Production, Characterization, and Application of Monoclonal Antibodies Which Distinguish Three Glucosyltransferases from *Streptococcus mutans*

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Thirty-three murine monoclonal antibodies (MAbs) against the three glucosyltransferases (GTFs) (GTF-I, -SI, and -S) from *Streptococcus mutans* were obtained by the fusion of murine myeloma cells (P3X63-Ag8-U1) with spleen cells of BALB/c mice immunized with pure GTF-S or partially purified GTF-I from serotype c *S. mutans* PS14. The immunoreactivities of these MAbs were tested by enzyme-linked immunosorbent assay and Western blotting (immunoblotting) with various GTF preparations. GTF-I and GTF-SI were expressed from two *Streptococcus milleri* or *Escherichia coli* transformants harboring *gtfB* or *gtfC*, respectively. All of the five MAbs raised against the GTF-S from PS14 reacted only with the homologous enzyme. All 28 MAbs obtained by using the GTF-I from PS14 also reacted only with the homologous enzymes. Of these, 8 MAbs reacted only with the *gtfB* gene product (GTF-I), 4 MAbs reacted only with the *gtfC* gene product (GTF-SI), and the remaining 16 MAbs reacted with both gene products. The existence of GTF-SI in the purified GTF-I from PS14 was demonstrated by Western blot analysis using the representative monospecific MAbs. Further, the relative levels of the three GTFs in the extracellular and cellular fractions of *S. mutans* clinical isolates were examined by immunoblot analysis. The findings indicated that the relative level of GTF-SI, unlike that of GTF-I or GTF-S, differed markedly among isolates although the three GTFs were synthesized extracellularly by all the strains.

Of the mutans group of oral streptococci, *Streptococcus mutans* and *Streptococcus sobrinus* have been considered the principal etiologic agents of dental caries in humans, and their abilities to synthesize adhesive water-insoluble glucans (WIG) from dietary sucrose have been demonstrated to be an especially important cariogenic property (16). The adherent glucans are synthesized by the combined action of two or more glucosyltransferases (GTFs) (EC 2.4.1.5) produced by these mutans streptococci and function in colonization of the cells to the tooth surface (9, 16). With *S. mutans*, a WIG-forming GTF (GTF-I) and a water-soluble-glucan-forming GTF (GTF-S) have been isolated from culture fluids or cell extracts and extensively characterized (2, 10, 15, 17). Recent molecular genetic studies (1, 12, 13, 21, 23, 26) have clearly indicated the existence of three genes on the *S. mutans* chromosome: a *gtfB* gene coding for GTF-I, a *gtfC* gene coding for another type of WIG-forming enzyme (termed GTF-SI [12]), and a *gtfD* gene coding for GTF-S. More recently, we have reported that the *gtfB* and *gtfC* gene products have similar catalytic properties, although the two genes may play distinctive roles in *S. mutans* virulence (8). However, it is not clear whether the *gtfC* gene is expressed in all strains of *S. mutans* and how this expression affects tooth colonization. In addition, the regulation of expression of this gene and the interaction of its gene product with other GTFs remain to be determined. To answer these questions, a simple means of detecting the *gtfC* gene product is absolutely necessary. Polyclonal antibodies (PAbs) cannot easily distinguish between GTF-I and GTF-SI, which have extensive amino acid homology (26). Therefore, the present investigation was carried out to produce monoclonal anti-

bodies (MAbs) which can distinguish the three GTFs and clarify whether fresh clinical isolates of *S. mutans* produce the GTF-SI enzyme.

MATERIALS AND METHODS

Bacterial strains. *S. mutans* PS14 (serotype c), *S. sobrinus* B13N (serotype d), *Streptococcus milleri* transformants KSB8 and KSC43 (8), and *Escherichia coli* transformants SU20 (23) and NH3 (12) were used to prepare antigens for immunization and/or immunoassay. The transformants harboring *S. mutans gtf* genes were kindly provided by H. K. Kuramitsu (University of Texas Health Sciences Center, San Antonio, Tex.). In addition, 13 clinical isolates of *S. mutans* were routinely obtained from the saliva of nine subjects and used in the enzyme expression studies.

