INFECTION AND IMMUNITY, Mar. 1980, p. 922-927
0019-9567/80/03-0922/06$02.00/0

Vol. 27, No. 3

Effect of Growth Conditions on Sucrose Phosphotransferase Activity of *Streptococcus mutans*

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Sucrose and glucose phosphoenolpyruvate-dependent phosphotransferase (PTS) activities were studied in growing cultures of *Streptococcus mutans* serotype *c* and *d/g* cells adapted to either glucose or sucrose. Both acid production and optical absorbance were used to monitor growth in pH-controlled defined growth medium. The sucrose PTS activity appeared to be significant only under conditions of substrate limitation or slow growth as a result of low environmental pH. However, under environmental conditions which permitted rapid growth, sucrose PTS activity appeared to be repressed, and only when the cells approached substrate-limited stationary phase after growth on high sucrose-supplemented medium was significant sucrose PTS activity again observed. A mutant apparently defective in sucrose PTS activity grew rapidly and produced acid under conditions of high environmental sucrose level but showed no sucrose PTS activity when the culture approached stationary phase. The mutant, however, after adaptation to glucose, demonstrated significant glucose PTS once the culture had attained the stationary growth phase. During diauxie growth in the presence of glucose and sucrose, there were sequential apparent inductions and repressions of glucose and sucrose PTS activities corresponding to decreases and increases of growth rate on the two substrates. Thus, *S. mutans* possesses at least two transport mechanisms for each substrate studied. One system (PTS) functions under conditions permitting slow growth and another functions under conditions permitting rapid growth.

The direct correlation of the frequency of dietary sucrose consumption with the incidence of dental caries associated with infection by *Streptococcus mutans* is both striking and well established (5, 10, 14). The biochemistry and physiology of *S. mutans* with respect to sucrose utilization appear to be central to the causation and thus to the prevention and control of dental caries (24). Sucrose dissimilation by these cariogenic bacteria may be viewed to involve at least two distinct processes, namely (i) hexosyltransferase activities mediated by extracellular or cell-associated enzymes (1), and (ii) the transport of sucrose to intracellular sites for subsequent catabolism. Catabolism of sucrose by these organisms yields primarily lactic acid (1) which is considered to be the prime agent in the dissolution of tooth enamel (1-3). Unfortunately, little is currently known regarding the permeation of sucrose into *S. mutans*.

Previous studies in our laboratories have demonstrated that at least one mode of sucrose transport by *S. mutans* is that mediated via an inducible phosphoenolpyruvate-dependent sucrose phosphotransferase system (PTS) (19). This PTS was seen among the five major *S. mutans* serotypes (20, 21). However, preliminary studies revealed that cells harvested during their exponential growth phase possessed minimal sucrose PTS activity. Thus, the effect of the phase of growth, growth rate, and growth conditions on sucrose PTS activity of two strains of *S. mutans* representative of the most common serotypes (*c* and *d/g*) found in humans (16, 18, 27) were examined. This paper reports that growth rate and growth conditions greatly affect sucrose PTS activity and indicates that *S. mutans* transports sucrose by at least two systems.

MATERIALS AND METHODS

Microorganisms. *S. mutans* serotype *c* strain representative NCTC 10449, its mutant 217-7:2, with an apparent defect of its sucrose PTS, and *S. mutans* serotype *d/g* strain representative 6715-13 were used in these studies. Stock cultures were maintained by monthly transfer in fluid thioglycollate (Difco) supplemented with 20% (vol/vol) beef extract and excess $CaCO_3$.

Isolation of presumptive sucrose PTS mutants. *S. mutans* NCTC 10449 was mutagenized by a slight modification of previously described techniques (6). Cultures were grown to an optical density at 600 nm (OD_{600}) of 1.0 (1-cm light path) in a complex growth medium (22) supplemented with 55.5 mM glucose. The cells were harvested by centrifugation (10,000 ×

