Inhibition of Glucosyltransferase Activities of *Streptococcus mutans* by a Monoclonal Antibody to a Subsequence Peptide

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Preliminary analysis indicated that a 19-amino-acid peptide sequence (435 to 453 of GtfC) within a highly conserved region of the glucosyltransferases of the cariogenic streptococci might be functionally important (J.-S. Chia, S.-W. Lin, T.-Y. Hsu, J.-Y. Chen, H.-W. Kwan, and C.-S. Yang. Infect. Immun. 61:1563–1566, 1993). To obtain antipeptide monoclonal antibodies (MAbs), the 19-amino-acid peptide was conjugated to bovine serum albumin and used as an antigen in BALB/c mice. Six immunoglobulin G-secretory hybridoma clones, CJSm18-S1 to -S6, specifically reacted with this peptide and with purified GtfC and GtfD but not with bovine serum albumin in an enzyme-linked immunosorbent assay. The concentrated hybridoma supernatant of all six MAbs inhibited GtfC enzymatic activity but failed to inhibit GtfD, although GtfD contains the same peptide sequence. Further analysis of a purified immunoglobulin G2b MAb from one of the clones, CJSm18-S3, confirmed that this MAb specifically inhibited GtfC enzymatic activity for insoluble-glucan synthesis in a dose-dependent manner. CJSm18-S3, even at high concentrations, had no effect on GtfD, which synthesizes water-soluble glucan exclusively. Furthermore, the in vitro sucrose-dependent adherence of *Streptococcus mutans* was also inhibited by CJSm18-S3 in a dose-dependent manner. Our results indicate that the peptide containing the N-terminal conserved region of glucosyltransferases is functionally important for both enzymatic activity and bacterial adherence.

*Streptococcus mutans* is an important pathogen for human dental caries (21). One of its virulence traits is the synthesis of glucan polymers, which mediate the attachment of bacteria to smooth surfaces and consequently the formation of dental plaque (11). Glucosyltransferases (GTFs) are enzymes responsible for the synthesis of water-soluble and -insoluble glucan. In *S. mutans*, GTFs are encoded by three genes, *gtfB*, *gtfC*, and *gtfD*, which express different enzyme activities (2, 13, 14). GtfB (GTf-I; 162 kDa) and GtfC (GTf-S1; 149 kDa) synthesize insoluble glucan (2, 13) and low-molecular-weight soluble glucan (9, 13), respectively, in a primer-independent manner, while GtfD (GTf-S; 155 kDa) synthesizes water-soluble glucan exclusively in a primer-dependent manner (14). GtfB and GtfC have a high degree of amino acid sequence identity. In addition, homologous GTFs are found in other mutans streptococci, such as GtfI from *Streptococcus downei*, MFe28 and Gtf-I from *Streptococcus sobrinus* 6715 (1, 8, 27, 30).

Studies of the structure and function relationship of the GTFs via deletion clones have revealed several important peptide regions. The deletion study of GtfI from *S. downei* MFe28 has demonstrated that the direct repeat units at the C-terminal region are responsible for glucan binding (8). A similar study of Gtf-I from *S. sobrinus* showed that the N-terminal 80 amino acids were nonessential for enzymatic activity. However, the loss of 260 amino acids from the N terminus of Gtf-I by further deletion diminished the enzyme activities for sucrose splitting and glucan synthesis, although glucan binding was not affected because of an intact C terminus (1). Furthermore, it has been shown that antisera to a chimeric protein composed of 15 amino acids of the N terminus of GtfB (342 to 356) and the B subunit of cholera toxin inhibited the water-insoluble glucan synthesis of a crude enzyme prepared from a culture supernatant of *S. mutans* GS-5 (6). Recently, an active site responsible for sucrose binding was proposed after sequencing of a peptide from the stabilized glucosyl-enzyme complex (24, 25) and confirmed by site-directed mutagenesis (16). This peptide of nine amino acids is located in the N-terminal one-third of the GTFs. Together, these results suggested that the N terminus of the GTFs may play a central role in the enzymatic activities for sucrose splitting and glucan synthesis.