Enzyme preparation. The GTF-S enzyme from *S. mutans* PS14 was purified to homogeneity from the culture fluid of dialyzed brain heart infusion medium by the method described by Baba et al. (2) and used as an antigen for immunization and immunoassay. The GTF-I enzyme from the same strain was prepared as follows. The PS14 cells were grown at 37°C for 30 h in 8 liters of M4 medium supplemented with 1% ammonium sulfate (25) and 10 μM *p*-aminophenylmethylsulfonyl fluoride. After centrifugation, the cell-free culture supernatant was concentrated with a Pellicon cassette system (Millipore, Tokyo, Japan) and brought to 60% saturation with ammonium sulfate. After standing overnight, the precipitate was collected, dissolved, and dialyzed against 50 mM potassium phosphate buffer (KPB), pH 7.5. The crude enzyme preparation was applied to a carboxymethyl cellulose column (2.9 by 13 cm) equilibrated with the dialyzing buffer. The adsorbed proteins were eluted with a linear gradient of 0 to 1 M NaCl in 50 mM KPB (400

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ml). Fractions possessing WIG synthetic activity, which were eluted at a NaCl concentration of approximately 0.7 M, were pooled, dialyzed against 50 mM KPB, and used in immunization, immunoassays, and inhibition tests. As GTF-I and GTF-SI antigens for immunoassay, the crude preparations of *gtfB* and *gtfC* gene products were prepared as previously reported (8) from the culture fluids of *S. milleri* transformants KSB8 and KSC43, respectively. Both gene products (crude extracts) from the *E. coli* transformants also were prepared, as described by Hanada and Kuramitsu (12). Three GTFs (GTF-I, GTF-S$_1$, and GTF-S$_2$) from *S. sobrinus* were purified from the culture fluids of strain B13N as previously reported (19), except that each GTF was further purified by rechromatography on DEAE Bio-Gel A.

Crude extracellular and cell-associated GTFs from human clinical isolates of *S. mutans* were prepared as follows. The isolates were grown anaerobically at 37°C to the mid-log phase (optical density at 660 nm, 0.6 to 0.7) in 100 ml of brain heart infusion broth supplemented with 1% glucose and 10 μM p-aminophenylmethylsulfonyl fluoride. The culture fluid obtained by centrifugation was concentrated to 3 ml by 60% saturated ammonium sulfate precipitation followed by dialysis, and the cells were suspended in 3 ml of phosphate-buffered saline (PBS). These samples were heat treated (100°C, 5 min) with an equal volume of 2% sodium dodecyl sulfate (SDS) sample buffer for SDS-polyacrylamide gel electrophoresis (PAGE) and centrifuged at 10,000 × g for 5 min. The supernatant fluids from the concentrated culture fluid and the cell suspension were subjected to an immunoblot analysis as crude preparations of extracellular and cell-associated enzymes, respectively.

Preparation of MAbs and PAbs. MAbs to GTFs were prepared by the method described previously (20) with modification. Briefly, 6-week-old female BALB/c mice were injected intraperitoneally with the purified enzymes (10 μg per animal) mixed with an equal volume of Freund's complete adjuvant three to five times at 2-week intervals. The final booster injection was given intravenously without adjuvant. Hyperimmune mice were killed 3 days after the final boost, and spleen cells were fused with P3X63-Ag8-U1 murine myeloma cells in the presence of polyethylene glycol 1500 (BMY, Tokyo, Japan). Hybridomas producing MAbs were detected by enzyme-linked immunosorbent assay (ELISA) with the antigen immobilized in 96-well microtiter plates (Linbro/Titertek, McLean, Va.). Positive hybridomas were cloned at least twice by the single-cell manipulation method, and 33 stable hybrid cell lines were established. The culture supernatant fluids of the hybridomas were used as MAbs in this investigation.

PAbs to GTF-I (PAb-I) and GTF-S (PAb-S) were prepared as follows. Each purified enzyme (100 μg per animal) was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously into rabbits three times at 2-week intervals. Antisera were collected from ear veins 10 days after the final injection. Serotype c-specific rabbit antiserum was prepared as described by Hamada et al. (11), by using whole cells of *S. mutans* Ingbritt (serotype c).

