INFECTION AND IMMUNITY, June 1990, p. 1509–1513
0019-9567/90/061509-05$02.00/0

Vol. 58, No. 6

Inhibition of *Streptococcus mutans* Glucosyltransferase Activity by Antiserum to a Subsequence Peptide

MARK T. DERTZBAUGH† AND FRANCIS L. MACRINA*

Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, Virginia 23298-0678

Received 14 November 1989/Accepted 21 February 1990

An antigenic 15-amino-acid peptide sequence (gtfB.1) from the glucosyltransferase B enzyme of the cariogenic bacterium *Streptococcus mutans* GS-5 was identified previously from the genetic fusion of this sequence to the B subunit of cholera toxin. The resulting chimeric protein was used to raise antiserum in rabbits. This antiserum was shown to recognize the native glucosyltransferase enzyme and to inhibit its activity. The antiserum inhibited the synthesis of water-soluble glucan by ~40% and the synthesis of water-insoluble glucan by >90%. The antiserum was shown to partially inhibit fructosyltransferase activity as well. The ability of this antipeptide antiserum to inhibit several enzymes from *S. mutans* suggests that these enzymes share an epitope related to enzymatic activity.

The glucosyltransferase enzymes of *Streptococcus mutans* catalyze the formation of glucan polymers from sucrose, and these polymers allow the bacterium to accumulate tenaciously on the tooth surface in sufficient quantities for acid demineralization of the enamel to occur (7). To date, three different glucosyltransferase genes have been cloned. The *gtfB* gene encodes a dextran primer-independent enzyme of ~166 kilodaltons (kDa) that synthesizes water-insoluble glucan (1). The product of the *gtfC* gene is ~153 kDa and primer independent and makes a mixture of both soluble and insoluble glucans (8). The *gtfD* gene encodes a primer-dependent enzyme of ~155 kDa that produces water-soluble glucan (9). The enzyme(s) that synthesizes water-insoluble glucan has been associated with the virulence of *S. mutans* (2), because these polymers mediate effective cell accumulation on the tooth surface in the aqueous environment of the mouth. For this reason, the product of the *gtfB* gene has been of great interest as a potential candidate for subunit vaccines (10, 11, 18).

From previous work, we had identified a region of the *gtfB* gene which encoded a peptide that was recognized by antiserum to the native glucosyltransferase B enzyme (3). This region was identified during construction of a fusion protein composed of the N-terminal third of the *gtfB* enzyme (~46 kDa) fused to the N-terminal end of the B subunit of cholera toxin (CTB). Proteolysis of this fusion protein in *Escherichia coli* consistently produced a 14.4-kDa degradation product that cross-reacted with antiserum to the *gtfB* gene product and to CTB. This product was later determined to be composed of ~270 amino acids from the C-terminal end of the truncated *gtfB* enzyme fused to CTB. A hydrophilic domain (denoted gtfB.1) which came from this region and which encoded 15 amino acids was genetically fused to CTB (4), and the resulting chimeric protein reacted strongly with antiserum to glucosyltransferase B. The antigenicity of the chimera led us to examine whether this protein could elicit antibody capable of inhibiting glucosyltransferase activity.

In this paper, we report the results of these studies. Rabbit antiserum to the V1782 chimera was able to inhibit the net synthesis of glucan polymer by an extracellular protein preparation of *S. mutans*. In particular, the synthesis of water-insoluble glucan was almost completely inhibited by the antiserum. This is the first time that antiserum to a peptide of a glucosyltransferase has been demonstrated to inhibit enzyme activity. This observation may be helpful for defining the active site of the enzyme and has important implications for vaccines directed towards glucosyltransferases of *S. mutans*.