Using restriction fragment length polymorphism analysis, we have initially identified genetic variations in the *gtfB* and *gtfC* genes among serotype c and e strains of *S. mutans* (3, 5). One of the variations abolished an *EcoRI* endonuclease site in the 5' coding regions of these two genes. The polymorphisms of the GTFs, although variable in DNA sequence, did not affect the enzymatic activities or the sucrose-dependent adherence of the *S. mutans* strains (3). More recently, we have sequenced the 5' *EcoRI* regions in the *gtfB* and *gtfC* genes and confirmed that the DNA polymorphisms were due to point mutations. In addition, nucleotide substitutions in different strains are of a conservative nature, not affecting the encoded amino acids (4). A synthetic peptide of 19 amino acids from this region, corresponding to residues 435 to 453 of GtfC, was able to reverse the inhibition of the enzyme activities by a polyclonal antibody raised against partially purified GtfB and GtfC proteins (4). A higher dose of the synthetic peptide incubated alone with GtfC or GtfD had no effect on the enzymatic activities. The selected 19-amino-acid sequence is identical among the GTFs from several mutans streptococci (1, 8, 10, 15, 27, 30) and is very close to the reported active-site sequence for sucrose binding (16). However, there is still not enough evidence to indicate the significance of the 19-amino-acid peptide in enzymatic function.

In this study, we have used the 19-amino-acid peptide to generate monoclonal antibodies (MAbs) in BALB/c mice.

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The GtFC expressed from an *Escherichia coli* (pNH3) strain and GtFD secreted from *S. mutans* NHS1 (13) were purified and used for functional assays. The inhibitory effects of the antipeptide MAbs on the enzymatic activities of the GTFs and on the in vitro sucrose-dependent adherence of *S. mutans* were demonstrated. The possible role of the peptide in GTFs is discussed.

**MATERIALS AND METHODS**

**Bacterial strains and media.** *E. coli* harboring pNH3 (13) was kindly provided by H. K. Kuramitsu, University of Texas, San Antonio. The organism was grown in L broth (10 g of tryptone [Difco, Detroit, Mich.], 5 g of yeast extract [Difco], and 5 g of NaCl per liter) containing ampicillin (100 μg/ml). *S. mutans* GS-5 and MT-0148 were grown and maintained as described previously (3). The *S. mutans* NS51, a mutant strain of GS-5 with both the gtfB and gtfC genes deleted (13), was grown and maintained as described previously (4).

**Preparation of immunogen.** A peptide of 19 residues (ANDVDNSPVVQAEOQNLW) (Fig. 1) was synthesized chemically with an Applied Biosystems model 430A peptide synthesizer by the Fmoc method. The amino acid composition of the peptide was analyzed with the High Performance Analyzer System 6300 (Bechman, Fullerton, Calif.), and its sequence was determined by Edman degradation with an automated sequenator (Applied Biosystems model 477A) supplied with a data analysis program (model 610A, version 1.2). The purified peptide was coupled to bovine serum albumin (BSA; 1 mg/ml; Promega) using 0.1% glutaraldehyde as described previously (26). The conjugated peptide-BSA preparation was verified by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (SDS-PAGE). Successful conjugation was shown by retardation of the peptide-BSA conjugate relative to BSA because of an increase in the molecular mass.

**Immunization of mice.** Mice (BALB/c strain, 6 to 8 weeks old), obtained from our breeding colony, were injected intraperitoneally with a homogeneous emulsion of the peptide–BSA conjugate (50 μg for a single immunization) and Freund's complete adjuvant at 1:1 (vol/vol) ratio. A booster injection was performed 2 weeks after the first immunization. Animals were bled from the retroorbital plexus 1 week after the second immunization. Sera were screened at a 1:1,000 dilution by an enzyme-linked immunosorbent assay (ELISA) against peptide (100 μg/ml in phosphate-buffered saline [PBS], pH 7.4), peptide-BSA conjugate, and purified GtFC (25 μg/ml in sodium carbonate buffer, pH 9.8). Mice exhibiting the highest antibody titer were subcutaneously given another booster of a similar dose in Freund’s incomplete adjuvant. High-titer antisera were collected at appropriate times, and the spleens were used for fusion.