ELISA. ELISA was performed essentially as described by Engvall and Perlmann (6). Briefly, the 96-well microtitration plates were coated with the crude GTF antigens (ca. 10 μg of protein per ml) or the purified GTF antigens (ca. 2 μg of protein per ml). After they were blocked with bovine serum albumin, the antigen-coated wells were incubated with 50 μl of hybridoma supernatant fluids (dilution, 1:10) or rabbit antisera (dilution, 1:500). To quantitate the immune reaction, horseradish peroxidase-conjugated goat anti-immunoglobu-

lin (anti-Ig) (Amersham, Amersham, United Kingdom) in PBS–Tween 20 was added. The horseradish peroxidase color development reagent was added thereafter.

Inhibition of WIG synthesis. The reaction mixture (625 μl) consisting of the purified GTF-I enzyme (6 mU), 50 μl of hybridoma supernatants (no dilution) or rabbit antisera (dilution, 1:500), 100 mM KPB (pH 6), and 0.01% sodium azide was preincubated at 37°C for 10 min, and the reaction was started by the addition of 125 μl of 300 mM sucrose in 100 mM KPB (pH 6). After incubation at 37°C for 16 h, the reaction mixture was sonicated (50 W, 3 s) to disperse the WIG formed, and the turbidity at 550 nm was measured by a spectrophotometer.

Other analytical procedures. SDS-PAGE and Western blot (immunoblot) analyses were carried out as previously described (8). The isotype of the MAbs was determined by ELISA with a monoclonal mouse Ig kit (PharMingen, San Diego, Calif.). Sucrase activity was measured as previously described (19), except that the reaction was carried out at 37°C. Protein was measured by the method of Bradford (3). Identification of the clinical isolates was accomplished by the method of Shklair and Keene (24) as well as an immunodiffusion test using the c type-specific rabbit antiserum. In vitro sucrose-dependent colonization of the isolates was achieved as previously described (8), except that vortexing was omitted.

RESULTS

Purification and homogeneity of extracellular GTFs. The GTF-S enzyme, which was purified by the method of Baba et al. (2) from the culture fluid of strain PS14 grown in dialyzed brain heart infusion broth, migrated as a single 145-kDa protein band expressing water-soluble glucan synthetic activity on SDS-PAGE gels. This preparation synthesized no WIG from sucrose and did not react with PAb-I antiserum in ELISA (Table 1) and Western blots. These results suggested that the purified GTF-S was homogeneous. On the other hand, the GTF-I enzyme was purified by ammonium sulfate fractionation and carboxymethyl cellulose column chromatography from the culture fluid of the same strain grown in 1% ammonium sulfate-supplemented M4 medium. The SDS-PAGE analysis of the purified enzyme (see Fig. 2) showed that it consisted of a major 158-kDa protein which had strong WIG synthetic activity, two minor proteins (147 and 135 kDa) which had lower WIG synthetic activity, and an enzymatically inactive minor protein (105 kDa). All of these protein bands were immunostained with the PAb-I antiserum but not the PAb-S antiserum. These results suggested that the purified GTF-I was not contaminated with the GTF-S enzyme although it is heterogeneous. The specific activities (by the sucrase assay) of the purified GTF-S and GTF-I enzymes were 8.2 and 35.8 U/mg of protein, respectively.

Production and characterization of MAbs. Thirty-three hybridomas, each secreting an antibody specific for GTF-S or GTF-I from *S. mutans* PS14, were selected by single-cell manipulation and ELISA techniques. Some characteristics of the MAbs obtained are described in Table 1. Twenty-nine of these MAbs were of the IgG1 type, one was IgG2a, one was IgG2b, and two were IgM. Of the thirty-one MAbs examined, 10 (P13, P20, P73, P126, P136, P141, P141, P158, P180, and P225) inhibited WIG-forming activity of the purified GTF-I enzyme. In particular, the P20 and P136 antibodies as well as the PAb-I antiserum produced marked inhibition. The immunoreactivities against various GTF preparations of these antibodies were tested by ELISA, in