MATERIALS AND METHODS

Media and reagents. Todd-Hewitt broth was purchased from Difco Laboratories (Ann Arbor, Mich.). [^{14}C-glucose]sucrose and [^{14}C-fructose]sucrose were obtained from Dupont, NEN Research Products (Boston, Mass.). CTB was purchased from Sigma Chemical Co. (St. Louis, Mo.). T10 dextran was purchased from Pharmacia, Inc. (Piscataway, N.J.). Goat anti-CTB was purchased from Calbiochem-Behring (La Jolla, Calif.). Phosphatase-labeled second antibodies and 5-bromo-4-chloro-indolylphosphate/Nitro Blue Tetrazolium (BCIP/NBT) substrate were purchased from Kirkegaard and Perry Laboratories (Gaithersburg, Md.). Pooled normal rabbit serum was purchased from Flow Laboratories (McClean, Va.). Polyclonal rabbit antiserum to recombinant glucosyltransferase B was kindly provided by H. K. Kuramitsu, University of Texas Health Science Center (San Antonio).

Bacteria and proteins. The gtfB.1::CTB fusion protein of *E. coli* V1782 was constructed, expressed, and purified as previously described (4). Briefly, the chimeric protein was created by replacing the 5′ end of the *ctxB* gene of *Vibrio cholerae*, encoding the leader sequence of CTB, with an oligonucleotide encoding 15 amino acids of the *gtfB* gene of *S. mutans*. This construct was fused to a portion of the *E. coli ompA* gene, which encoded the leader peptide sequence of this protein, in order to cause the chimera to be exported to the periplasm of *E. coli* V1782. Expression of the chimera was inducible via the *lac* promoter. Strain GS-5, a Bratthall serotype c isolate of *S. mutans* (7), was used to make an extracellular protein preparation containing glucosyltransferase and fructosyltransferase enzymes.

Enzyme preparation. Extracellular proteins of *S. mutans* were obtained as follows. Strain GS-5 was grown in 3 liters of Todd-Hewitt broth, under anaerobic conditions, to an

* Corresponding author.
† Present address: Division of Gastroenterology, University of Alabama at Birmingham, Birmingham, AL 35294.

optical density at 660 nm of 0.6. The cells were removed by centrifugation, and ammonium sulfate was added to the supernatant to 60% saturation. The precipitate containing extracellular proteins was collected by centrifugation, suspended in 300 ml of phosphate-buffered saline (PBS; 0.1 M, pH 7.3), and then dialyzed three times against PBS. Approximately 30 ml of this material was divided evenly and stored at −70°C. This material was used for all enzyme assays. The remaining sample was diluted in PBS plus 6 M urea and subjected to diafiltration through a YM-100 ultrafiltration membrane (Amicon, Danvers, Mass.). The clarified sample was concentrated by ultrafiltration to 20 ml and then dialyzed in PBS in order to remove the urea. The samples were divided evenly and stored at −70°C. Although devoid of activity, this concentrated enzyme preparation was useful for immunoblotting analyses.

Antiserum. The following protocol was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University (Richmond). Antiserum to the purified, monomeric form of the V1782 chimeric protein was raised in female New Zealand White rabbits. One milligram of protein was emulsified in complete Freund adjuvant (Difco) and injected subcutaneously into the hindquarters of the animal. Three weeks later, the rabbit was given a booster injection with the protein emulsified in incomplete Freund adjuvant. One week after the second immunization, the rabbit was bled. The serum was collected, divided evenly, and stored at −20°C.

Immunoblotting analysis. Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (14) and then electrophoretically transferred to nitrocellulose sheets (19). Nonspecific binding of antibody was prevented by blocking the sheets for 1 h at 25°C with a 5% solution of chicken serum in Tris-buffered saline (TBS; 20 mM Tris–500 mM NaCl [pH 7.5]). The sheets were incubated for 16 h in antisera diluted 1:2,000 in TBS with 5% chicken serum. The sheets were washed three times for 10 min in TBS with 0.05% Tween 20 (TTBS) and then incubated for 2 h in enzyme-labeled second antibody diluted 1:2,000 in TBS. After being washed again three times in TTBS, the sheets were developed with BCIP/NBT substrate.