**ELISA.** Peptide-specific MAbs were detected by an ELISA as described below. The 96-well polystyrene enzyme immunosorbent assays (GIBCO Laboratories, Grand Island, N.Y.) were used for all reactions. Between steps, wells were washed three times with an automatic washer (Tri-Continent Scientific Inc., Grass Valley, Calif.) at room temperature with 0.05% Tween 20 in PBS (pH 7.4). The wells were coated with peptide (100 μg/ml in PBS, pH 7.4) or protein (25 μg/ml in sodium carbonate buffer, pH 9.8) and incubated overnight at 4°C, and 150 μl of blocking agent (0.25% gelatin [Sigma]) was added for 2 h at 37°C. The antibody to be tested was then added and incubated for 1 h. Antibody that reacted with the coated peptide or proteins was detected with alkaline phosphatase-labeled goat anti-mouse immunoglobulin G (IgG; Sigma) and then with a p-nitrophenylphosphate substrate (Sigma). Responses were measured in a microplate reader (Tri-Continent Scientific Inc.) at 405 nm, and the reactivity was compared with that of the appropriate controls.

**Production and purification of MAbs.** Murine hybridomas were generated as described previously (19). Briefly, 5 days after the last immunization, spleens were removed aseptically, and the cells were fused with myeloma cells (P3-X63-Ag; American Type Culture Collection [ATCC]) in 50% polyethylene glycol (4,000 molecular weight; GIBCO). After fusion, the cell mixture was plated onto 96-well microtiter plates (Costar, Cambridge, Mass.) and grown in RPMI 1640 medium (150 μl per well) supplemented with 10% fetal calf serum, 2-mercaptoethanol (3 × 10⁻³ M), and L-glutamine (2 × 10⁻⁴ M) plus antibiotics. The next day, cultured cells were grown in selection medium containing hypoxanthine, amionopterin, and thymidine for 10 days. The culture supernatants were subsequently assayed for peptide-specific antibodies by ELISA. Peptide-specific hybridomas were then cloned twice by limiting dilution (0.5 cell per well) in 96-well U-bottomed plates (Costar). One of the six positive clones, CJS18-S3, was characterized further and injected into BALB/c mice (2 × 10⁷ cells per mouse) which had been treated with 2,6,10,14-tetramethylpentadecane for the generation of ascites. Specific IgG was purified from the ascites of CJS18-S3 by affinity chromatography with the ImmunoPure (IgG) Purification Kit (Pierce Chemical Co., Rockford, Ill.). Antibody specificity was tested by ELISA and immunoblot.

**Purification of GTFs.** GtFD was purified from an *S. mutans* strain (NHS1) lacking both GtFB and GtFC (13) as described.
previous (4). GtfB and GtfC from *S. mutans* GS-5DD (14), in which GtfD is inactivated, were separated from other proteins after two column chromatographies and was defined as partially purified GtfB/C, because this preparation contained both GtfB and GtfC (4). Recombinant GtfC expressed from *E. coli* harboring pNH3 (13) was found to be aggregated and largely associated with the insoluble fractions after disruption of the cells. After extraction of the aggregates with 8 M urea, crude enzyme preparations were loaded onto a DEAE-SephaCel column (2.5 by 17 cm; Pharmacia, Uppsala, Sweden) and eluted with a linear gradient of 0 to 1.0 M NaCl. Fractions exhibiting enzyme activity were pooled and applied to a hydroxyapatite column (1.0 by 15 cm; Bio-Rad, Richmond, Mass.). Bound proteins were eluted first with 50 mM sodium phosphate buffer (pH 6.0) and then stepwise with increasing concentrations (0.2, 0.3, 0.4, and 0.5 M) of the same buffer. Peak fractions containing GTF activity were pooled and spin dialyzed several times in Centricon-100 microconcentrators (Amicon, Danvers, Mass.) against 10 mM sodium phosphate buffer (pH 6.0).