g for 5 min at 4°C) and washed twice with M-9 buffer at pH 7.0 (12). The resultant pellet was resuspended in M-9 at pH 7.0 and subjected to ultrasonic vibration (model 185W, Heat Systems-Ultrasonics, Inc.) at low output (setting 2.5) for short periods (four 5-s bursts). This treatment was required to disrupt bacterial clumps and chains (95% as viewed by direct microscopy) without appreciably affecting cell viability. The culture was then incubated with 0.15 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine for 60 min at 37°C in a candle extinction jar. The cells were harvested by centrifugation (10,000 × *g* for 5 min at 4°C) and washed three times with M-9 buffer. The final cell pellet was resuspended in a complex medium (22) containing 55.5 mM glucose and incubated at 37°C for 5 h. The culture was then briefly subjected to ultrasonic vibration as described above, diluted, and plated on the same complex medium modified by the omission of yeast extract and by supplementation with 55.5 mM glucose and 1.5% agar. After incubation for 48 h at 37°C, colonies were replica plated with sterile velveteen pads to the medium supplemented with either glucose (55.5 mM) or sucrose (1 mM). After incubation at 37°C for 72 h, colonies which grew on the glucose-containing medium but not on low sucrose medium were picked and grown in the liquid complex medium supplemented with 55.5 mM glucose. These cultures were examined for ability to ferment glucose, fructose, and raffinose, all at a final concentration of 10 mM, and sucrose at both 2 or 10 mM. Cultures unable to grow or produce acid from the low concentration of sucrose but which could ferment both glucose and fructose and which gave growth kinetics in glucose-supplemented broth similar to the wild-type parent strain were examined for sucrose and glucose PTS activities and for invertase activity by techniques previously described (9, 19, 20, 22). Isolates whose glucose PTS activity and invertase activity were comparable to that of the parent strain but which failed to exhibit sucrose PTS activity were identified as sucrose PTS-defective mutants, one of which, 217-7:2, was used in these studies. Twenty-two nonsibling mutants so isolated and identified had the same fermentation phenotype as the wild-type progenitor except for their inability to grow on and ferment low concentrations of sucrose and the absence of sucrose PTS activities.

Growth medium and growth conditions for PTS studies. After two successive transfers in defined medium (FMC, 26) supplemented with 5 mM glucose, cells for experimental use were grown at 37°C for 18 h in screw-capped 100-ml Erlenmeyer flasks with no headspace. Subsequently, cells were harvested by centrifugation (10,000 × *g* for 5 min at 4°C) and washed once with 250-ml sterile 25 mM potassium phosphate buffer, pH 7.0, supplemented with 10 mM magnesium sulfate and were resuspended such that a 10-fold dilution in buffer had an OD_{600} of 0.35. A 10-ml amount of the undiluted cell suspension was used to inoculate 1,000 ml of FMC which had been supplemented with the required carbohydrate at the required pH. The medium was contained in a double side-armed, water-jacketed flask (Bellco Spinner, Bellco, Vineland, N.J.) at 37°C and stirred with a magnetic impeller. Constant pH was maintained by the automatic addition of standardized 0.5 N NaOH with a pH-stat system (model 26 pH meter, type TTT11b titrator, and autoburette ABU 13, Radiometer, Copenhagen, Denmark). The volume of titrant consumed was monitored continuously with a strip chart recorder adapted to the autoburette.

Cell harvest and sugar PTS assay. A 20-ml amount of culture was sampled periodically during the growth cycle and its optical absorbance was determined, at an OD_{600} (1-cm light path) with a Gilford 300-N spectrophotometer before freezing at −20°C for further study. After thawing, the cell pellets were washed three times with 25 mM phosphate buffer (pH 7.0) supplemented with 10 mM magnesium sulfate, and sugar PTS activities were determined.

Sugar transport via the phosphoenylpyruvate-dependent PTS was assayed by a modification (19) of the method of Kornberg and Reeves (11). Cells were made permeable (decryptified) by the addition of 0.01 volume of toluene-acetone (1:4) by techniques previously described (19). The standard assay mixture for the sugar PTS assay contained the following: 1.0 mM phosphoenolpyruvate; 0.1 mM reduced nicotinamide adenine dinucleotide (NADH); 1.0 U of lactate dehydrogenase per ml; 1.0 mM $MgSO_4$; 10 mM NaF; and 25 mM potassium phosphate buffer, pH 7.0. Decryptified cells, at a final concentration of 2.0 mg of bacterial protein per ml, were added, and the reaction mixture was preincubated at 37°C for 5 min. The carbohydrate of interest was then added to a final concentration of 0.05 mM, and the oxidation of NADH was monitored as previously described (19). Sugar PTS activity was expressed as micromoles of NADH oxidized per milligram of bacterial protein per minute.

Growth rate. Growth was measured turbidimetrically at OD_{600} with a Gilford 300N spectrophotometer. Also, for homolactic fermenters like *S. mutans* (23, 25) the rate of growth is determined by the rate of net adenosine 5'-triphosphate production via glycolysis (8). Consequently, the rate of acid production was used as an indirect measure of growth rate (8, 22). The validity of this index of growth has been established, specifically, with *S. mutans* (17, 22).

Protein determinations. The method of Lowry et al. (12) was used to estimate the protein content of decryptified cells with bovine serum albumin as standard.

