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Preferential Utilization of the Glucosyl Moiety of Sucrose by a Cariogenic Strain of *Streptococcus mutans*

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The utilization of sucrose by a cariogenic strain of *Streptococcus mutans* was studied. The soluble and cell-bound sucrose-dependent, polymer-forming sucrase activities constitutively produced by the bacteria during growth on glucose were measured. About eight times more dextransucrase activity was present than levansucrase activity. During various states of growth on sucrose, *S. mutans* accumulated two to five times more insoluble and water-soluble dextran than levan. Although more of the fructosyl moiety of sucrose was therefore available to the cells, the glucosyl portion of the disaccharide was preferentially incorporated into cellular macromolecules. Glucose was shown to inhibit the utilization of fructose by *S. mutans*.

The interaction of *Streptococcus mutans* and sucrose results in the production of extracellular polysaccharides which appear to play a major role in the formation of dental caries on the smooth surfaces of teeth (5, 11, 22). Although data are available concerning cleavage of the sucrose molecule by specific *S. mutans* sucrases (1, 8), resulting in the formation of extracellular dextrans and levans (3, 7, 9, 20), there is little information concerning the metabolic fate of the nonpolymerized glucosyl and fructosyl moieties of sucrose released by this process.

In this communication, studies are presented which (i) compare the amount of dextransucrase (α-1,6-glucan:D-fructose 2-glucosyl-transferase, E.C. 2.4.1.5) and levansucrase (β-2,6-fructan:D-glucose 6-fructosyltransferase, E.C. 2.4.1.10) produced by *S. mutans*, (ii) analyze the fate of the glucosyl and fructosyl moieties of sucrose during growth of this organism and (ii) demonstrate the preferential utilization of glucose over fructose by this bacterium.

MATERIALS AND METHODS

Bacteria and growth conditions. *S. mutans* strain 6715 is a streptomycin-resistant cariogenic bacterium (4) which produces both levans and dextrans when grown with sucrose (16). For growth experiments, tubes fitted with vaccine caps were inoculated and flushed with a mixture of nitrogen (95%) and carbon dioxide (2%) through needles inserted in the caps. Growth (increase in cell mass) was followed by turbidity measurements with a Klett-Summerson colorimeter (no. 66 filter) with uninoculated medium as a blank. For isotope incorporation studies, cells were grown as described previously (16).

Sucrose utilization. Sucrose labeled in the fructosyl moiety with ^3H, sucrose labeled in the glucosyl moiety with ^{14}C, and uniformly labeled ^{14}C-sucrose were used to analyze the fate of the individual hexoses during growth on 58.4 mM sucrose. The isolation of water-soluble and insoluble or cell-bound polysaccharides and cell fractionation were performed as described elsewhere (16).

Glucose and fructose utilization. For the sugar competition studies, cells were grown for 18 hr with either 0.11 M glucose or 0.11 M fructose and diluted 200-fold into fresh medium containing the same sugar labeled with either ^{14}C or ^3H. Various concentrations of the competing nonradioactive hexose were added, and the cultures were incubated for 24 hr. The labeled cells were collected by centrifugation (10,000 $\times$ *g* for 10 min) and washed twice with standard saline-citrate (0.15 M NaCl, 0.015 M sodium citrate). Total isotope incorporation was measured by trichloroacetic acid precipitation and fractionation as described elsewhere (17).

Enzyme assays. Cultures grown for 18 hr with glucose or sucrose were centrifuged at 10,000 $\times$ *g* for 10 min at 4 C. The sedimented cells were suspended in 0.05 M potassium phosphate buffer (*p*H 6.8), collected by centrifugation, and suspended in the same buffer to a concentration equal to the cell density in the original culture. The culture supernatant fluid and washed cells were dialyzed against the phosphate buffer for 18 to 24 hr at 4 C. The assay for sucrose-dependent polysaccharide-forming activity was designed by S. A. Robrish and M. I. Krichevsky at the