Enzyme assays. Glucosyltransferase activity was determined by measuring the amount of [^{14}C]glucose incorporated into glucan polymer from specifically labeled sucrose. Fructosyltransferase activity was measured similarly, except that sucrose labeled with ^{14}C in the fructose moiety was used as the substrate instead. The reaction mixture was incubated at 37°C and consisted of 100 μl of extracellular protein preparation, 50 μl of T10 dextran primer (5 mg/ml), 50 μl of labeled substrate (261 mCi/mmol, 20 μCi/ml), and 800 μl of substrate buffer (10 mM imidazole, 10 mM sucrose, 0.02% sodium azide [pH 6.5]). A 100-μl sample of the reaction mixture was pipeted onto 2.4-cm glass fiber filters (Whatman GFA, Maidstone, England). All sampling was performed in triplicate. Total polymer was collected onto the filters by precipitation in methanol. The filters were washed six times with methanol by using a vacuum manifold apparatus (Millipore Corp., Bedford, Mass.). Water-insoluble polymer was collected by washing the filters four times with deionized water and then twice with methanol. The filters were air dried, placed into vials with 5 ml of scintillation cocktail (Amersham OCS, Arlington Heights, Ill.), and then counted for 2 min in a scintillation counter (LS-1800; Beckman Instruments, Inc., Fullerton, Calif.). The mean counts per minute of each sample were subtracted from a background control containing PBS. For inhibition assays, enzyme was incubated with an equal volume of serum for 1 h at 37°C before being added to the reaction mixture. For use as a positive control, the enzyme preparation was incubated with an equal volume of PBS. Serum was diluted in PBS, when required.

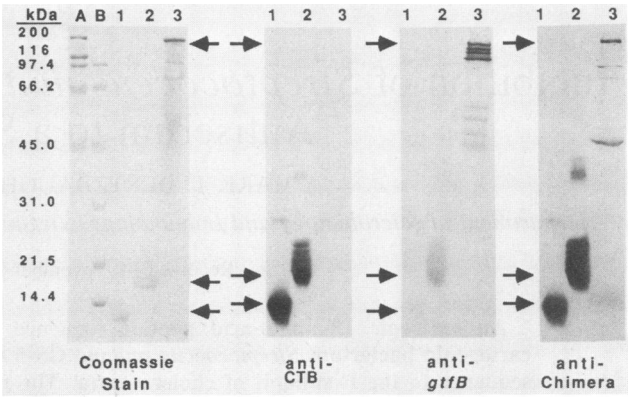

FIG. 1. Immunogenicity of the chimera. Protein samples were fractionated on 10% discontinuous polyacrylamide gels containing 0.1% sodium dodecyl sulfate and then electrophoretically transferred to nitrocellulose sheets. Replicate blots were probed with antiserum to either CTB (anti-CTB), glucosyltransferase B (anti-*gtfB*), or the chimera (anti-Chimera). Total protein was visualized in a replicate gel by using Coomassie blue. Molecular masses are listed on the left. Arrows denote immunoreactive proteins of interest. Lanes: A, high-molecular-weight standards (Bio-Rad Laboratories, Richmond, Calif.); B, low-molecular-weight standards (Bio-Rad); 1, CTB (0.6 μg); 2, V1782 chimera (0.6 μg); 3, extracellular proteins from *S. mutans* GS-5 (16 μg).

RESULTS

Immunoreactivity of the chimera. Previous immunoblots had demonstrated that the V1782 chimera was recognized by antisera either to CTB or to glucosyltransferase B (4). However, it was not clear that this protein was immunogenic or that antiserum raised to the chimera would recognize the glucosyltransferase B enzyme. In order to determine this, replicate blots containing CTB, chimera, and extracellular protein from *S. mutans* GS-5 were prepared. The blots were probed with antiserum to either CTB, glucosyltransferase B, or chimera (Fig. 1). These results demonstrated that the chimera was recognized by antisera raised against the native proteins from which it was derived. The blots also showed the specificity of the chimera-elicited antiserum, which reacted with CTB and a high-molecular-weight protein of ~155 kDa found in the extracellular preparation of *S. mutans*.

