INFECTION AND IMMUNITY, Oct. 1975, p. 934-937

Vol. 12, No. 4
Printed in U.S.A.

Production of Elevated Levels of Dextransucrase by a Mutant of *Streptococcus mutans*

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Received for publication 12 June 1975

A mutant (S19) of *Streptococcus mutans* strain 6715 which produces elevated levels of dextransucrase (EC 2.4.1.5) was isolated. Soluble enzyme in culture supernatant solutions from S19 polymerized the glucosyl moiety of sucrose into alcohol-insoluble and water-insoluble glucans at a rate three to six times greater than that of the parent strain. Washed-cell suspensions of S19 also contained increased amounts of cell-associated enzyme. Adherence of S19 to glass in the presence of sucrose occurred at twice the rate of strain 6715. The K_m values for sucrose and primer dextran were similar for the mutant and parent enzymes. Mutant S19 should facilitate studies on the mechanism of adherence of *S. mutans* and the control of dextransucrase production by this bacterium.

The cariogenicity of *Streptococcus mutans* has been related to its ability to convert the glucosyl moiety of sucrose into extracellular adherent glucans (7, 8, 10, 11, 13, 18). Strong support for this relationship has recently been obtained utilizing mutants of *S. mutans* which produce structurally altered or decreased amounts of glucan when grown in the presence of sucrose and which also have lost the ability to form adhesive microbial plaques in vitro (3, 4, 14, 19). Some of the variants exhibited markedly decreased virulence when tested in rats (3, 22). In this communication we report the isolation of a mutant of *S. mutans* 6715 which produces elevated levels of dextransucrase and demonstrate the effect of this phenotypic change on glucan production from sucrose and sucrose-stimulated adherence of cells to smooth glass surfaces.

An exponentially growing anaerobic 37 C culture of *S. mutans* 6715 in Trypticase soy broth (BBL, Cockeysville, Md.) supplemented with 0.5% (wt/vol) yeast extract (Difco, Detroit, Mich.) was diluted with an equal volume of broth (final volume 10 ml) and poured into a sterile 100-mm petri dish. The culture was vigorously stirred magnetically and irradiated at 254 nm with a dose rate of 400 erg/mm^2 per s to a total dose of 1.2×10^4 erg/mm^2 (uncorrected for sample absorption). These irradiation conditions resulted in a 99% decrease in colony-forming units as determined by plating on sucrose-supplemented (5%) Trypticase soy agar (BBL). After overnight incubation normal-appearing rough colonies were picked and inoculated into broth supplemented with glucose (2.5%, wt/vol) and $NaKPO_4$ buffer (0.1 M, pH 6.8). Cultures were incubated anaerobically at 37 C overnight. Aliquots of these cultures were inoculated into fresh broth and grown overnight. Since microscopic examination of the culture to be irradiated revealed that the bacteria were in chains, the above culturing procedures were used to allow segregation of mutants into individual plating units. Finally, the last broth subcultures were diluted and plated on mitis-salivarius agar (BBL). One colonial variant, designated S19, was identified as a small, highly granular, dark colony which produced large amounts of liquid extracellular exudate. Isolate S19 was shown to carry the streptomycin resistance marker of the parent (9) and to have retained the ability to agglutinate upon addition of either sucrose or high-molecular-weight dextran (8).

Cell-free supernatants from overnight cultures of 6715 and mutant S19 were prepared as described elsewhere (2). Production of methanol-insoluble polysaccharide was quantitated by a previously described method (6), utilizing sucrose labeled in the fructosyl moiety with [^{3}H]- and uniformly labeled [^{14}C]sucrose. Only small quantities of fructan were produced by either cell supernatant during prolonged incubation with the specifically labeled sucrose. With both enzyme preparations, less than 3% of the total polysaccharide produced during a 90-min incubation was derived from the fructosyl portion of sucrose. This is consistent with our previous finding (21) that under our growth conditions strain 6715 produces only low levels of levansucrase (EC 2.4.1.10). Mutant S19 ap-

pears to have retained this characteristic. When the ability of the supernatants to produce glucan from sucrose was monitored, it was demonstrated (Fig. 1) that the mutant preparation produced glucan at a two- to threefold greater rate than the 6715 preparation when assayed either in the presence or absence of primer (5). The rates of water-insoluble glucan production from sucrose were also determined (21). It was found that synthesis of this material proceeded at a rate approximately six times greater in the S19 preparation (Fig. 2) than in the 6715 preparation.

