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Use of Specifically Labeled Sucrose for Comparison of Extracellular Glucan and Fructan Metabolism by Oral Streptococci

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Sucrose labeled in the fructosyl (^3H) and glucosyl (^{14}C) moieties was used to quantitate extracellular polysaccharide production and degradation by cariogenic and noncariogenic oral streptococci. All of the strains produced glucan and fructan. *Streptococcus salivarius* produced primarily fructan, whereas *S. mutans* and *S. sanguis* produced more glucan than fructan. The cariogenic streptococci could degrade the fructan produced by noncariogenic strains. Although the soluble glucans from all of the strains were sensitive to dextranase, the insoluble glucan from *S. mutans* could be distinguished from the *S. sanguis* insoluble glucan by its greater resistance to this enzyme.

There are at least two enzymatic mechanisms by which oral streptococci can attack sucrose. Dextransucrases (α-1,6-glucan:D-fructose 2-glucosyl-transferase, EC 2.4.1.5) catalyze dextran formation with the release of free fructose, and levansucrases (β-2,6-fructan:D-glucose 6-fructosyltransferase, EC 2.4.1.10) cause levan formation with the release of glucose. The polysaccharide products of these extracellular constitutive enzymes play definitive roles in dental caries formation in that dextrans can induce aggregation of bacteria on the tooth surface (7, 9) and form part of the stable matrix of dental plaque (3, 6), whereas levans can serve as an extracellular storage polysaccharide capable of being hydrolyzed to metabolizable fructose by plaque bacteria (4, 23). At this time there is little data which allow direct comparison of the glucan- and fructan-producing capabilities of oral streptococci under defined conditions, and the organisms responsible for fructan production and degradation in plaque have not been clearly defined.

In this note, studies are presented in which sucrose labeled in the fructosyl moiety with ^3H and sucrose labeled in the glucosyl moiety with ^{14}C were utilized to quantitate simultaneously glucan and fructan production by oral streptococci. In addition, the dextranase sensitivity of the radioactive polysaccharides was tested, and the ability of oral streptococci to degrade homologous and heterologous levan was determined.

The human cariogenic strains of *Streptococcus mutans* AHT (reference 24, serological group I) and BHT (reference 13, serological group II) and the cariogenic rat strain FA-1 (reference 5, serological group II) were obtained from A. S. Bleiweiss (University of Florida, Gainesville). *S. mutans* strain 6715 (5) and *S. sanguis* H7P were obtained from W. F. Liljemark (Forsyth Dental Center, Boston, Mass.). *S. salivarius* strain C1 is a lab isolate recognized by its characteristic growth on Mitis salivarius agar (21). Bacteria were inoculated into basal broth (9) supplemented with 2% sucrose and grown anaerobically (BBL, GasPak anaerobic system) for 16 hr at 37 C.The cultures were diluted 50-fold into fresh 2% sucrose broth containing radioactive sucrose (sucrose-fructose-*1-^3H*, 5.6 Ci/mmole, 1.0 μCi/ml and sucrose-D-glucose-*U-^{14}C*, 243 mCi/mmole, 0.1 μCi/ml; New England Nuclear Corp., Boston). After anaerobic incubation at 37 C for 32 hr, the cultures were vigorously dispersed and centrifuged (Sorval RC-2 centrifuge, 30 min, 20,000 $\times$ *g*) to sediment the cells and insoluble polysaccharides.

Soluble polysaccharides were precipitated from the supernatant fluid by the addition of ethanol to a concentration of 90%. The polymers were collected by centrifugation, washed twice with 90% ethanol, dissolved in water, and assayed for ^{14}C and ^3H as described elsewhere (19). Bacterial cell material was separated from the water-insoluble polysaccharides by mild sonic treatment and washing with alkali, as described by Gibbons and Nygaard (9). Each streptococcal strain varied in its response to this treatment. With *S. mutans* strains 6715 and AHT, it was

eventually necessary to dissolve the cellular components with 0.3 N NaOH (37 C, 18 hr) and then to remove the insoluble polysaccharides by centrifugation. Trichloroacetic acid fractionation (20) of the supernatant fluid revealed that virtually all of the cellular deoxyribonucleic acid and protein was solubilized by this treatment. The low levels of radioactivity in the 0.3 N NaOH supernatant fluid and the lack of ethanol-precipitable counts indicated that little depolymerization of the insoluble polysaccharides occurred during this process and that the bacteria contained little amylopectin-type polysaccharide (C. F. Schachtele, *unpublished data*). The alkaline washes were combined with the residual insoluble polysaccharides, and the NaOH concentration was increased to 5 N. Extensive heating was used to dissolve the polysaccharide, and the amount of ^{14}C and ^{3}H was determined.