Inhibition of enzyme activity. Antiserum to the chimera was evaluated for its potential to inhibit glucosyltransferase activity in vitro. Incubation of the enzyme in undiluted antiserum to either the chimera or glucosyltransferase B, prior to the addition of substrate, resulted in a ~50% reduction in total glucan synthesis (water-soluble and water-insoluble glucans) relative to the synthesis in the control (Fig. 2). This reduction did not appear to change over the time interval assayed. In order to exclude the possibility of nonspecific inhibition by a component of the rabbit serum, an identical control was prepared using pooled normal rabbit serum. The results demonstrated that normal rabbit serum did not inhibit enzyme activity.

Differential inhibition of glucan synthesis. *S. mutans* encodes three gene products—*gtfB*, *gtfC*, and *gtfD*—which

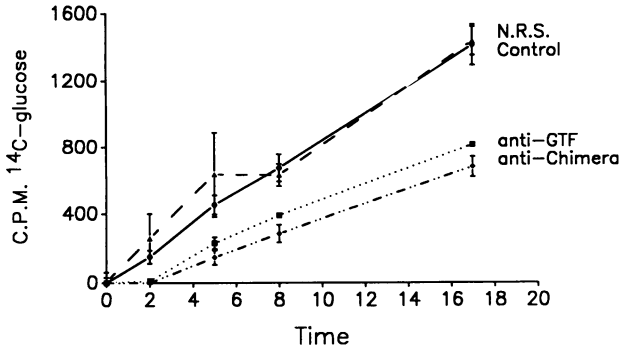

FIG. 2. Inhibition of enzyme activity by specific antisera. The enzyme preparation was diluted 1:2 in either PBS (control), pooled normal rabbit serum (N.R.S.), rabbit antiserum to glucosyltransferase B (anti-GTF), or rabbit antiserum to the chimera (anti-Chimera). Total glucan synthesis was measured over 18 h.

have glucosyltransferase activity. These enzymes differ in the type of glucan polymer produced, and their activities can be partially differentiated on the basis of the solubility of their reaction products in water. In order to determine whether the antisera had any effect on the type of glucan polymer being made, the amounts of water-insoluble and -soluble glucans were quantitated (Fig. 3). The results clearly demonstrated a dramatic effect on the synthesis of water-insoluble glucan. Water-soluble glucan made up a significant portion of the total polymer synthesized, and the antisera inhibited the synthesis of this product by approximately 40%. However, the formation of water-insoluble glucan was inhibited >90% by the antisera. The inhibition occurred regardless of whether antiserum to the chimera or to glucosyltransferase B was used.

Inhibition of fructan synthesis. To confirm the specificity of the antisera, an assay for fructosyltransferase activity was performed (Fig. 4). Surprisingly, antiserum to either the peptide or the intact glucosyltransferase B enzyme was also able to inhibit fructan synthesis, although much less than it inhibited glucan synthesis. In this case, normal rabbit serum inhibited fructan synthesis ~40% relative to synthesis in the

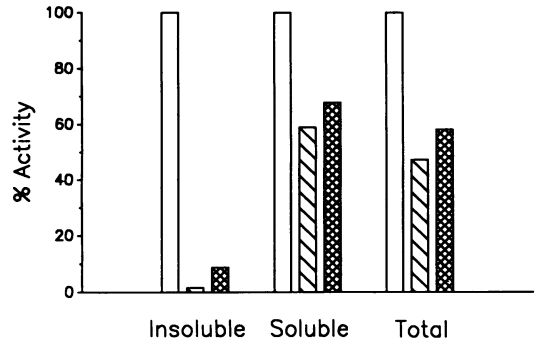

FIG. 3. Differential inhibition of glucan synthesis. The enzyme preparation was incubated in a 1:2 dilution of either PBS (□), antiserum to glucosyltransferase B (▨), or antiserum to the chimera (▩) and then assayed for differential synthesis of glucan. Samples were incubated in substrate containing [^{14}C-glucose]sucrose for 8 h at 37°C before activity was measured. The amount of water-soluble glucan produced was determined by subtracting the amount of insoluble glucan from the amount of total glucan. The activity of the samples in each assay was determined as a percentage of activity in the controls, which was normalized to 100%.