National Institute of Dental Research, National Institutes of Health, Bethesda, Md. (*personal communication*). It involves the conversion of radioactive sucrose into an alcohol-insoluble polymer which can be trapped and counted on glass-fiber filter discs. The assay, as modified for this study, is described in detail below. The enzyme reaction mix consisted of 0.3 ml of 0.05 M phosphate buffer (*p*H 6.8) containing 150 μg of NaF and 1.75 μmoles of sucrose labeled with the appropriate isotope. Total polysaccharide synthesis was determined with uniformly labeled ^{14}C-sucrose (1.0 μCi/μmole), dextran was measured with sucrose: D-glucose-*U*-^{14}*C* (0.1 μCi/μmole), and levan was assayed with sucrose:fructose-*1*-^{3}*H* (5 μCi/μmole). Reactions were initiated with 0.2 ml of enzyme sample and were normally run in duplicate at 37 C for 10 min. Termination of the reaction involved the addition of 5 ml of 95% ethanol. The resulting precipitate was collected and washed with ethanol on 2.4-cm Whatman GF/A glass-fiber filters. Radioactivity retained on the discs was measured as described below.

Radioisotope assay procedure. For counting of radioactive compounds, 0.01-ml samples were placed on Whatman 3MM or GF/A filter discs mounted on stick pins and allowed to dry. Dried discs were placed in glass counting vials containing 10 ml of scintillation fluid, which consisted of 100 mg of 1,4-bis-2(5-phenyloxazolyl)-benzene (POPOP) and 4 g of 2,5-diphenyloxazole (PPO) per liter of toluene. Samples were counted with a Packard Tri-Carb 2425 liquid scintillation spectrometer.

Protein determination. The amount of protein in the various preparations was determined by the method of Lowry et al. (12).

Chemicals and radioisotopes. All of the sugars used were the best grades available from Mann Research Laboratories, New York, N.Y., or from Calbiochem, Los Angeles, Calif. All of the radioactive sugars were from New England Nuclear Corp., Boston, Mass., and included sucrose-*U*-^{14}*C* (346 mCi/mmole), sucrose: fructose-*1*-^{3}*H* (5.6 Ci/mmole), sucrose:glucose-*U*-^{14}*C* (243 mCi/mmole, fructose-*U*-^{14}*C* (121 mCi/mmole), and glucose-*6*-^{3}*H* (5.9 Ci/mmole).

RESULTS

Polysaccharide-forming activity in cultures. Figure 1 illustrates the ability of the culture supernatant fluid and washed cells from a glucose-grown *S. mutans* strain 6715 culture to polymerize the hexoses from sucrose into alcohol-insoluble polymers. With both enzyme sources, polysaccharide production was linear with time for at least 30 min. Most of the sucrase activity (80 to 90%) was found in the culture supernatant fluid. Specifically labeled sucroses were used to differentiate the above activities into dextran and levan production. Table 1 presents the results of this study. The specifically labeled sucroses clearly allow differentiation of the total polymerizing activity into dextran and levan production. *S. mutans* produces about eight times more dextran than levan, and a portion of both of the polymer-forming activities remains attached to the washed cells. Thus, *S. mutans* strain 6715 constitutively produces soluble and cell-bound sucrases which make primarily dextran when incubated with sucrose.