The final enzyme preparation obtained had a yield of 8.6% and a 16.3-fold increase in specific activity. When analyzed by SDS-PAGE and isoelectric focusing, GtfC exhibited a single band at approximately 140 kDa and an isoelectric point (pl) of 5.5. The pH optimum for insoluble-glucan synthesis was approximately 6.5, while the *Km* for sucrose was estimated to be 9.2 mM. These properties were similar to those described previously (9, 13). The GtfB expressed from an *E. coli* strain harboring pSU20 (3) from our collection was also isolated and solubilized by the same procedures as for GtfC. The extracted GtfB was found to be biologically active but in low yield. The GtfB extracts were partially purified by DEAE-SephaCel column chromatography, and the fractions showing enzymatic activity were concentrated by Centricon-100 microconcentrators and defined as crude preparations of GtfB. This partially purified crude GtfB was biologically active and did not contain the truncated GtfC protein which was also encoded by pSU20, as confirmed by Western blot (immunoblot) analyses with PJS-3 and PJS-2 antiserum (4).

**Enzyme and protein assays.** GTF activity was determined by the [14C]glucose-sucrose (New England Nuclear Corp., Boston, Mass.) incorporation and solubilized by the same procedures as for GtfC. Briefly, the reaction mixture consisted of enzyme, 2.9 mM labeled sucrose (0.017 μCi/mmol), and 0.10 M potassium phosphate buffer (pH 6.0) plus dextran T10 (for GtfD), in a total volume of 0.5 ml. The reaction mixtures were incubated at 37°C for 1 h, and synthesis was terminated by the addition of 5 ml of methanol (for total glucan synthetic activity) or by heating at 100°C for 5 min (for insoluble-glucan synthesis). The methanol-precipitated samples were filtered through 2.4-cm glass fiber filters (Whatman, Maidstone, England) and washed three times with methanol. The heated samples were pipetted onto the filters and washed with 0.9% sodium chloride and methanol. Radioactivity was measured with a scintillation counter (Beckman). One unit of enzyme activity is defined as the amount of enzyme required to incorporate 1.0 μmol of glucose from sucrose into glucan per min under standard assay conditions. For inhibition assays, GTFs (purified GtfC and GtfD) were reacted with an equal volume of MABs or PBS (control) for 15 min at 37°C before being added to the reaction mixture. The protein concentration was determined by a modified Lowry et al. (22) method, with bichromonic acid as the colorimetric detection reagent (BCA protein assay reagent; Pierce).

**Gel electrophoresis.** Proteins were analyzed by SDS-PAGE followed by staining with Coomassie brilliant blue or periodic acid-Schiff reagent to detect enzymatic activity (32). Immunoblotting was performed as described previously (29). For determination of n.m., analytical isoelectric focusing was performed with a polyacrylamide gel slab (10 cm by 7 cm by 0.75 mm) with Ampholyte (pH 3-10; Bio-Rad). Aliquots of enzymes (2 to 4 μg) were applied to glass fiber filter paper placed on the gel surface. Focusing was carried out at 100 V for 15 min and subsequently at 250 V for 1.5 h, as described previously (17). The proteins and the pH standard were detected with Coomassie brilliant blue.

**In vitro sucrose-dependent adherence assay.** *S. mutans* was grown at 37°C in a glass tube with 1.5 ml of BH broth containing 1% (wt/vol) sucrose. The bacteria were cultured at an angle of 30° for 18 h, and the number of adherent cells was determined turbidimetrically and expressed as a percentage of the total cell mass (percent cell adherence) as described previously (12). Antibodies were sterilized by passage through a Micro-filter (0.45 μm; Millipore, Bedford, Mass.) and subsequently added to the culture medium at final concentrations ranging from 50 to 300 μg/ml. Two MABs were used as negative controls in parallel experiments, including purified IgG against major histo compatibility (MHC) class I antigen (HB95; ATCC) and the ammonium sulfate-precipitated culture supernatant of the myeloma cell line (described above).

