Cloning and Expression of Two *Streptococcus mutans* Glucosyltransferases in *Escherichia coli* K-12

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Chromosomal DNA from *Streptococcus mutans* strain MFe28 (serotype *h*) was cloned in the bacteriophage vector λ L47.1. Two classes of recombinants were found which expressed glucosyltransferase activity in phage plaques: (i) *gtfS*, which expressed a glucosyltransferase synthesizing a water-soluble, dextranase-sensitive glucan, and (ii) *gtfI*, which expressed a primer-dependent glucosyltransferase synthesizing an insoluble glucan.

Streptococcus mutans has been shown to be the major organism responsible for dental caries (10). The cariogenicity of *S. mutans* lies, at least in part, in its ability to contribute to plaque formation by producing extracellular glucans which mediate cell-to-cell and cell-to-tooth adherence (8, 10). The enzymes responsible for the synthesis of these polymers are known as glucosyltransferases (GTFs; EC 2.4.1.5.). Studies of GTFs have proved difficult (3), notably because enzyme preparations often contain more than one enzyme activity and these commonly occur in high-molecular-weight aggregates. Recently, the techniques of genetic engineering have been used with some success in the study of *S. mutans* enzymes (12, 18). We report here the cloning and expression of two distinct *S. mutans* GTF genes in *Escherichia coli* K-12.

MATERIALS AND METHODS

Construction of gene bank. The DNA manipulation techniques used here have been described in detail elsewhere (13, 22). Briefly, chromosomal DNA from *S. mutans* strain MFe28 (serotype *h*) was partially digested with restriction endonuclease *Sau*3A to give fragments which were cloned into the replacement bacteriophage vector λ L47.1 (16) and packaged into phage particles in vitro.

Screening. The gene bank of recombinant phages was screened by two methods as follows.

(i) Screening for polymer-forming phage plaques was done on M9 minimal salts agar medium containing 0.4% (wt/vol) sucrose, dextran T10 (0.1 mg/ml), and the supplements thiamine (1 μg/ml), leucine (8 μg/ml), and threonine (8 μg/ml) required by *E. coli* strain C600 for growth. Presumptive recombinant phages were plated with *E. coli* C600 in soft agar (made with Luria broth, which contains tryptone [10 g/liter], yeast extract [5 g/liter], and NaCl [10 g/liter]) to give approximately 500 plaques per petri dish and incubated at 37°C for 48 h. *E. coli* C600 cannot utilize sucrose as a carbon source, so only a weak lawn of growth was formed from the nutrients in the Luria broth soft agar overlay. Approximately 1 in every 400 plaques produced a sticky, slightly cloudy, watery substance which was at first concentrated at the edge of the plaque, but with prolonged incubation (3 to 4 days) at room temperature gave domes resembling bacterial colonies (Fig. 1a). Growth of *E. coli* was stimulated around these recombinant plaques, suggesting that monosaccharides were being released by the action of cloned *S.*

mutans enzymes. Thirteen such plaques (recombinants M1 to M13) were picked and purified by streaking on *E. coli* C600, and single plaques were picked and stored over chloroform at 4°C.

(ii) For immunological screening, recombinant phages were plated on *E. coli* C600 to give about 500 plaques per plate. Replica blots on disks of nitrocellulose were prepared and probed with rabbit antiserum as described recently (22). The antiserum was raised against GTF purified from *S. mutans* strain K1 (serotype *g*) as previously described (21). One plaque of about 10,000 screened gave a positive reaction with the serum, indicating that an *S. mutans* antigen was present. When this recombinant phage (MFeA) was plated on *E. coli* C600 on sucrose M9 minimal agar and incubated as described above, it gave plaques which resembled those of M1 to M13 except that these plaques were less watery, more opaque and white, and at first had a characteristic "ring doughnut" appearance (Fig. 1b).

To study the cloned enzymes, bulk lysates were prepared by plating recombinant phage on *E. coli* C600 so as to yield confluent lysis after overnight incubation of the plates at 37°C. The soft agar layer from each plate was scraped off, macerated, and briefly subjected to ultrasonic treatment. The lysates were then centrifuged at 20,000 rpm for 20 min, and the supernatants were collected and stored at 4°C until use.

Characterization of enzymes from recombinant clones. The appearance of two different kinds of plaques on sucrose-containing plates suggested that two different types of polymer were being formed. Since no polymer was produced on plates containing raffinose, which can act as a substrate for fructosyltransferase, we suspected that the polymers were glucans and not fructans. This was confirmed by showing glucose to be the only sugar found on thin-layer chromatograms of acid-hydrolyzed polymer (21) and by assaying GTF activity in crude lysates using methods essentially as described by Kuramitsu (15) to follow the incorporation of radioactivity from sucrose labeled in the glucose moiety (New England Nuclear Corp., Boston, Mass.) into water-insoluble and methanol-insoluble polymers (Table 1). The enzyme activity in all of the recombinants M1 to M13 had the same characteristics of producing a water-soluble glucan and not being dependent upon the presence of dextran T10 as a primer. The polymer formed was destroyed by overnight incubation at 37°C in 0.05 M phosphate (pH 6.5) containing dextranase (1 mg/ml; BDH Chemicals, Poole, England). We designated the gene for this enzyme *gtfS*. The
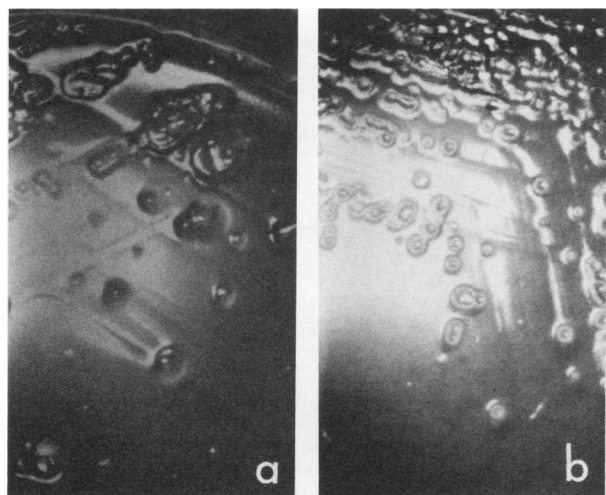
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FIG. 1. Appearance of plaques of λ recombinants carrying (a) *gtfS* or (b) *gtfI* plated on *E. coli* C600 on sucrose minimal medium.
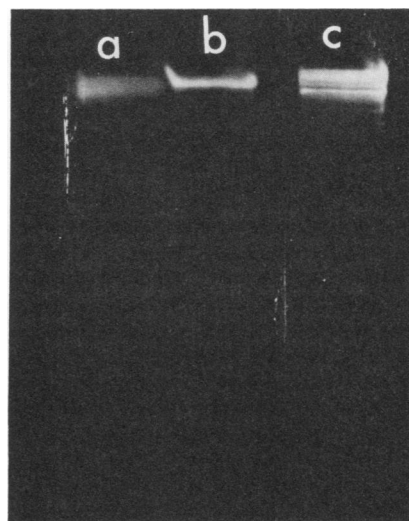