Chemicals. All biochemicals for the defined growth medium and NTG were purchased from Sigma Chemical Co., St. Louis, Mo. Sucrose, glucose, and fructose were purchased from J. T. Baker Chemical Co., Phillipsburg, N.J.

RESULTS

Logarithmic-phase *S. mutans* NCTC 10449, adapted to FMC supplemented with glucose, upon inoculation into the same medium supplemented with 5 mM sucrose, exhibited a brief lag period followed by rapid growth as measured both by optical absorbance and acid production (Fig. 1). During exponential growth (30 to 180 min) phosphoenolpyruvate-dependent sucrose-PTS activity was minimal (<0.5 μmol of NADH oxidized per mg of bacterial protein per min).

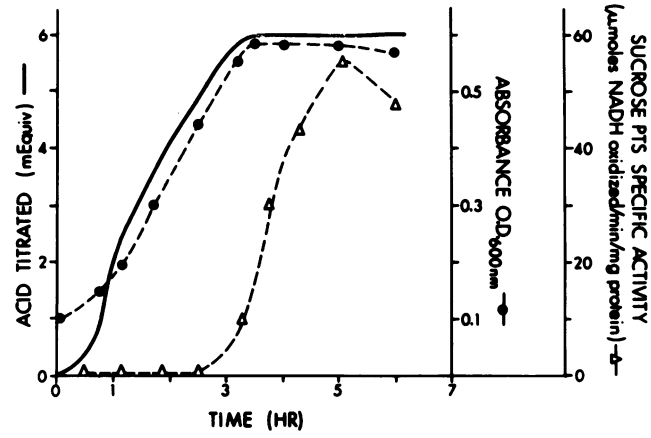

FIG. 1. *Plot of growth and sucrose PTS activity as a function of time for glucose-adapted S. mutans NCTC 10449. A 500-ml amount of defined medium supplemented with 5 mM sucrose was maintained by a pH stat at pH 7.0.*

However, as the termination of exponential growth was approached, sucrose PTS activity increased significantly and reached a maximum (55 μmol of NADH oxidized per mg of protein per min) during stationary phase. The final sucrose PTS specific activity was similar to those previously reported (19, 20). Analogous results were obtained when *S. mutans* 6715-13 was studied. These data suggested that there are at least two transport systems responsible for sucrose permeation in *S. mutans*: one which is induced under conditions of slow growth or limiting substrate concentration, the other system(s) which operates during periods of rapid growth or abundance of substrate (exponential phase).

To be certain that the low sucrose PTS specific activity during exponential growth was not merely the result of failure of the decryptification procedure for log-phase cells, sucrose PTS activity was tested in cell sonicates (7). Thus, cells grown and harvested under the same conditions as described above were studied in both exponential and stationary phases and, subsequent to washing, were disrupted by ultrasonic vibration at maximum output for 6 min while continually cooled by circulating ice water. Sucrose PTS activity could be detected in sonically disrupted cells which had been harvested in stationary but not in exponential phase.

If indeed there be two transport mechanisms for sucrose, then a mutant which possesses a defect in its sucrose PTS should still be able to transport sucrose at concentrations permitting rapid growth and production of acid. Studies were conducted with mutant 217-7:2 which failed to elicit the oxidation of NADH in the presence of sucrose and phosphoenolpyruvate but which promoted the oxidation of NADH in the presence of glucose and phosphoenolpyruvate. This apparent sucrose PTS mutant produced acid and grew in FMC supplemented with 5 mM sucrose but showed no detectable PTS activity throughout the growth cycle. However, it produced acid when grown on FMC supplemented with 5 mM glucose and had glucose PTS activity once the cells had attained late log and stationary phase (Fig. 2). Furthermore, it failed to produce acid and to grow in FMC supplemented with a lower level (2.0 mM, data not shown) of sucrose, in contrast to the parent strain incubated under identical conditions (Fig. 3).

These data suggested that at low substrate levels the sucrose PTS assumes major importance in the transport of sucrose. To investigate this further, glucose-FMC-adapted wild-type cells of *S. mutans* NCTC 10449 were grown in FMC supplemented with 2.0 mM sucrose, sampled, and assayed for PTS activity. Figure 3 indicates that at this sucrose concentration (or at 1.0 mM, not shown) the growth rate was slower than for these cells grown in 5 mM sucrose-supplemented medium (as shown above in Fig. 1). However, sucrose PTS activity was induced early in the growth cycle and ascended to and remained at a high level through the stationary phase (Fig. 3). The addition of more sucrose to the culture such as to elevate its concentration to 20 mM promoted rapid growth and suppressed sucrose PTS activity. Sucrose PTS activity remained at undetectable levels until the cells again approached stationary growth phase.