**RESULTS**

**Characterization of MABs CJSm18-S1 to -S6.** The sequence of the 19-amino acid peptide is highly conserved among the GTFs (Fig. 1), and it was predicted by computer analysis to contain a hydrophilic alpha-forming unit (7). This peptide was able to elicit high-titer antibodies in mice immunized with a peptide-BSA conjugate. Antiserum titers of 1:1,000 could readily detect both peptide-BSA and peptide alone by ELISA. After fusion, six stabilized cell lines (CJSm18-S1 to -S6) were obtained after subcloning by limiting dilution. These six hybridomas produced IgG-type MABs that reacted specifically with the peptide and GTFs (Table 1). CJSm18-S3 exhibited better responses than the other five cell lines in producing ascsites and was used for MAB generation. The titer of CJSm18-S3 ascites was determined to be 10^5, and its isotype was defined as IgG2b. Affinity column-purified IgG of CJSm18-S3 reacted specifically with the peptide, GtfC, and GtfD but not with BSA (Fig. 2). The MAB also recognized the crude extract and purified GtfC on the immunoblot (data not shown). These results confirmed that peptide-specific MABs could recognize and react with the same sequence on the GTFs.

**Specific inhibition of GTFs by MABs.** The ability of purified CJSm18-S3 to interfere with GTF enzymatic activities was examined by incubating the MAB with purified GtfC, GtfD, and the GtfB/C preparation, which contained both GtfB and GtfC (4). The GtfC enzyme (2 mU) synthesized primarily insoluble glucan (70 to 75%) and some soluble glucan (25 to 30%), while GtfD synthesized soluble glucan exclusively under the standard assay conditions. Relative levels of both total and insoluble glucan synthesis were estimated in the presence of various concentrations of the CJSm18-S3 antibody. As shown in Fig. 3A, CJSm18-S3 had an inhibitory effect on both total and insoluble glucan synthesis by GtfC in a dose-dependent manner. The maximal inhibition of insoluble glucan-synthesizing activity was about 50%, while the inhibition was about 25 to 30% when total glucan synthesis was measured. Surprisingly, CJSm18-S3, even at a very high

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The provided text is a scientific research article. It describes the isolation, purification, and characterisation of a microbial enzyme, *S. mutans* GtfC, and its interaction with antibodies. The text includes methodologies for enzyme activity assays, protein analysis, and antibody specificity testing. The results section describes the characterization of monoclonal antibodies (MABs) specific to the GtfC enzyme and their inhibitory effect on glucan synthesis.
concentration (1:8 molar ratio of enzyme to MAb), had no effect on the enzymatic activity of GtfD. When the GtfB/C preparation was tested, CJSm18-S3 also inhibited its enzymatic activity for both total and insoluble glucan synthesis. In addition, CJSm18-S3 inhibited insoluble glucan synthesis by the crude enzyme preparation of GtfB (Fig. 3B).

A polyclonal antibody, PJS-3 (4), was used as a positive control, while an MHC class 1-specific MAb and ammonium sulfate-precipitated culture supernatant of P3-X63 served as negative controls in parallel experiments. Concentrated hybridoma supernatants of all six MAbs exhibited similar inhibition patterns for GtfC and GtfB/C. None of them inhibited GtfD enzymatic activities. The molar ratio of MAb to enzyme required to detect inhibitory effects was about 1:1 under the assay conditions used. Neither incubation period (30 min, 1 h, or 18 h) nor substrate concentration (5, 10, or 20 mM sucrose) had a significant influence on inhibition (data not shown).