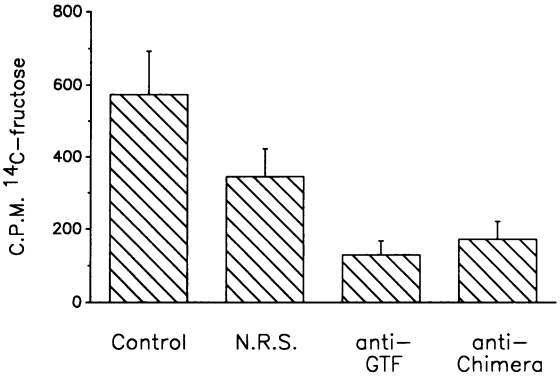

FIG. 4. Inhibition of fructan synthesis. Extracellular protein from *S. mutans* GS-5 was incubated for 1 h at 37°C in a 1:2 dilution of either PBS (control), normal rabbit serum (N.R.S.), antiserum to glucosyltransferase B (anti-GTF), or antiserum to the chimera (anti-Chimera). The samples were incubated in substrate containing [^{14}C-fructose]sucrose for 8 h at 37°C.

control, but inhibition was still not as great as that observed for the glucosyltransferase-specific antisera. After subtracting out the inhibition observed for the normal rabbit serum, antiglucosyltransferase and antipeptide antisera inhibited fructan synthesis 35 and 31%, respectively.

DISCUSSION

The gtfB.1 peptide was derived from the nucleotide sequence of the *gtfB* gene (17), the product of which catalyzes the formation of water-insoluble glucan from sucrose. Immunoblotting analysis and enzyme inhibition studies have shown that the gtfB.1 peptide was immunogenic and that antiserum to the peptide could recognize some of the same-size proteins recognized by antiserum to the purified, recombinant form of glucosyltransferase B. The high-molecular-weight proteins that appear in the extracellular preparations from *S. mutans* appear to be various forms of glucosyltransferases which were generated as a result of proteolysis in the preparation. Compared with the antiserum raised against the *gtfB* enzyme, antiserum to the chimera was able to react with only a limited number of forms of these enzymes. It is not known whether these differences are conformational or whether they are due to the absence of the gtfB.1 peptide sequence in these proteins.

To our surprise, antiserum to gtfB.1 inhibited the synthesis of glucan polymer from sucrose. The ability to inhibit *S. mutans* glucosyltransferase activity by specific antisera is not new (5). However, this is the first time that antipeptide antiserum has been demonstrated to inhibit glucosyltransferase activity in vitro. As the kinetics study indicated, the antisera did not prevent the net synthesis of glucan from occurring but merely reduced the rate of synthesis. However, upon examination of the type of glucan synthesized, it was clear that the antisera had a much greater effect on the production of insoluble glucan. And, as reflected in Fig. 3, insoluble glucan made up a much smaller percentage of the total glucan synthesized by *S. mutans* than soluble glucan did. Therefore, the antisera appeared to have some specificity and, consequently, a greater inhibitory effect on the synthesis of insoluble glucan than on the synthesis of soluble glucan.