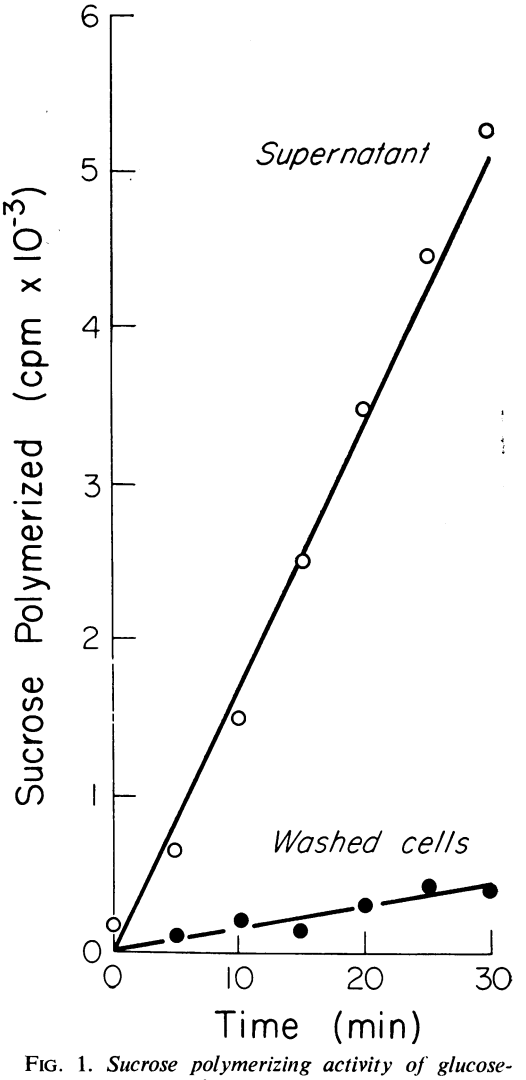

FIG. 1. *Sucrose polymerizing activity of glucose-grown S. mutans. The incorporation of the hexoses from sucrose into alcohol-insoluble polysaccharides was determined with a cell-free culture supernatant fluid and washed cells obtained from a culture grown for 18 hr with 0.11 M glucose. The washed cells were suspended in buffer to the same concentration as in the original culture so the activity of the two enzyme sources could be directly compared.*

Growth of S. mutans on various sugars. Figure 2 demonstrates the growth of *S. mutans* on glucose, fructose, and sucrose. The growth rate and final cell yield were essentially the same with each of the hexoses. However, incubation with sucrose resulted in a 3- to 4-hr lag, and then the cells grew linearly. Linear growth with *S. mutans* in sucrose

TABLE 1. *Polysaccharide-forming activity of glucose-grown Streptococcus mutans strain 6715*

Enzyme source	Amt of hexose polymerized (pmoles per 10 min per μg of cell protein)[a]		
	Total polysaccharide	dextran	levan
Cell supernatant fluid	847.1	532.2	284.6
Washed cells	74.2	65.8	7.7

[a] Cell supernatant fluid activity was calculated by using the amount of cell protein which was present prior to removal of the bacteria.

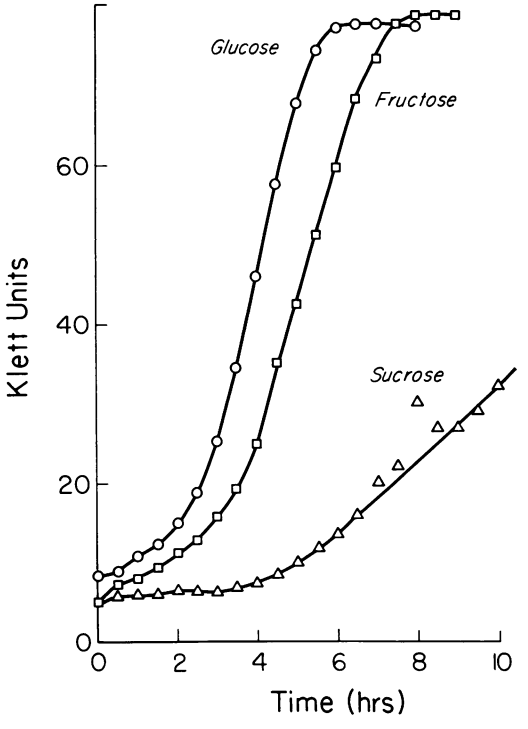

FIG. 2. *Growth of S. mutans with various sugars. Bacteria were grown for 24 hr in medium containing either 0.11 M glucose, 0.11 M fructose, or 58.4 mM sucrose. After a 50-fold dilution of each culture into fresh medium containing the same sugar, growth was followed by measuring the turbidity at various intervals as described in Materials and Methods.*

appears to be the consequence of extracellular dextran production (19). The scattering of the turbidity readings in the sucrose culture reflected dextran induced cellular aggregation (6) and adherence of the bacteria to the walls of the culture flask. Thus, *S. mutans* grows equally well on either glucose or fructose, but growth with double the amount of hexose available as the disaccharide sucrose is inhibited by extracellular polysaccharide formation.