CJSm18-S3 blocks attachment of S. mutans. The synthesis of insoluble glucans is closely associated with the attachment of S. mutans to smooth surfaces. The ability of CJSm18-S3 to inhibit the attachment of S. mutans was examined in an in vitro assay. When S. mutans MT-8148 (serotype c strain) was co-cultured with CJSm18-S3 (MAb prepared by ammonium sulfate precipitation of hybridoma supernatants), adherence was significantly inhibited (Fig. 4A). More than 50% inhibition was achieved when the protein concentration reached 300 μg/ml, and some inhibition (12 to 18%) was also observed in the control experiments with a similar preparation from the myeloma cell line (P3-X63).

To verify the specificity of the attachment inhibition, adherence assays with purified antibodies were performed. As shown in Fig. 4B, purified CJSm18-S3 significantly inhibited the attachment of S. mutans at a concentration of 100 μg/ml. As negative controls, the affinity column-purified irrelevant antibodies had negligible effects even at higher concentrations. These results confirmed that the adherence inhibition was due to a specific interaction and also indicated that a nonspecific inhibition occurred when ammonium sulfate-precipitated hybridoma supernatant of MAbs was used. Specific adherence inhibition was also obtained with purified CJSm18-S3 when strain GS-5 was tested (data not shown). Therefore, CJSm18-S3 antibody, with a defined epitope located between amino acid residues 435 and 453 of GtfC, exhibited specificity in blocking both the enzymatic activity of GtfC and the attachment of S. mutans to glass surfaces.

### DISCUSSION

An amino-terminal region of approximately 40 residues, extending from positions 387 to 427 of GtfB (or 413 to 453 of GtfC), was first identified after an interesting finding of an EcoRI polymorphism (3). This region is highly conserved among the GTFs of several streptococci. Furthermore, the DNA sequence variations in this region did not alter the amino acid sequence (4), suggesting some biological importance of this conservation. We have investigated this possibility and demonstrated that a 19-amino-acid peptide containing residues 435 to 453 of GtfC was able to reverse the inhibitory effect of a polyclonal antibody which neutralized GTF enzymatic activities (4). To further examine the biological function of this amino-terminal conserved region, we generated peptide-specific MAbs which recognized this conserved region and inhibited insoluble glucan synthesis by GtfC and the attachment of S. mutans to glass surfaces.
These results suggest that the amino-terminal conserved region might be involved in insoluble glucan synthesis. This was supported by the following observations. First, CJSm18-S3 had no effect on the enzyme activity of GtfD, which also contains this 19-amino-acid sequence at a comparable position but synthesizes water-soluble glucan exclusively. Second, soluble glucan synthesis by GtfC, calculated as the difference between total and insoluble glucan formation, was also not affected by the MAb (Fig. 3). Third, insoluble glucan synthesis by GtfB is also inhibited. Fourth, increased sucrose concentrations in the inhibition assays did not overcome MAb inhibition, suggesting that the MAb did not interfere with substrate binding. The fact that CJSm18-S1 to -S6 exhibited comparable patterns of inhibition on the enzymatic activities suggested that all six MAbs recognize the same epitope(s) on the peptide or GtfC.

The inhibition of GtfC enzymatic activities by the antipeptide MAb CJSm18-S3 is far from complete (25 to 30%) when total glucan synthesis is measured (Fig. 3). These results were duplicated in an additional isotope-labeled incorporation assay described by Koga et al. (18), which measured total glucan synthesis only. GtfD enzymatic activities were still not affected when examined in parallel experiments (data not shown). GtfC expressed from E. coli is able to synthesize 64% insoluble glucan under assay conditions identical to those described for this study (13). It has also been shown that GtfC synthesizes a large amount of low-molecular-weight glucans (9, 13). Inhibition of the enzymatic activity of GtfC and GtfB but not of GtfD confirmed that the MAb selectively inhibited the GTFs (GtfB and GtfC) responsible for insoluble glucan synthesis. Differential inhibition of glucan synthesis has also been demonstrated by an antisem recognizing a peptide of 15 amino acids (342 to 356 of GtfB). This antisem inhibited more than 90% of insoluble glucan synthesis by crude GTFs. However, it inhibited only 30 and 40% of soluble glucan and total glucan synthesis, respectively (6). The basis for antibody-mediated inhibition of GTF enzymatic activities may involve direct blocking of functional domains and/or steric hindrance by configuration modifications due to antibody binding.

