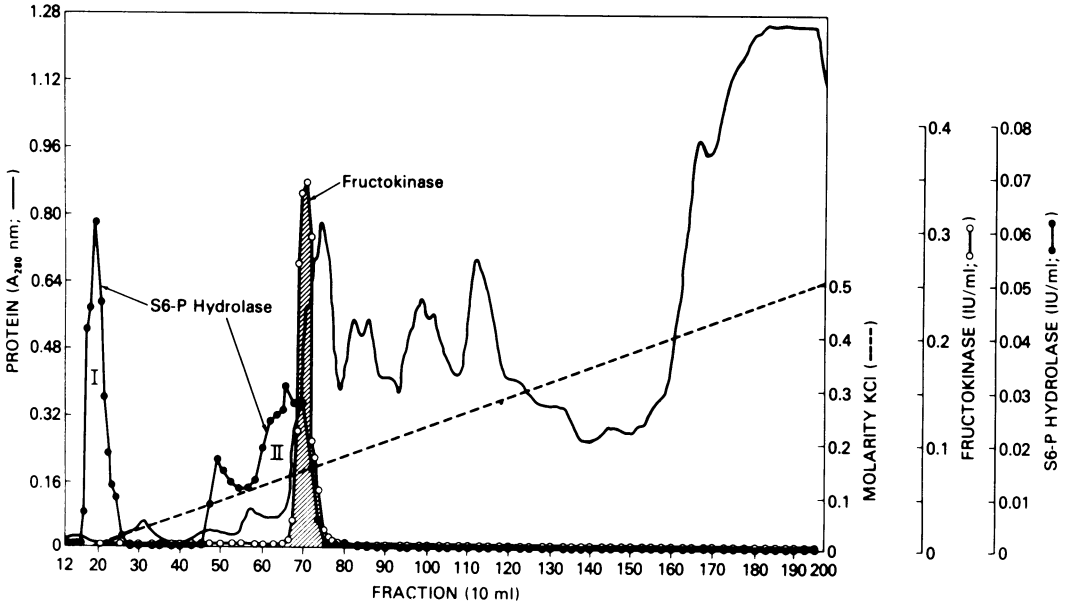

FIG. 3. *Identification of sucrose 6-phosphate hydrolase and fructokinase activities present in crude cell extracts of S. lactis K1. The high-speed supernatant fluid obtained from disrupted S. lactis K1 (cells from a 4-liter culture) was concentrated to 110 ml and loaded onto a column (2.5 by 40 cm) of DEAE-Sephacel equilibrated with 0.1 M HEPES buffer (pH 7.5) containing 1 mM DTT. The flow rate was ca. 1.6 ml* min^{-1}. *Proteins were eluted by using a linear gradient of KCl and were monitored by 280-nm absorbance* (A_{280}). *Fractions were assayed for sucrose 6-phosphate hydrolase* (●) *and fructokinase* (○) *as described in the text.*

TABLE 3. *Purification of sucrose 6-phosphate hydrolase and fructokinase from S. lactis K1*

Preparative stage	Sucrose 6-phosphate hydrolase[a]			Fructokinase[a]		
	Sp act[b]	Fold purification	% Recovery	Sp act[b]	Fold purification	% Recovery
Crude extract	0.028		100	0.043		100
DEAE-Sephacel						
Peak I	6.86	245	29	0.774	18.16	84
Peak II	3.00	107	54			
Sephacryl-S-200						
Peak I	10.3	368	22	10.91	256.1	66
Peak II	9.3	332	43			

[a] Enzymes were assayed as described in the text.
[b] Specific activities as micromoles per minute per milligram of protein.

tained two major and five minor bands. Activity staining indicated that the fructokinase activity corresponded with one of the major bands comprising approximately 20% of the total protein (data not shown).

Characterization of sucrose 6-phosphate hydrolase from *S. lactis* K1. The two sucrose 6-phosphate hydrolase preparations, purified through the gel filtration step, each had an approximate M_r of 28,000. No contaminating glucokinase or fructokinase was detected. Peak I exhibited a K_m of 0.11 mM, and peak II had a K_m of 0.10 mM for sucrose 6-phosphate. Both fractions hydrolyzed sucrose at a much higher K_m (101 and 99 mM, respectively), but with essentially the same relative V_{max}. Sucrose was a competitive inhibitor of sucrose 6-phosphate hydrolysis (K_i, 12 mM). These parameters are similar to those reported for the sucrose 6-phosphate hydrolases found in *S. mutans* (8; E. V. Porter and B. M. Chassy, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K134, p. 159), although the molecular weights were significantly lower (28,000 for *S. lactis* versus 38,000 to 43,000 for *S. mutans*). The similar size and kinetic constants of the two sucrose 6-phosphate hydrolase peaks isolated from *S. lactis* K1 extracts suggest that they represented the same protein which chromatographed in a nonideal manner on DEAE-Sephacel. Finally, it was found that sucrose 6′-phosphate was not hydrolyzed by either crude extracts or the purified sucrose 6-phosphate hydrolase preparations isolated from *S. lactis* K1.