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of lysates of recombinants carrying (a) *gtfS* or (b) *gtfI*. Bands of glucan formed after overnight incubation in sucrose can be seen. Lane c, analysis of a 10-fold concentrated culture supernatant of *S. mutans* MFe28.

enzyme in lysates of *E. coli* infected with MFeA was very different, being dependent upon primer and synthesizing a water-insoluble and dextranase-resistant glucan (Table 1). We designated the gene for this enzyme *gtfI*. The amount of GTF activity in the crude lysates was approximately 40% of that found in overnight cultures of *S. mutans* MFe28.

RESULTS AND DISCUSSION

To determine the molecular weight of the cloned GTF, the crude lysates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and GTF activity was detected by incubation with sucrose in the presence of 1% Triton X-100 to overcome the inhibition by sodium dodecyl sulfate (20, 21). An opaque white band of polymer was formed by the water-insoluble GTF (GTF-I), whereas the water-soluble GTF (GTF-S) formed a more translucent polymer (Fig. 2). Lysates of both GTFs also contained a number of weaker bands of activity just below the major bands. Multiple bands in the same region of the gel were obtained with *S. mutans* MFe28. Comparison with Coomassie blue-stained gels on which RNA polymerase (subunit molecular weights of 39,000, 155,000, and 165,000) was

TABLE 1. Incorporation of radioactivity from [^{14}C]glucose-sucrose into polymer

Recombinant	Assay type	Methanol-insoluble cpm	Water-insoluble cpm (%)^a
GTF-S	Standard assay^b	3,272	124 (4)
	No primer dextran	3,265	107
	Dextranase-treated	130	70
GTF-I	Standard assay	5,841	5,705 (97)
	No primer dextran	0	0
	Dextranase-treated	5,140	5,275

^{a} Water-insoluble counts per minute expressed as a percentage of total, methanol-insoluble counts per minute.

^{b} Crude lysates of *E. coli* infected with recombinants M7 (*gtfS*) or MFeA (*gtfI*) were incubated for 3 h at 37°C in a reaction mixture (total volume, 0.4 ml) containing [^{14}C]glucose-sucrose (0.1 µCi/ml)–0.5% sucrose–0.2% dextran T10 (Pharmacia Fine Chemicals, Piscataway, N.J.) in 0.05 M phosphate buffer (pH 6.5).

included showed that GTF-S and GTF-I were between 150,000 and 160,000, with the major GTF-I band found to be consistently higher than that of GTF-S.

S. mutans serotype *h* strains have been shown to be closely related to strains of serotypes *d* and *g*, although there are distinct differences in some of their physiological characteristics and in serotype polysaccharide (2, 17). DNA homology studies, which would clarify the relationship, have not yet been published. No reports of the properties of GTFs from serotype *h* strains have appeared, but a number of workers have studied strains of serotype *d* and *g* and have described the existence of GTFs with properties, including molecular weight, type of glucan produced, and the primer dependency of enzyme activity, that closely resemble those found here in recombinants expressing *gtfS* and *gtfI* (4–7, 11, 14, 19, 23). We have also found (unpublished observations) that GTFs of serotype *h* strains have isoelectric points in the range pH 4 to 5, as do those of serotypes *d* and *g* (14, 23, 24). We also report here that antiserum raised against a serotype *g* GTF preparation reacts with cloned serotype *h* GTF-I, and indeed it was this cross-reaction that allowed us to detect the *gtfI* recombinant originally. Recombinant plaques or lysates in which *gtfS* is expressed do not react with the same serum in immunoblotting experiments, indicating that in serotype *h* GTF-S and GTF-I are antigenically distinct. GTF-S and GTF-I of *S. mutans* 6715 (serotype *g*) have also been shown to be antigenically distinct (7).

Although each of the cloned GTFs gave several bands of enzyme activity on sodium dodecyl sulfate-polyacrylamide gels, we suspect that only a single GTF gene is present in each recombinant since such multiple banding of pure GTF is a common occurrence (1, 9). This can only be conclusively demonstrated by subcloning the recombinant DNA to ensure that only a single gene is present. We are now attempting such subcloning experiments and hope that the availability of specific DNA sequences for *gtfS* and *gtfI* will be of great value in helping to elucidate the extent to which the two genes are related to each other and to the GTF genes of other bacteria.

ACKNOWLEDGMENTS

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