Table 1 shows the amount of soluble and insoluble glucan and fructan produced during growth on sucrose by each of the streptococci. Several significant findings are apparent when the various strains are compared. As expected (18), *S. salivarius* produced large quantities of soluble ^{3}H-labeled polysaccharide, presumably levan, and only a very small quantity of ^{14}C-labeled glucan. It is important that this organism is not found in high proportions in dental plaque but is found mainly in saliva and on the tongue (1, 15) and is probably not a contributor to plaque fructan. In contrast, *S. sanguis* and *S. mutans* can make up high percentages of the streptococci found in plaque (2, 15), and these organisms appear capable of contributing to plaque fructan. Under the conditions of our experiment, *S. sanguis* consistently produced two to five times as much fructan as any of the *S. mutans* strains.

TABLE 1. *Glucan and fructan production from specifically labeled sucrose by oral streptococci*

Organism	Amt of hexose (μmoles/μg of protein)[a]				Total glucan/fructan
	^{14}C-glucan		^{3}H-fructan		
	Soluble	Insoluble	Soluble	Insoluble	
Streptococcus mutans 6715	10.5	7.2	5.6	1.5	2.5
S. mutans FA-1	22.8	3.0	11.2	1.3	2.1
S. mutans AHT	15.3	7.3	8.2	1.5	2.3
S. mutans BHT	15.8	3.2	16.7	1.8	1.0
S. sanguis H7P	180.3	5.4	37.0	1.6	4.8
S. salivarius C1	0.0	2.0	107.4	5.0	0.1

[a] Cellular protein was determined by the method of Lowry et al. (17).

TABLE 2. *Fructan hydrolysis by oral streptococci*

Source of fructan hydrolase[a]	Source of radioactive fructan[b]	Per cent hydrolysis[c]
Streptococcus mutans 6715	*Streptococcus mutans* 6715	74.0
S. mutans 6715 (heated)[d]	*S. mutans* 6715	2.3
S. mutans FA-1	*S. mutans* FA-1	86.2
S. mutans AHT	*S. mutans* AHT	81.5
S. mutans BHT	*S. mutans* BHT	85.6
S. saguis H7P	*S. sanguis* H7P	78.0
S. salivarius C1	*S. salivarius* C1	85.0
S. mutans 6515	*S. sanguis* H7P	88.1
S. mutans FA-1	*S. sanguis* H7P	87.2
S. mutans AHT	*S. sanguis* H7P	86.2
S. mutans BHT	*S. sanguis* H7P	89.0
S. salivarius C1	*S. sanguis* H70	85.3

[a] Bacteria were grown for 36 hr in broth containing 2% sucrose. The enzyme-containing culture supernatant fluids were obtained by centrifugation (10,000 × *g*, 10 min), dialyzed at 4 C against 0.1 M acetate buffer (*p*H 6.0), and stored at −20 C.

[b] Tritium-labeled fructans were the soluble polysaccharides prepared in the previous experiment (Table 1).

[c] Assay conditions: 0.1 M acetate buffer (*p*H 6.0), radioactive substrate (1,000 to 5,000 counts/min), culture supernatant fluid in a final volume of 0.7 ml, 37 C, 60 min. Ethanol-insoluble counts were determined (14, 21) before and after incubation of reaction mixture.

[d] Culture supernatant fluid was heated (100 C, 5 min) prior to assay.

A large proportion of human plaque streptococci is capable of producing the inducible enzyme fructan hydrolase when grown in the presence of levan or sucrose (4). The ability to synthesize this enzyme and to utilize fructan as a carbon and energy source may be important for the survival of bacteria in plaque, and the metabolism of fructose could play a role in acid production and consequently contribute to dental caries formation. It was of interest to utilize the ^{3}H-labeled fructans prepared above to assess fructan hydrolase production by the specific strains of oral streptococci and to analyze the ability of these bacteria to degrade fructans produced by themselves and by related strains. Table 2 shows that, when grown with 2% sucrose, all of the streptococci studied produced a heat-labile fructan-hydrolyzing activity capable of degrading a large percentage of its own fructan. Since noncariogenic *S. sanguis* constitutes a large

proportion of the streptococci found in plaque (2) and appears capable of being a primary contributor to plaque fructan, the soluble polysaccharide from this bacterium was treated with culture supernatant fluids from each of the *S. mutans* strains. Both the human and rat cariogenic strains produce enzymatic activity capable of hydrolyzing most of the *S. sanguis* fructan. In addition, in experiments not presented here, we have shown that *S. sanguis* can produce activity capable of hydrolyzing fructan from several *S. mutans* strains. This lack of strain specificity in the fructan-hydrolyzing activities clearly indicates that the availability of plaque fructan is not restricted to the organism producing the polysaccharide and that cariogenic streptococci can readily utilize fructan produced by noncariogenic strains.

