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NOTES

Evaluation of Dextranase Production by the Cariogenic Bacterium *Streptococcus mutans*

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A recently developed blue dextran-agar assay was utilized to detect dextranase production by a large number of strains of *Streptococcus mutans*. An extract obtained from *S. mutans* strain B2 hydrolyzed high-molecular-weight radioactive dextran to oligosaccharides. There are several possible roles for this enzyme activity in the metabolism of *S. mutans* dextrans.

During the course of our analysis of supragingival dental plaque for the presence of dextranase (α-1,6-glucan 6-glucanohydrolase, EC 3.2.1.11)-producing microorganisms (16), we isolated many gram-positive cocci that were capable of producing this enzyme. Microbiological and biochemical identification of several of these organisms indicated that they resembled the cariogenic bacterium *Streptococcus mutans*. Although the work of Mäkinen and Paunio, using blue dextran as an enzyme substrate (13), suggested the presence of dextranase in one strain of *S. mutans*, Walker, using reducing sugar assays, was unable to detect this enzyme in *S. mutans* (18). The importance of dextran production from sucrose for the initiation of dental caries by this organism (6, 7) encouraged us to evaluate more critically the ability of various strains of *S. mutans* to produce dextranase. In this note, we confirm the presence of a dextranase activity in several strains of *S. mutans* and discuss its possible physiological function.

Defined strains of the different genetic groups of *S. mutans* (3, 4) were obtained from A. L. Coykendall (Naval Medical Research Institute, Bethesda, Md.). Laboratory strains of *S. mutans* were obtained by plating diluted suspensions of the dental plaque from elementary school children on mitis-salivarius agar(BBL, Cockeysville, Md.). After anaerobic incubation for 24 h at 37 C and aerobic incubation for 24 h at room temperature, typical colonies of *S. mutans* (10) were picked and cloned. The isolates were subsequently checked for their ability to ferment mannitol or sorbitol broths to pH 5.6 or lower (2, 10). Those organisms giving positive results were considered *S. mutans*.

The ability of strains of *S. mutans* to produce dextranase was determined by streaking the bacteria on the previously described blue dextran medium (14, 16). After anaerobic incubation, the presence of a clear zone around isolated colonies indicated the production of dextran-degrading activity. Table 1 shows that 10 of the 13 defined *S. mutans* strains had detectable dextranase activity. The survey of freshly isolated strains of *S. mutans* revealed that 90% were positive for dextranase production. Of the characterized strains, *S. mutans* B2 and GS5 (group 1) showed the greatest hydrolysis of the blue dextran medium.

S. mutans strain B2 was chosen for further study. The organism was grown anaerobically for 48 h at 37 C in Trypticase soy broth (BBL) supplemented with 1% dextran T10 (Pharmacia Inc., Piscataway, N.J.). The cells were harvested by centrifugation (Sorvall RC-2 centrifuge, 15 min, 10,000 $\times$ *g*), washed twice with 0.05 M sodium citrate buffer (pH 5.6), suspended in the same buffer, and disrupted in a French pressure cell (American Instrument Co., Silver Spring, Md.). Particulate material was sedimented by centrifugation (Sorvall OTD centrifuge, 30 min, 50,000 $\times$ *g*). At this point, all cell and medium fractions were tested for dextranase activity by the blue dextran-agar diffusion assay (16). Activity was detected in the soluble fraction of the disrupted cells. This fraction was used as the enzyme source in a typical dextranase assay in which the presence of reducing sugar was measured (17). The

TABLE 1. *Evaluation of dextranase production by S. mutans*

Genetic group	Strain of *S. mutans*	Dextranase activity[a]
1	Ingbritt	+
	10449	+
	LM7	+
	B2	+
	GS5	+
2	BHT	+
	FA1	+
3	K1R	+
	SL1	−
4	E49	+
	HS6	−
	AHT	−
	OMZ61	+
Unknown	Lab isolates	35+, 4−

[a] Bacteria were grown anaerobically for 18 h at 37 C and then streaked onto blue dextran plates. After anaerobic incubation for 24 h at 37 C, the plates were evaluated for dextranase activity(+, clearing of blue dextran; −, no clearing).