Fate of the glucosyl and fructosyl moieties of sucrose. A prediction from our data on the *S. mutans* polysaccharide-forming activities was that this bacterium would produce more dextran than levan when grown with sucrose. Cells were inoculated into medium containing the two specifically labeled sucroses. At various times, replicate cultures were fractionated to determine the location of the radioactive hexoses. Figure 3 illustrates the amount of dextran and levan present at various times after inoculation. Dextran was accumulated at a faster rate than levan. After 32 hr of growth, the cells had polymerized about five times as much glucose as fructose. Data obtained from several experiments demonstarted that, of the total sucrose available to *S. mutans*, about 4.5 to 6% of the glucosyl moiety was polymerized into dextran. One to 1.2% of the fructosyl moiety was polymerized into levan. About two-thirds of the dextran synthesized by *S. mutans* was in a water-insoluble form, and a similar fraction of the total levan was found in the insoluble polysaccharide fraction. The results of additional experiments with *S. mutans* grown on sucrose have shown that the ratio of soluble to insoluble dextran can vary greatly depending on the sucrose concentration and the duration of incubation at 37 C (Schachtele, *unpublished data*), However, a very consistent finding from these studies was that the constitutive ability of *S. mutans* to produce more dextransucrase than levansucrase is reflected by the synthesis of more insoluble and soluble dextran, in comparison to levan, during growth with sucrose.

A direct consequence of the greater polymerization of the glucosyl moiety of sucrose by dextran-

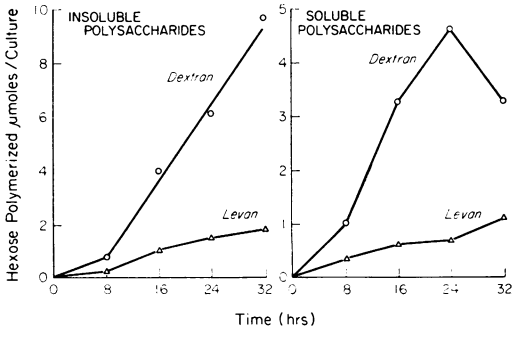

FIG. 3. *Polysaccharide production by S. mutans during growth with sucrose. Incorporation of the glucosyl (^{14}C) and fructosyl (^{3}H) moieties of sucrose into soluble and insoluble dextran and levan was measured at various intervals as described in Materials and Methods.*

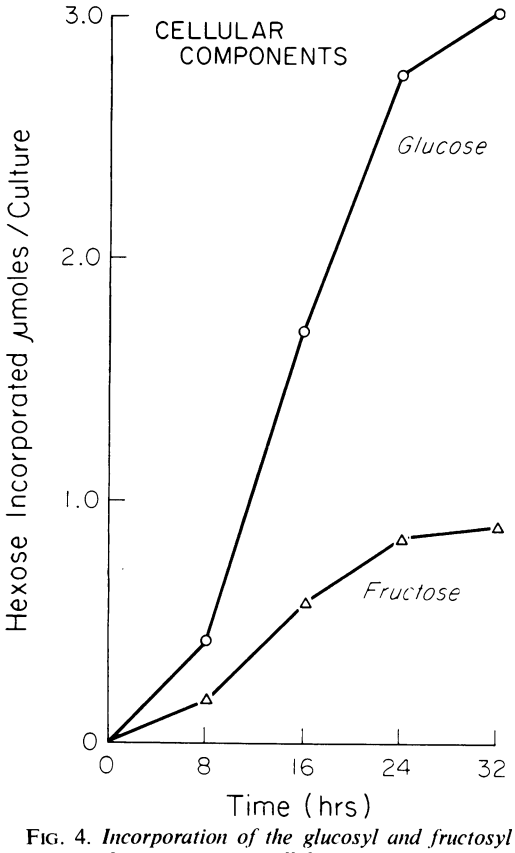

FIG. 4. *Incorporation of the glucosyl and fructosyl moieties of sucrose into cellular components. The amount of each hexose in trichloroacetic acid-precipitable cellular components was measured with sucrose labeled with ^{14}C in the glucosyl portion and sucrose labeled in the fructosyl portion with ^{3}H as described in Materials and Methods.*

sucrase would be the net release of more free fructose than glucose for utilization by the growing cells. Figure 4 illustrates incorporation of the radio-labeled glucosyl and fructosyl moieties of sucrose into trichloroacetic acid-precipitable cellular components during growth with the disaccharide. It is clear that the glucosyl moiety of sucrose was incorporated more readily than the fructosyl portion. After 32 hr of growth, three times as much glucose was incorporated into acid-insoluble cellular components.