Another feature of polysaccharide production by oral streptococci presented in Table 1 is that under identical conditions *S. sanguis* produces about 10 times more soluble glucan than any of the *S. mutans* strains and thus would be expected to be a primary source of plaque glucan. Of special interest was the finding that *S. sanguis* produced as much insoluble glucan as the cariogenic strains of *S. mutans*. Since there is evidence (8, 12) that water-insoluble glucans may play a key role in the potent cariogenicity of *S. mutans*, it is likely that there are additional differences

TABLE 3. *Dextranase sensitivity of insoluble glucans from Streptococcus mutans 6715 and S. sanguis H7P*

Source of ^{14}C-labeled insoluble glucan[a]	Per cent degradation[b]	
	Bacterial dextranase[c]	Fungal dextranase[d]
Streptococcus mutans 6715	30.0	26.7
S. sanguis H7P	86.0	85.8

[a] Bacteria were grown overnight in broth containing 2% glucose. Washed cells were incubated in broth containing 500 μg of NaF per ml and 2% radioactive sucrose (sucrose-D-glucose-*U*-^{14}C, 243 mCi/mmole, 0.1 μCi/ml), washed three times with standard saline citrate (0.15 M NaCl, 0.015 M sodium citrate), and suspended in 0.1 M acetate buffer (*p*H 4.8).

[b] Calculated from the amount of ethanol-insoluble radioactivity (14, 22) remaining after enzyme treatment.

[c] Calbiochem, Los Angeles, Calif., lot no. 001621. Assay conditions: 0.1 M acetate buffer (*p*H 4.8), 10 units of enzyme per ml, 40 C, 20 min.

[d] Nutritional Biochemicals Corp., Cleveland, Ohio, control no. 9313. Assay conditions: same as above.

TABLE 4. *Dextranase sensitivity of soluble glucans from Streptococcus mutans and S. sanguis*

Source of radioactive glucan[a]	Per cent degradation[b]	
	Fungal dextranase	Bacterial dextranase
Streptococcus mutans 6715	70.1	78.3
S. mutans FA-1	76.1	82.6
S. mutans AHT	71.9	79.2
S. mutans BHT	59.4	62.4
S. sanguis H7P	73.6	81.4

[a] ^{14}C-labeled glucans were the soluble polysaccharides prepared previously (Table 1).

[b] See Table 3 for assay conditions.

in the glucans from *S. mutans* and *S. sanguis*. An immediate difference in the insoluble glucans from these bacteria was demonstrated when the enzyme dextranase (α-1,6-glucan 6-glucanohydrolase, EC 3.2.1.11) was tested against these polysaccharides. Table 3 clearly demonstrates that the *S. mutans* glucans are far less sensitive to both fungal and bacterial dextranase when compared to the glucans of *S. sanguis*. Either the *S. mutans* insoluble glucans contain fewer α-1,6 linkages or more branching, or the physical nature of the cell-dextran aggregates limits enzyme accessibility. Evidence for the physical heterogeneity of *S. mutans* insoluble glucans has been presented (11), although the structural differences in the polymers have not been determined.

The soluble glucans from each of the oral streptococci were tested for dextranase sensitivity (Table 4), and it is clear that the polysaccharides from all of the strains contain 1,6-linkages which are readily hydrolyzed by the enzyme. It is of interest that the soluble glucans from the cariogenic *S. mutans* E49 contain 69% 1,6-like linkages, 13% either 1,2- or 1,4-linkages, and 18% 1,3-linkages (16).

Specifically labeled sucrose allows simultaneous and accurate evaluation of extracellular polysaccharide production by oral microorganisms under defined conditions. Use of this technique for analysis of sucrose metabolism under various conditions (e.g., nongrowing cells or mixed cultures, or both) should provide useful information concerning the catabolism of sucrose in the oral environment. The availability of specifically labeled glucans (^{14}C) and fructans (^{3}H) will allow rapid comparative studies on the physical and metabolic properties of these polymers.

The metabolic fate of the glucosyl and fructosyl moieties of sucrose employing specific isotopic tags has recently been studied in nongrowing

(Tanzer, Chassy, and Krichevsky, *in press*) and actively growing *S. mutans* (C. F. Schachtele et al., Bacteriol. Proc., p. 140, 1971). In addition, sucrose labeled in the glucosyl moiety has been used to study the metabolic relationships between soluble and insoluble dextran formation by oral streptococci (C. F. Schachtele et al., *manuscript in preparation*).

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