To further characterize the system, the induc-

tion of the sucrose PTS during growth on two PTS-transportable (19, 20) substrates, glucose, and sucrose, was studied. Figure 4 illustrates that after inoculation of stationary-phase glucose-adapted cells to FMC medium containing 5 mM glucose and 5 mM sucrose, both sucrose and glucose PTS activities could be demonstrated for *S. mutans* NCTC 10449 during a prolonged lag phase. However, at the commencement of exponential growth, both sucrose and glucose PTS specific activities declined to undectable levels. When the majority of the glucose of the medium had been consumed, glucose PTS activity increased dramatically. After the adaptation to growth on sucrose as revealed by renewed growth after a diauxic lag (15, 22), glucose PTS activity redeclined to an insignificant level and its specific activity remained low during the second rapid growth phase. However, when the culture again approached the stationary phase, sucrose PTS activity was observed to increase dramatically followed by glucose PTS activity. Similar data (not shown) were obtained for *S. mutans* 6715-13.

Under conditions of slow growth prompted by maintaining the medium pH at 5, even with a sucrose concentration of 5 mM, sucrose PTS activity could be detected throughout the resultant slow growth cycle (Fig. 5). The addition of more sucrose to the culture so as to raise its concentration 10 mM did not affect the rate of acid production or the sucrose PTS specific activity. However, when the pH of the medium was readjusted to 7.0, the rate of acid production and absorbance (OD_{600}) rapidly increased and the sucrose PTS specific activity decreased to

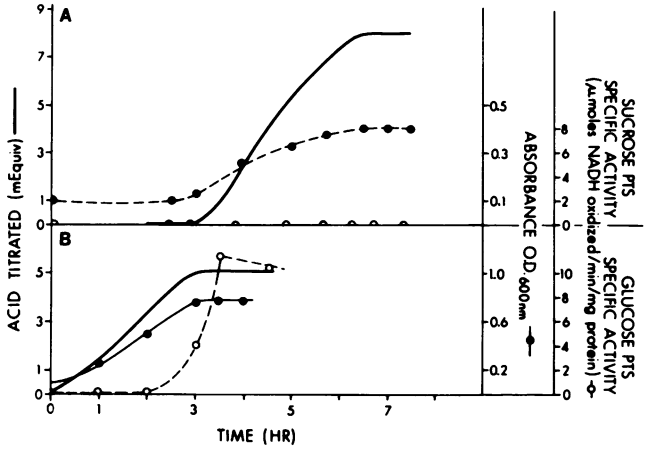

FIG. 2. *Plot of growth and PTS activity (sucrose PTS activity, A; glucose PTS activity, B) as a function of time for glucose-adapted PTS-defective mutant 217-7:2. A 1,000-ml amount of defined medium (FMC) supplemented with either 5 mM sucrose (A) or 5 mM glucose (B) was maintained by a pH stat at pH 7.0.*

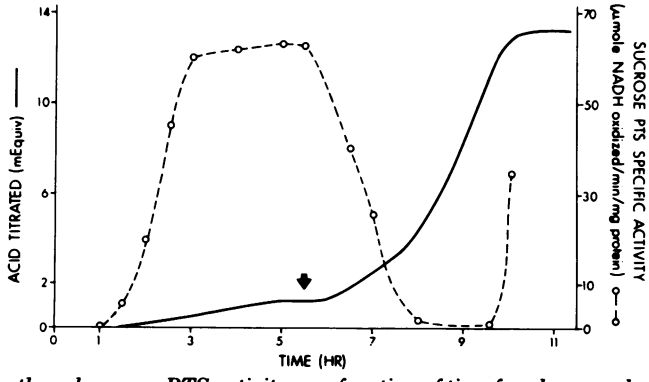

FIG. 3. *Plot of growth and sucrose-PTS activity as a function of time for glucose-adapted S. mutans NCTC 10449. A 1,000-ml amount of defined medium (FMC) supplemented initially with 2 mM sucrose was used; at the time indicated by the arrow the concentration of sucrose was increased to 20 mM. pH was maintained at 7.0 by a pH stat.*