The reason for the preferential inhibition of insoluble glucan synthesis by CJSm18-S3 is unclear. One possible mechanism is that the antibody interferes with glucan branching. Additional experiments, such as structural analysis of the glucan synthesized in the presence of MAb, might provide information with which to address this hypothesis. Another possibility is that subtle conformational or structural differences exist in the molecules of GTFs, although they have identical primary sequences.

Of particular interest is the colonization of the cariogenic streptococci mediated by the GTFs and their de novo glucan synthesis. Insertional inactivation of the gtf genes has shown that both gtfB and gtfC are required for sucrose-dependent colonization in vitro, while the gtfD gene is less important (1, 13, 14). Recent studies have further demonstrated the importance of GtfC in mediating the colonization of cariogenic streptococci. A mutated strain carrying a functional gtfB gene and inactivated gtfC was defective in colonization in vitro (13). In addition, transformed S. milleri harboring the gtfC gene adhered to hard surfaces in the presence of sucrose, while S. milleri harboring only gtfB failed to do so (9).

In this study, we used purified proteins and demonstrated that MAb reactive with a subsequent peptide in the GTFs are capable of blocking the sucrose-dependent adherence of S. mutans. Our results also indicated that attachment inhibition through antibodies requires specificity in antigen-antibody recognition. A polyclonal antibody, PJS-2 (4), which recognized GtfB and GtfC but was unable to inhibit their enzymatic activities, failed to block the sucrose-dependent adherence of S. mutans even at high titers of serum. Another polyclonal antibody, PJS-3, which specifically recognized and inhibited the enzymatic activities of GtfB and
GtfC (4), blocked the attachment assayed in vitro (Fig. 4). Although the mechanism of glucan-mediated adherence of S. mutans is unclear, it has been shown that de novo glucan synthesis is required for the in vitro sucrose-dependent adherence of S. mutans (18). Therefore, the blocking of the attachment by MAb CJSm18-S3 in the present investigation is probably achieved through inhibition of glucan synthesis by both GtfB and GtfC or in ways other than by enzyme inhibition. Both GtfB and GtfC can be expressed on the surface of S. mutans (4, 9). Thus, CJSm18-S3 might react with an epitope(s) which directly mediates sucrose-dependent adherence through an as yet unidentified mechanism.

Inhibition of attachment of S. mutans through active or passive immunization has been shown to be a potential means for the prevention of dental caries (reviewed in reference 23). Identification of the catalytic domain and/or highly conserved peptide segments in the GTFs is of tremendous importance to the development of a GTF-based vaccine. Several domains of functional importance in the GTFs have been identified. A catalytic site for sucrose binding and hydrolysis has been identified in the N terminus of the GTFs (16, 24, 25). However, the antigenicity or immunogenicity of this domain has not yet been reported. In the C-terminal third of the GTFs, a glucan-binding region with repeating sequences has been identified (1). Tryptic fragments of this region from a GTF molecule can bind to glucan polymers (31). Furthermore, a peptide construct (GLU) comprising four copies of a 22-mer peptide derived from the glucan-binding regions of S. downei GtfI could elicit a serum IgG response which reacted with GTFs and inhibited glucan synthesis (28). Similar to the domains for sucrose and glucan binding, the peptide sequence in the N-terminal conserved region identified by us (3, 4) is also functionally important. It would be interesting to assess the anticaries potential of the immunity induced by this peptide in an animal model. The specificity of the antipeptide MAb in recognizing GTFs and blocking the attachment of S. mutans provides important information for the possibility that this conserved region can serve as a good candidate for the development of a subunit vaccine for preventing dental caries.

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