It was possible that the above observation reflected a difference in the metabolism of the specific isotopes by the bacteria. In these studies, the fructosyl portion of sucrose was labeled in carbon-1 with ^{9}H, whereas the glucosyl moiety was uniformly labeled with ^{14}C. To confirm the preferential utilization of the glucosyl moiety of sucrose, one culture of *S. mutans* was grown with uniformly labeled ^{14}C-sucrose, and another culture was grown with sucrose uniformly labeled in only the glucosyl portion with ^{14}C. If the hexoses derived from sucrose were equally incorporated by the bacteria, the former culture would incorporate exactly twice as much radioactive hexose into acid-precipitable material as the latter. When the two cultures are compared (Table 2), it is evident that nearly 70% of the hexose incorporated by the bacteria was derived from the glucosyl portion of sucrose. This result compares very well with the data obtained with the differentially labeled sucroses (Fig. 4). In addition, the data in Table 2 confirm our observation (Fig. 3) that a large portion of the total polysaccharide synthesized from sucrose is derived from the glucosyl moiety of sucrose.

Glucose inhibition of fructose utilization by S. mutans. To confirm directly the preferential utilization of glucose over fructose by *S. mutans*, a competition experiment was performed in which the cellular incorporation of each hexose was studied in the presence of increasing amounts of the other sugar. Figure 5 illustrates the results of this study. First, the incorporation of ^{3}H-glucose is insensitive to the presence of fructose when the concentration of the ketohexose is approximately equal to the concentration of glucose. When the amount of fructose available to the cells is twice as great as the amount of glucose, there is only a 30% reduction in the amount of glucose utilized. Second, the incorporation of ^{14}C-fructose is very sensitive to the presence of glucose, and very low levels of glucose effectively inhibit incorporation of the ketohexose. When the amount of glucose available is one-fourth the amount of fructose, greater than 80% inhibition of fructose incorporation was observed. Thus, *S. mutans* will preferentially utilize glucose over fructose when both sugars are avilable.

TABLE 2. *Utilization of specifically labeled sucrose by Streptococcus mutans*[a]

Isotope	Amt of ^{14}C-hexose incorporated (μmoles/μg of cell protein)	
	Total polysaccharide	Cellular components
Sucrose-*U*-^{14}C	36.2	7.3
Sucrose:glucose-*U*-^{14}C	25.0	4.9

[a] Cells were grown for 24 hr in 2% sucrose cultures containing either sucrose-*U*-^{14}C (0.03 μCi/μmole) or sucrose:glucose-*U*-^{14}C (0.3 μCi/μmole).

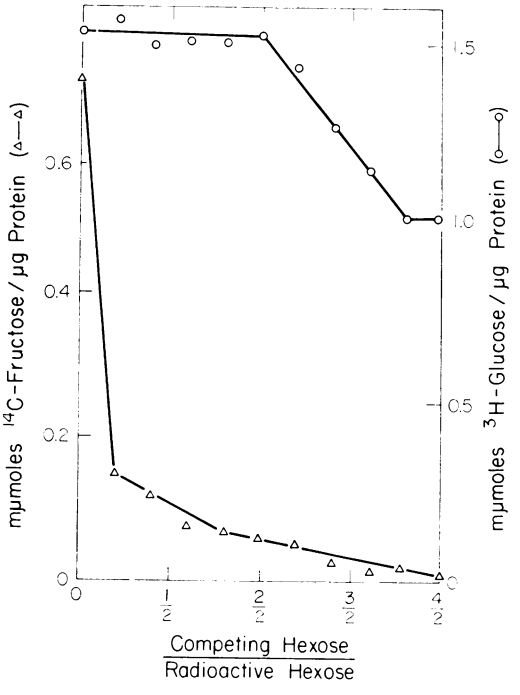

FIG. 5. *Competitive utilization of glucose and fructose by S. mutans. Cultures containing 1 μCi of ^{3}H-glucose (final concentration 0.11 M) per ml and cultures containing 0.5 μCi of ^{14}C-fructose (final concentration 0.11 M) per ml were grown in the presence of increasing amounts of the nonradioactive competing hexose. Incorporation of label into the cells was determined as described in Materials and Methods.*