results of this experiment indicated that no additional reducing groups were liberated during incubation. A possible explanation for this lack of reducing sugar production was that, although dextran hydrolysis was actually occurring, the products of the reaction were functioning as acceptor molecules for residual *S. mutans* dextransucrase (α-1,6-glucan:D-fructose 2-glucosyltransferase, EC 2.4.1.5) activity (18). To eliminate this possible masking effect, we designed an experiment which would saturate the dextransucrase with the alternate acceptor molecule maltose (18) while we determined dextranase activity by the cleavage of ^{3}H-labeled dextran. The reaction mixture contained 0.5 ml of the soluble cell fraction, 1.5 ml of 0.05 M sodium citrate buffer, 20 mg of maltose per ml, and 1 μCi of [^{3}H]dextran (molecular weight; 77,000 Amersham-Searle, Chicago, Ill.). After incubation for 18 h at 37 C, the radioactive products of the reaction were separated by passage through a Bio-Gel P-100 filtration column (1.5 by 30 cm; Bio-Rad Laboratories, Richmond, Calif.) (Fig. 1). The boiled-enzyme control elution pattern (open circles) indicated little nonenzymic hydrolysis of the dextran. The enzymatically active fraction (closed circles) degraded the high-molecular-weight dextrans into randomly mixed oligosaccharides. The appearance of these labeled oligosaccharides in conjunction with the disappearance of the original dextran confirms the presence of dextranase in this cell fraction. The data also suggest that the enzyme activity was endohydrolytic, since no significant peak appeared at the elution volume of glucose and isomaltose (fractions 49 and 50).

There are several possible functions for the production of dextranase activity by an organism which is capable of synthesizing large quantities of dextran (7, 8). Utilization of extracellular, soluble dextran as an energy and carbon source during periods of nutritional deprivation is one potential function. This would be analogous to the inducible fructan hydrolase system which allows *S. mutans* to degrade its own extracellular levan (15). However, *S. mutans* does not produce acid during incubation in broth containing dextran as the fermentable substrate (2). This is in agreement with our data (Fig. 1), which demonstrate that cleavage of dextran by the *S. mutans* dextranase is endohydrolytic and no free glucose is released.

Another possible function for the *S. mutans* dextranase activity may be in the formation of the highly branched dextrans (8, 11, 12) produced from sucrose by the dextransucrase activity of this organism (6–8). Ebert and Brosche (5) presented data with the dextransucrases of *Leuconostoc mesenteroides* which indicated that low-molecular-weight dextrans could act as acceptor molecules or chain terminators and could be "inserted" into α-(1→6)-linked chains as α-(1→3)-linked branches. If such a reaction scheme is valid for *S. mutans*, as suggested by the data of Walker (18, 19) for the alteration of

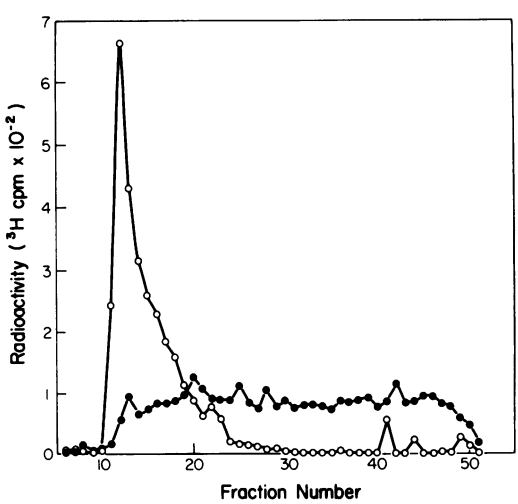

FIG. 1. *Bio-Gel P-100 column chromatography of the reaction products resulting from the enzymatic degradation of ^{3}H-labeled dextran with the soluble extract preparation from S. mutans B2. Symbols: ●, active enzyme preparation; ○, heat-inactivated (95 C, 10 min) enzyme preparation.*

dextran production by *S. mutans* with dextranase from *S. mitis*, then a low level of endohydrolytic dextranase activity could influence polymer formation by hydrolyzing soluble dextrans to oligosaccharides. The resulting oligosaccharides would then be incorporated by the *S. mutans* dextransucrase into the dextran as a branch. The result of such an integrated system would be the production of a highly branched polymer. This is consistant with the known structure (11, 12) and dextranase resistance (1, 8, 15) of the *S. mutans* dextrans.

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