In addition to the soluble dextransucrase found in *S. mutans* culture supernatants, enzyme activity has also been shown to occur in a cell-associated form (10, 11, 15, 17, 18). Cell-associated activity was also compared in washed-cell suspensions of strains 6715 and S19. Bacteria were washed and suspended in sodium acetate buffer (0.05 M, pH 5.5) containing NaF (1 mM). [^{14}C]sucrose (final concentration 33 mM, 90 μCi/mmol; New England Nuclear Corp., Boston) was added to reaction tubes containing the organisms at a concentration equivalent to 12 mg of cell protein (16), and the 0.25-ml samples were incubated for various intervals at 37 C. The reaction was terminated by adding a 20-fold excess of buffer and pouring the diluted reaction mixture over a membrane filter (0.2 μm, Millipore Filter Corp., Bedford, Mass.). The filters were washed with 80 ml of buffer to remove nonpolymerized sugar and then counted for radioactivity as described previously (20). Figure 3 illustrates the time course of cell-associated glucan production by the cells and it is clear that the mutant bacteria accumulate glucan more rapidly than the control 6715 cells.

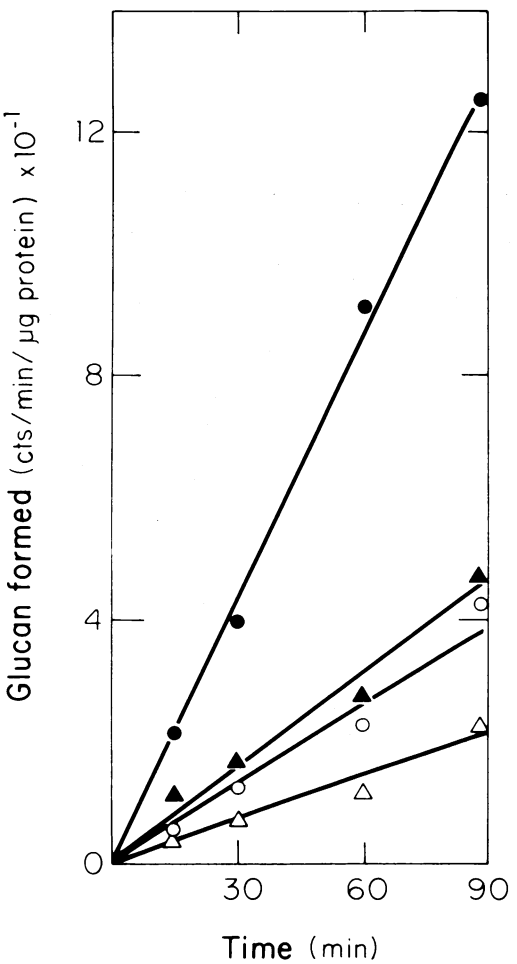

FIG. 1. *Time course of glucan production from sucrose by crude dextransucrase from S. mutans 6715 and mutant strain S19 in the presence and absence of primer. Symbols:* (○) *6715 plus primer;* (△) *6715 minus primer;* (●) *S19 plus primer; and* (▲) *S19 minus primer.*

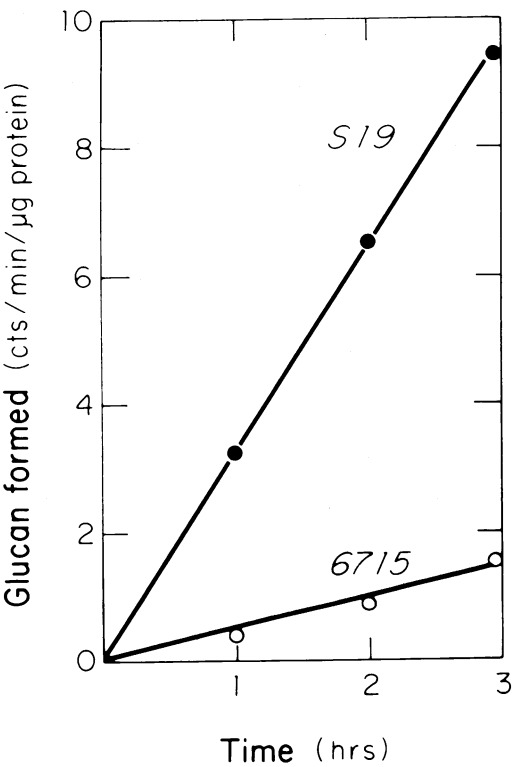

FIG. 2. *Time course of water-insoluble glucan production from sucrose by crude dextransucrase from S. mutans 6715* (○) *and mutant S19* (●).

When [^{3}H]thymidine-labeled washed-cell preparations of the mutant and parent strains were prepared and sucrose-stimulated adherence to glass was monitored (21), it was observed that cells of the mutant strain became fixed more rapidly than 6715 cells (Fig. 4). The quantity of cells attached to glass after 18 h of incubation (final level) was twofold higher with the mutant bacteria (data not shown).