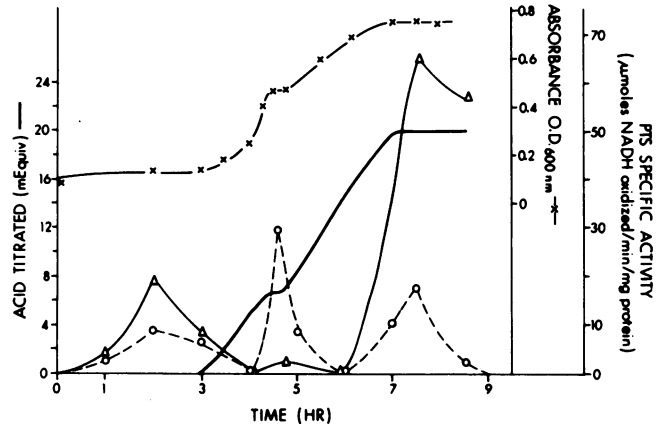

FIG. 4. *Diauxie growth and PTS activity of glucose-adapted S. mutans NCTC 10449 inoculated into 1,000 ml of defined medium (FMC) supplemented with 5 mM glucose and 5 mM sucrose and maintained at pH 7.0 by a pH stat. Symbols: △, sucrose-PTS specific activity; ○, glucose-PTS specific activity.*

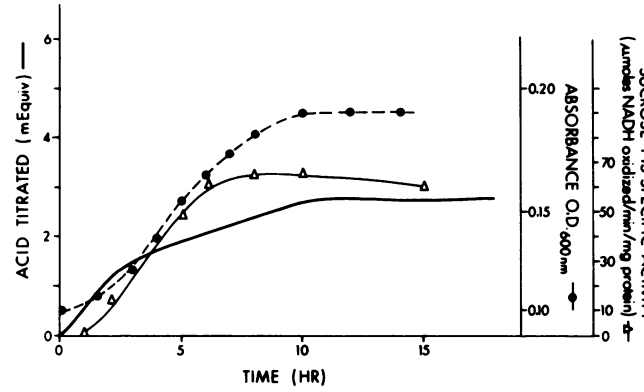

FIG. 5. *Plot of growth and sucrose-PTS activity of glucose-adapted S. mutans NCTC 10449 inoculated into 1,000 ml of defined medium (FMC) supplemented with 5 mM sucrose. A pH stat maintained the pH at 5.0.*

undectable levels. The PTS activity was only detected again when the cells approached stationary phase. Thus, the sucrose PTS appears to be activated at low pH, whereas the second, unidentified sucrose transport system appears to be inhibited by low pH of the milieu.

DISCUSSION

It is apparent that the transport of sucrose by *S. mutans* serotype *c* and *d/g* cells is mediated by at least two systems. The sucrose PTS is significant only under conditions of substrate limitation or slow growth; under environmental conditions permitting rapid growth, sucrose PTS activity is repressed. Only when the cells approach substrate-limited stationary phase after growth in high sucrose-supplemented medium is significant sucrose PTS activity observed, except for the sucrose PTS-defective mutant 217-7:2. Similar findings were also noted for the induction of glucose PTS activity by the wild-type cell. The sucrose PTS-defective mutant 217-7:2 was fully capable of induction of glucose PTS activity. The variation in the observed specific activities of sucrose and glucose PTSs as a function of growth rate cannot be ascribed to a failure of the decryptification procedure to permeabilize logarithmically growing cells because sonically ruptured cells show similar patterns of low and high PTS activities as a function of growth.

Consistent with the present findings, previous chemostat culture investigations by Ellwood et al. (4) reported glucose PTS activity of the serotype *c* strain Ingbritt 1600 to be dependent on growth rate. Under conditions of substrate limitation, glucose PTS activity was significantly higher than under glucose excess. These workers concluded that this organism must have at least two transport systems for glucose (4).

The variation of PTS activity with growth conditions suggests that the sucrose-specific and glucose-specific PTS activities of *S. mutans* supply substrate under limiting or unfavorable growth conditions. Such conditions probably occur in vivo when either the pH levels in dental plaque are low as a result of carbohydrate catabolism or when carbohydrate supplies are limited, as occurs after the cessation of food intake by the host. The high affinities for sucrose and for glucose of these PTSs, as revealed by their apparent K_m's (18–20), support such a view, as do the growth studies at various substrate concentrations and environmental pH values described here.

Because both sucrose and glucose PTS activities are repressed during rapid growth in substrate abundance, *S. mutans* must, therefore, rely on another mechanism(s) to provide rapid sucrose or glucose uptake during such growth. The rapid growth of the sucrose PTS-defective mutant 217-7:2 in 5 mM sucrose also strongly supports the view that there are at least two sucrose permeation systems in *S. mutans*. The actual nature of such a second system is currently unknown and is the subject of other ongoing studies in our laboratories.

ACKNOWLEDGMENTS

This study was supported by a grant SA-1-064/77 from the Sugar Association, Inc., Washington, D.C. to A.M.S. and by Public Health Service grant DE-04758 from the National Institutes of Health to J.M.T.

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