Several conclusions regarding the structure of glucosyltransferases may be drawn by comparing the nucleotide sequences of these enzymes with the information obtained

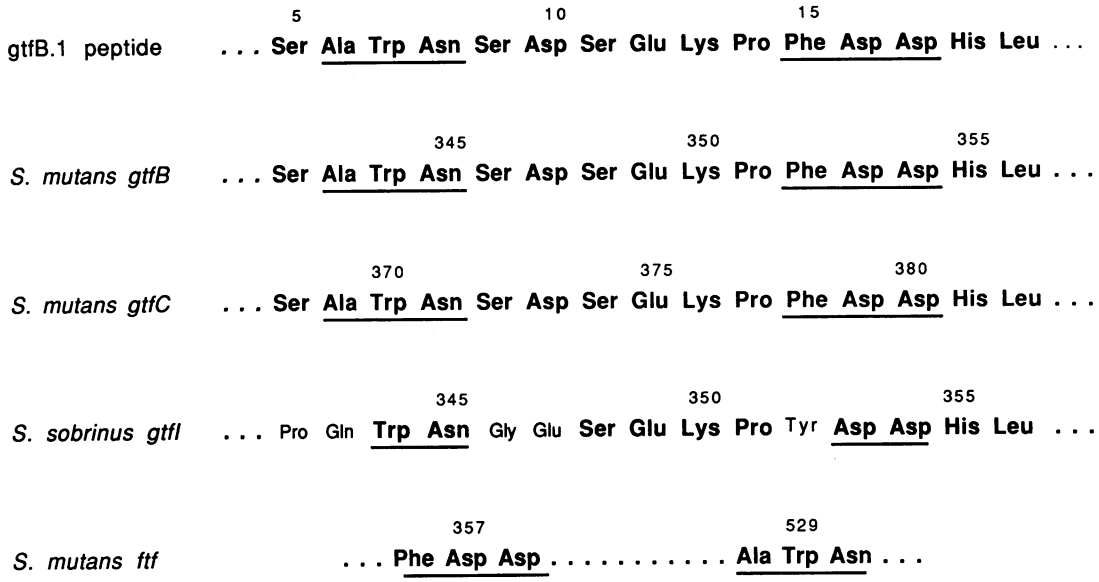

FIG. 5. Comparison of the gtfB.1 peptide sequence from glucosyltransferase B with related sequences found in other enzymes of the cariogenic streptococci. The hosts and genes of origin are listed on the left. Protein sequences are based on codons specified by the corresponding genes. The number of amino acids encoded by the unprocessed form of each gene product are as follows: *gtfB*, 1,475 (17); *gtfC*, 1,375 (20); *gtfI*, 1,597 (6); *ftf*, ~728 (16). Homologous residues are shown in boldface type. Numbers above the sequences indicate the position of the amino acid residues in the unprocessed form of each protein. Underlined residues indicate sequences conserved between all the enzymes, which may be important for the catalytic activities of these enzymes.

from the antibody inhibition studies. Soluble glucan is produced by the product of the *gtfD* gene, which has recently been cloned (9) but not yet sequenced. Another enzyme, the product of the *gtfC* gene, synthesizes a mixture of both soluble and insoluble glucans (8). Comparison of the nucleotide sequence data for *gtfB* and *gtfC* (17, 20) shows that the gtfB.1 peptide is located in similar regions of both gene products (Fig. 5) and that these regions share significant homology with one another. The slight decrease observed in soluble glucan synthesis may be due to inhibition of the *gtfC* gene product by the antiserum, since the gtfB.1 peptide sequence is located in this enzyme. Although the sequence of the *gtfD* gene is currently not known, restriction mapping data and Southern blotting analyses have indicated that *gtfD* has little detectable homology with other glucosyltransferases from *S. mutans* (9). Thus, the gtfB.1 peptide may not exist in *gtfD*, which would explain why soluble glucan synthesis was inhibited only minimally. Alternatively, the gtfB.1 sequence may be present in *gtfD*, but the enzyme is so different in structure from the other two that the peptide plays no role in the activity of the enzyme. Confirmation of the existence of the gtfB.1 peptide in the *gtfD* gene product will have to wait until the gene is sequenced or until the gene products described above are individually examined for their level of inhibition by the gtfB.1-specific antiserum.