DISCUSSION

Although the dextransucrases (8) and levansucrase (1) produced by various strains of *S. mutans* have been partially purified and characterized, there are little data available comparing the relative amounts of these enzymes produced by this organism. Since the activity of both of these enzymes is normally measured in 0.05 M phosphate buffer, *p*H 6.8 (1, 8, 13), we used this buffer to obtain our comparative data on the production of dextrans and levans by *S. mutans*. The fact that the soluble glucosyltransferase activity from several oral streptococci can be fractionated into several enzymes with different isoelectric points and with different *p*H optima (8) makes it unlikely that our value for dextran production reflects the exact amount of this activity present. However, the relatively broad pH ranges of the various glucosyltransferase activities (8) indicate that all of these enzymes were functioning under the conditions of our assay.

As indicated in Table 1, we found that a small fraction of the dextran- and levan-producing activities was found in a cell-bound form. On the basis of immunological studies, it was suggested (8) that the cell-bound glucosyltransferases are identical to the soluble enzymes. However, the finding that the dextran products of these enzymes differ in their sensitivity to dextranase (16) and the observation that the cell-bound enzyme produces a dextran that remains attached to the cells and which cannot be "chased" into a soluble form by the addition of excess sucrose (Schachtele, *manuscript in preparation*) indicate that additional comparative studies on the *S. mutans* cell-bound and soluble glucosyltransferase might be informative.

Other workers (3, 7, 20) have demonstrated that various strains of *S. mutans* produce more dextran than levan when this organism is grown on sucrose. In our present studies, it was shown that, depending on the duration of growth, *S. mutans* strain 6715 produces two to five times more dextran than levan. A large proportion of the total dextran was water-insoluble. The probable role of insoluble dextrans in the cariogenicity of *S. mutans* has been discussed (5, 6). Since there is no evidence for the binding of levans to *S. mutans*, it is likely that the "insoluble" levan found in our experiments represented polymers which were trapped during cell-bound dextran production and cellular aggregation.

Tanzer, Chassy, and Krichevsky (Biochim. Biophys. Acta, *in press*) have analyzed the metabolism of specifically labeled sucrose by washed nongrowing *S. mutans*. Under the experimental conditions employed by these investigators, about 18% of the glucosyl and 3% of the fructosyl portions of sucrose were polymerized into dextrans and levans, respectively. The remainder of the glucose and fructose derived from sucrose were fermented to identical products typical of homofermentative bacteria. Our data indicate that cells growing on sucrose produce dextrans and levans to a level comparable to the amount produced by incubating washed cells in sucrose and buffer. However, all of the soluble sucrases are discarded in studying washed cells and, as demonstrated in this manuscript, this could represent greater than 80% of the enzyme activity. Although only a small percentage of the total sucrose available to the cells in our experiments was converted to alcohol-insoluble polysaccharides, extensive cell clumping and adherence to the sides of the culture tubes were observed. This was expected since Gibbons and Fitzgerald (6) had demonstrated that *S. mutans* will agglutinate in the presence of extremely small amounts of dextran.

It is of interest that the data of Tanzer et al. (*in press*) indicate that *S. mutans* strain SL-1 produces an invertase. Although we have not checked for this activity in *S. mutans* strain 6715, the presence of this enzyme would not markedly affect the results presented in this manuscript since invertase would release equal amounts of the two hexoses from sucrose.

The preferential utilization of one sugar over another is a common phenomenon observed with many bacteria. Catabolite repression by glucose of fructose utilization has been demonstrated with *Aerobacter aerogenes* and involved specific inhibition of fructokinase production by glucose (15). The metabolism of glucose by *S. mutans* has been studied (18, 21), but fructose utilization by this organism has not been investigated. There are several mechanisms by which glucose could block fructose utilization in *S. mutans*. Fructose transport into the cell and the enzymes involved in the initial steps of fructose metabolism are possible sites for the inhibitory action of glucose.

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