Characterization of the fructokinase isolated from *S. lactis* K1. Since crude extracts of *S. lactis* K1 were able to phosphorylate free fructose, the nature of the enzyme responsible for the ATP-dependent phosphorylation of fructose was investigated. The activity demonstrated in crude extracts was purified over 240-fold. Though not homogeneous, the fraction was sufficiently pure to allow a number of important conclusions to be drawn: (i) the fructose-phosphorylating activity was due to a specific ATP-dependent kinase since partially purified preparations could not phosphorylate glucose, galactose, allose, N-acetylglucosamine, glucosamine, or tagatose; (ii) mannose was a substrate for the enzyme (mannose K_m, 0.24 mM; fructose K_m, 0.33 mM); and (iii) mannose was a competitive inhibitor toward fructose (K_i 0.12 mM). In addition, the mannokinase activity cochromatographed with the fructokinase activity on ion-exchange and gel filtration columns. It was concluded from these results that the same protein catalyzed the phosphorylation of both mannose and fructose, as has been found previously with *S. mutans* (23). The apparent molecular weights of the mannofructokinase isolated from *S. lactis* (42,000) and *S. mutans* (47,000) were similar. The K_m for ATP was found to be 0.22 mM; though no K_m for ATP has been reported for the enzyme isolated from *S. mutans*, the fructose and mannose K_m values were comparable. Finally, the product of fructose phosphorylation by the ATP-dependent mannofructokinase was fructose 6-phosphate and not fructose 1-phosphate, since: (i) activity in the coupled assay was completely dependent on added phosphohexoseisomerase, Mg^{2+}, and ATP; and (ii) fructose 1-phosphate could not substitute for fructose when added to mannofructokinase in the coupled assay system.

DISCUSSION

Growth of *S. lactis* K1 on sucrose resulted in the induction of a sucrose-specific PTS and sucrose 6-phosphate hydrolase. Cells grown on this disaccharide also contained high levels of mannofructokinase. The product of the group translocation process via the sucrose-PTS was a sucrose monophosphate, and the phosphorylation

occurred at C-6 of the glucosyl moiety of the disaccharide. This conclusion was supported by chromatographic and enzymatic analysis of the transport products and by the observation that sucrose 6-phosphate and not sucrose 6'-phosphate was the substrate for the sucrose phosphate hydrolase prepared from *S. lactis* K1. Sucrose 6-phosphate hydrolase isolated from *S. mutans* is also incapable of utilizing sucrose 6'-phosphate as a substrate (8). Neither sucrose 6-phosphate hydrolase nor fructokinase has been reported previously in *S. lactis*. The PEP-dependent sucrose-PTS has been demonstrated in permeabilized cells (25, 26), but has not previously been studied in intact cells. In *S. lactis* K1, the properties of the three enzymes required for transport and mobilization of sucrose (Fig. 4) are similar to those described in *S. mutans*. It is not known how widely the pathway proposed in Fig. 4 is distributed among bacteria.

The sucrose-PTS has not been characterized in any organism, although a preliminary report concerning the fractionation of the sucrose-PTS in *S. mutans* has appeared (G. R. Jacobson, W. L. Ran, and P. J. Scott, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K122, p. 157). It is not clear whether the PTS of *S. lactis* K1 is comprised of an enzyme II B^{suc} and enzyme III^{suc} (as in the lac-PTS [15]) or if it resembles the enzyme II A/B glucose-PTS in this organism (31). The sucrose 6-phosphate hydrolases of *S. lactis* K1 and *S. mutans* have similar kinetic properties, but the *S. lactis* enzyme may be somewhat smaller than its counterpart in *S. mutans*. Sucrose 6-phosphate hydrolase has been shown to be loosely associated with the membrane of *S. mutans* (3). It is possible that the multiple peaks observed on DEAE-Sephacel chromatography resulted from the presence of aggregated forms of the enzyme or that one or more of the activity peaks is due to association of the enzyme with membrane fragments. Regardless of the causes of the nonideal chromatographic separation, the final sucrose 6-phosphate hydrolase preparations exhibited similar kinetic characteristics and relative molecular weights, suggesting that the two activity peaks contained the same enzyme. Comparison of the mannofructokinases found in *S. lactis* and *S. mutans* revealed an equally close relationship in terms of substrate specificity, molecular size, and kinetic parameters. As is the case with *S. mutans*, *S. lactis* appears to possess not only a sucrose-PTS, but reasonable activities of specific enzymes (sucrose 6-phosphate hydrolase and mannofructokinase) that are efficient enough, as judged by their K_m's, to facilitate further metabolism of sucrose after its import into the cell. Cleavage of sucrose 6-phosphate by sucrose 6-phosphate hydrolase yields glucose 6-phosphate and fructose, and the latter must be phosphorylated before entry into the glycolytic pathway. We cannot exclude the possibility that fructose exits the cell and becomes phosphorylated during reentry by a fructose-specific PTS or that fructose is phosphorylated intracellularly by the PTS (10). However, high intracellular levels of free fructose were found by enzymatic analysis of trichloroacetic acid extracts of cells that had transported sucrose (Table 1), and broken-cell extracts contained an enzyme capable of phosphorylating this sugar. Therefore, it seems likely