This study may have important implications for the development of an anticaries vaccine. The ability of this peptide to generate antiserum with inhibitory activity against a number of enzymes from *S. mutans* suggests that the construction of such a subunit vaccine may be feasible. A vaccine with this property would clearly be advantageous for preventing the accumulation of *S. mutans* on the tooth surface. However, attempts to demonstrate this effect in vitro were unsuccessful. Using a method similar to one used to study an inhibitor of *S. mutans* adherence (13), we were unable to inhibit the sucrose-dependent adherence of cells to plastic with the gtfB.1 antiserum. However, we did inhibit the adherence of the bacterium with antiserum to glucosyltransferase B over several dilutions (data not shown). The mutans streptococci may have simply produced more glucosyltransferase than there was antipeptide antibody available to inhibit it. Dilution studies have shown that the antipeptide antiserum was not of as high a titer as the antiserum to the entire glucosyltransferase B enzyme (data not shown). Antisera raised in different rabbits may have improved the response observed in these studies and in vivo, antibody may not be as limiting, since secretory antibodies are continuously being released into the oral cavity. A systematic study of the glucosyltransferase B enzyme has not been undertaken, and there may be other peptides which elicit better antibody responses or that elicit antibodies that are more inhibitory than gtfB.1. Furthermore, the gtfB.1 antiserum probably recognized the primary and secondary structure of the enzyme rather than its tertiary structure. By constructing a longer peptide or shifting the span of the gtfB.1 sequence, it may be possible to elicit antibody with greater inhibitory activity. Nevertheless, closer scrutiny of this peptide may prove beneficial towards its use in an anticaries vaccine. We plan to assess the anticaries potential of the V1782 chimera in an animal model.

The results of this study may prove beneficial in identifying amino acid residues that define the active sites of glucosyltransferases and other related enzymes. The ability of the gtfB.1 antiserum to inhibit glucan synthesis suggested that this peptide may be near the active site of the enzyme(s). Antibody binding to this sequence must have altered the conformation of the active site significantly in order to affect catalysis. Inhibition of fructosyltransferase activity by the glucosyltransferase antisera suggested that all or part of the gtfB.1 sequence may exist in this enzyme as well. Upon close comparison of the amino acid sequence data for gtfB.1 and fructosyltransferase (Fig. 5), two nonhomologous tripeptide sequences (Phe-Asp-Asp; Ala-Trp-Asn) which were shared by both enzymes were found. The antiserum to the chimera may recognize one or both of these

sequences, which may define a conserved region of these sucrose-utilizing enzymes that is of common importance to their function. These tripeptides may also define the location of the epitope(s) recognized by the inhibitory antibody, which could be used to make monoclonal antibodies with inhibitory function.

Very little is actually known about the active sites of these streptococcal enzymes. The gtfB.1 sequence may denote a region of the glucosyltransferase enzyme that would be a good target for site-directed mutagenesis. Mooser and Iwaoka (12) have isolated a reaction intermediate composed of the active site of the *gtfI* gene product of *Streptococcus sobrinus* complexed with glucose. However, they did not report the composition of the active site. The *gtfI* gene encodes an insoluble glucosyltransferase that shares 57% homology with the *gtfB* gene product of *S. mutans* (15). It also encodes a peptide sequence highly homologous to gtfB.1 (Fig. 5). Because of the significant homology between *gtfB* and *gtfI*, our antibody data may be useful for identifying some of the residues that define the active site of this enzyme. It would also be interesting to determine whether our antipeptide antibody recognizes the reaction intermediate that Mooser and Iwaoka have isolated. If so, then our hypothesis regarding the common role which the gtfB.1 peptide plays in the active site of these enzymes would be substantiated.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant R37 DE04224 from the National Institutes of Health to F.L.M.