The K_m values for sucrose and primer dextran with a molecular weight of 10^4 did not differ significantly for the 6715 (3 mM, sucrose; 2 μM, dextran) and S19 (4 mM, sucrose; 1.5 μM, dextran) dextransucrase. In addition, differences between the enzymes with respect to storage stability or behavior in purification procedures have not been noted. Thus it appears that mutant S19 simply produces greater quantities of the typical dextransucrase than does 6715.

The *S mutans* dextransucrase has been considered to be synthesized constitutively due to its production by cells growing in the absence of the substrate, sucrose (10, 12). Studies on the regulation of production of this enzyme have primarily involved differential analysis of the cell-associated and soluble forms of the enzyme (12, 15, 17), and no detailed investigations on the control of production of this enzyme by *S. mutans* have been presented. Work with *S. sanguis* has demonstrated that the level of dextransucrase activity can be markedly altered by varying the cell growth rate and culture medium, and it would appear that the regulation of this enzme is complex (1). In addition to genetic modifications which cause decreased production of enzyme activity (3, 4, 14, 19), the data presented in this communication demonstrate that variants can also be obtained which

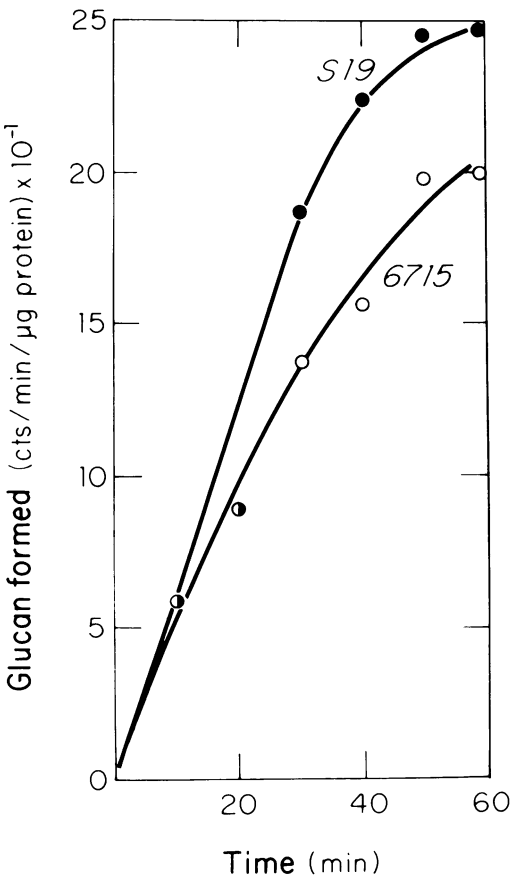

FIG. 3. *Time course of glucan synthesis from sucrose by S. mutans 6715* (○) *and mutant S19* (●) *cell-associated dextransucrase.*

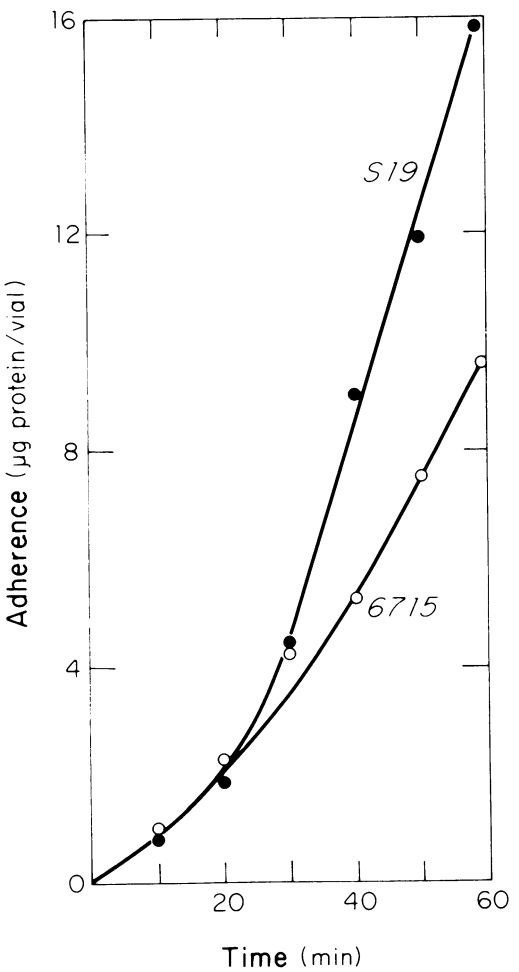

FIG. 4. *Time course of sucrose-stimulated adherence of S. mutans 6715* (○) *and mutant S19* (●) *to smooth glass surfaces.*

produce elevated levels of dextransucrase. Studies with mutants of this type should allow further evaluation of the control of dextransucrase production and the role of this enzyme in *S. mutans* adherence to smooth surfaces.

This work was supported by Public Health Service grant DE 02654 from the National Institute of Dental Research. C. F. S. was the recipient of Public Health Service career development award K4-DE-42,859.

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