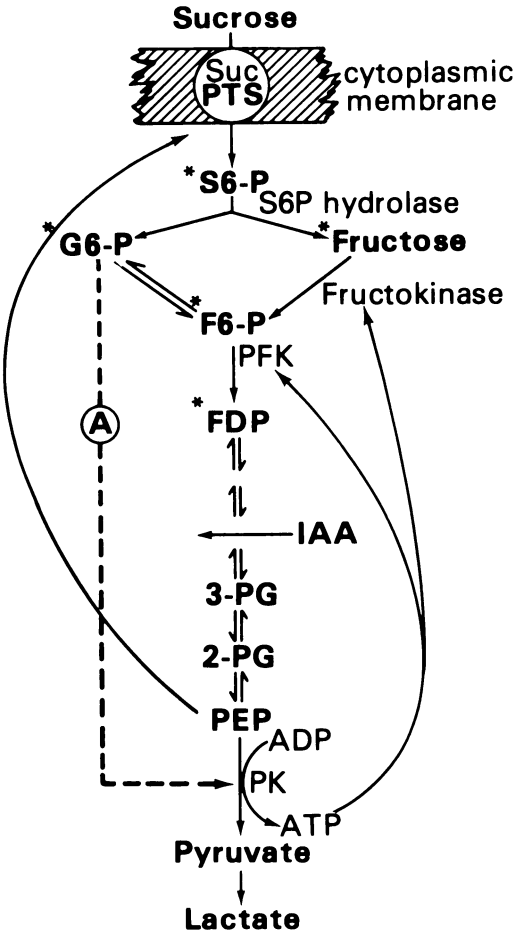

FIG. 4. *Proposed pathways for transport and metabolism of sucrose by S. lactis K1. The three glycolytic intermediates 3-phosphoglycerate (3-PG), 2-phosphoglycerate (2-PG), and phosphoenolpyruvate (PEP) comprise the endogenous PEP potential of starved cells. Asterisks designate compounds detected by enzymatic analysis and autoradiography after accumulation of sucrose by iodoacetate-inhibited starved cells. A, activator; PK, pyruvate kinase; PFK, phosphofructokinase; and suc-PTS, sucrose-PTS.*

that fructose is phosphorylated intracellularly to fructose 6-phosphate via the ATP-dependent mannofructokinase (Fig. 4).

Iodoacetate prevented extensive metabolism of accumulated sugar and also blocked ATP generation from glycolysis in intact cells (29, 30). However, the presence of high levels of fructose 1,6-diphosphate (Table 1) showed that ATP had been generated under the conditions described. We suggest that ATP required for the phosphorylation of fructose is generated from the endogenous PEP potential initially present in starved cells. It should be recalled that retention of the three metabolites comprising this potential (3-phosphoglyceric acid, 2-phosphoglyceric acid, and phosphoenolpyruvic acid) is a consequence of high levels of P_i (20) and depletion of glycolytic activators of pyruvate kinase (27, 29, 32). In vitro studies demonstrated that glucose 6-phosphate was a potent allosteric effector of enzyme activity in *S. lactis* (27) and *S. mutans* (33). We suggest that glucose 6-phosphate formed upon cleavage of sucrose 6-phosphate can activate pyruvate kinase. The ATP generated from the PEP potential (Fig. 4; 30) could then be used for phosphorylation of fructose to fructose 6-phosphate and fructose 1,6-diphosphate by ATP-dependent mannofructokinase and phosphofructokinase, respectively.

The three enzyme activities required for the metabolism of sucrose were present at high levels in cells of *S. lactis* K1 grown previously on the disaccharide, but not in cells grown previously on either glucose or fructose (Table 2). This data initially suggested that sucrose (or possibly sucrose 6-phosphate) served as the inducer for the coordinate expression of the sucrose-PTS, sucrose 6-phosphate hydrolase, and mannofructokinase. However, mannofructokinase activity, unlike the other activities, could also be expressed in the absence of sucrose. The high levels of this enzyme observed after growth on maltose, galactose, ribose, or arginine seem superfluous since it is unlikely that significant levels of intracellular fructose are formed during growth on these substrates. Mannofructokinase is apparently regulated independently of the genes coding for the sucrose-PTS and sucrose 6-phosphate hydrolase, but the mechanism of induction (or derepression) of the enzyme is not clear. It may be significant that high levels of mannofructokinase were found only after growth of the cells on non-PTS sugars (with the exception of sucrose) or on sugars poorly transported by the PTS (e.g., galactose). Cells of *S. lactis* K1 grown on lactose or glucose (Table 2) possessed little, if any, mannofructokinase activity. These findings suggest that expression of the mannofructokinase gene may be negatively regulated by a component (or a product) of the PTS.

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