We thank H. K. Kuramitsu for the kind gift of glucosyltransferase B antiserum used in these studies.

LITERATURE CITED

1. **Aoki, H., T. Shiroza, M. Hayakawa, S. Sato, and H. K. Kuramitsu.** 1986. Cloning of a *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. Infect. Immun. **53**:587–594.
2. **Asem, K., A. J. Cornish-Bowden, and J. A. Cole.** 1986. A comparative study of the extracellular glucosyl- and fructosyltransferases from cariogenic and non-cariogenic *Streptococcus mutans* strains of two different serotypes. Microbios **47**:53–66.
3. **Dertzbaugh, M. T., and F. L. Macrina.** 1989. Plasmid vectors for constructing translational fusions to the B subunit of cholera toxin. Gene **82**:335–343.
4. **Dertzbaugh, M. T., D. L. Peterson, and F. L. Macrina.** 1990. Cholera toxin B subunit gene fusion: structural and functional analysis of the chimeric protein. Infect. Immun. **58**:70–79.
5. **Evans, R. T., and R. J. Genco.** 1973. Inhibition of glucosyltransferase activity by antisera to known serotypes of *Streptococcus mutans*. Infect. Immun. **7**:237–241.
6. **Ferretti, J. J., M. L. Gilpin, and R. R. B. Russell.** 1987. Nucleotide sequence of a glucosyltransferase gene from *Streptococcus sobrinus* MFe28. J. Bacteriol. **169**:4271–4278.
7. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44**: 331–384.
8. **Hanada, N., and H. K. Kuramitsu.** 1988. Isolation and characterization of the *Streptococcus mutans gtfC* gene, coding for synthesis of both soluble and insoluble glucans. Infect. Immun. **56**:1999–2005.
9. **Hanada, N., and H. K. Kuramitsu.** 1989. Isolation and characterization of the *Streptococcus mutans gtfD* gene, coding for primer-dependent soluble glucan synthesis. Infect. Immun. **57**: 2079–2085.
10. **Krasse, B., C. G. Emilson, and L. Gahnberg.** 1987. An anticaries vaccine: report on the status of research. Caries Res. **21**: 255–276.
11. **McGhee, J. R., and S. M. Michalek.** 1981. Immunobiology of dental caries: microbial aspects and local immunity. Annu. Rev. Microbiol. **35**:595–638.
12. **Mooser, G., and K. R. Iwaoka.** 1989. Sucrose 6-alpha-D-glucosyltransferase from *Streptococcus sobrinus*: characterization of a glucosyl-enzyme complex. Biochemistry **28**:443–449.
13. **Murchison, H., S. Larrimore, and R. Curtiss III.** 1985. In vitro inhibition of adherence of *Streptococcus mutans* strains by nonadherent mutants of *S. mutans* 6715. Infect. Immun. **50**: 826–832.
14. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
15. **Russell, R. R. B., T. Shiroza, H. K. Kuramitsu, and J. J. Ferretti.** 1988. Homology of glucosyltransferase gene and protein sequences from *Streptococcus sobrinus* and *Streptococcus mutans*. J. Dent. Res. **67**:543–547.
16. **Shiroza, T., and H. K. Kuramitsu.** 1988. Sequence analysis of the *Streptococcus mutans* fructosyltransferase gene and flanking regions. J. Bacteriol. **170**:810–816.
17. **Shiroza, T., S. Ueda, and H. K. Kuramitsu.** 1987. Sequence analysis of the *gtfB* gene from *Streptococcus mutans*. J. Bacteriol. **169**:4263–4270.
18. **Smith, D. J., and M. A. Taubman.** 1987. Oral immunization of humans with *Streptococcus sobrinus* glucosyltransferase. Infect. Immun. **55**:2562–2569.
19. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
20. **Ueda, S., T. Shiroza, and H. K. Kuramitsu.** 1988. Sequence analysis of the *gtfC* gene from *Streptococcus mutans* GS-5. Gene **69**:101–109.