TABLE 1. Characterization of MAbs and PAbs raised against *S. mutans* GTFs[a]

| Antigen | Antibody | Isotype (Ig) | Immunoreactivity[b] | | | | Inhibition of WIG synthesis by GTF-I[c] |
| | | | S. mutans | | S. milleri | | |
			GTF-I	GTF-S	GTF-I	GTF-SI	
GTF-S	PAb-S		−	+++	−	−	−
GTF-I	PAb-I		+++	−	+++	+++	+++
GTF-S	MAbs						
	P4	G2b	−	+++	−	−	−
	P22	G1	−	+++	−	−	−
	P31	G1	−	+++	−	−	−
	P47	G1	−	+++	−	−	−
	P49	G1	−	+++	−	−	−
GTF-I	MAbs						
	P14	G1	+++	−	++	−	−
	P18	G1	+++	−	+++	−	−
	P60	G1	++	−	++	−	−
	P72	G1	+++	−	+++	−	−
	P126	G1	+++	−	++	−	++
	P136	G1	+++	−	+++	−	+++
	P142	G1	+++	−	+++	−	−
	P225	G1	+++	−	+++	−	+
	P1	G1	+	−	−	++	ND[d]
	P9	G1	+	−	−	++	ND
	P12	G1	+	−	−	++	−
	P32	M	++	−	−	+++	−
	P11	G1	++	−	+	+++	−
	P13	G1	+++	−	+++	++	++
	P20	G1	+++	−	+++	+	+++
	P25	M	+++	−	++	+	−
	P49	G1	+++	−	++	+	−
	P58	G1	++	−	+	+++	−
	P66	G1	+	−	+	+	−
	P73	G1	+++	−	+++	+	+
	P108	G1	+++	−	+++	+++	−
	P123	G1	+++	−	+++	+++	−
	P135	G1	+++	−	+	+++	−
	P141	G2a	+	−	+	+	++
	P144	G1	++	−	++	+	++
	P155	G1	+	−	++	+	−
	P158	G1	+++	−	+++	+	++
	P180	G1	+	−	+	+	+

[a] Enzymes: partially purified GTF-I from *S. mutans* PS14, pure GTF-S from *S. mutans* PS14, crude GTF-I from an *S. milleri* transformant expressing the *gtfB* gene, and crude GTF-SI from an *S. milleri* transformant expressing the *gtfC* gene.

[b] Tested by an ELISA with hybridoma supernatants at a 1:10 dilution or antisera at a 1:300 dilution. A_{492}: +++, ≥1.5; ++, ≥0.7; +, ≥0.2; −, <0.2.

[c] Estimated turbidimetrically as described in the text. +++, ≥90%; ++, ≥50%; +, ≥10%; −, <10%.

[d] ND, not determined.

which the purified GTF-I and -S enzymes from PS14 and the crude GTF-I and -SI enzymes from the two *S. milleri* transformants were used as coating antigens. All five MAbs, P4, P22, P31, P47, and P49, raised against GTF-S from PS14 reacted only with the homologous enzyme. All 28 MAbs raised against GTF-I from PS14 also reacted only with the homologous enzyme. Of these, eight MAbs (P14, P18, P60, P72, P126, P136, P142, and P225) reacted only with the *gtfB* gene product (GTF-I) while four MAbs (P1, P9, P12, and P32) reacted only with the *gtfC* gene product (GTF-SI). The remaining 16 antibodies reacted with both GTF-I and GTF-SI. When the three GTFs from *S. sobrinus* B13N (GTF-I, -S₁, and -S₂) were used as antigens, no reactivities of these MAbs were observed (data not shown). Essentially similar results regarding the MAb immunochemical specificity were obtained by Western blot analysis using the crude GTFs from *E. coli* transformants and the pure GTF-S from PS14.

Typical immunoblotting patterns with representative MAbs are shown in Fig. 1. As indicated, the P72 antibody reacted only with the cell extract from *E. coli* SU20 expressing GTF-I, the P32 antibody reacted solely with the extract from *E. coli* NH3 expressing GTF-SI, the P13 antibody reacted with both cell extracts, and the P4 antibody reacted only with the GTF-S enzyme. The partially purified GTF-I enzyme, which was used for immunization in this study, was also analyzed by Western blotting with these four MAbs. The major 158-kDa protein was immunostained with the P72 and P13 antibodies, and the three minor proteins were immunostained with the P32 and P13 antibodies (Fig. 2). These results suggested that the 158-kDa protein is primarily enzyme GTF-I and that the minor components in this preparation are primarily GTF-SI enzyme derivatives.

Immunoblot analysis of the GTFs produced by *S. mutans* clinical isolates. By utilizing the P72, P32, and P4 antibodies
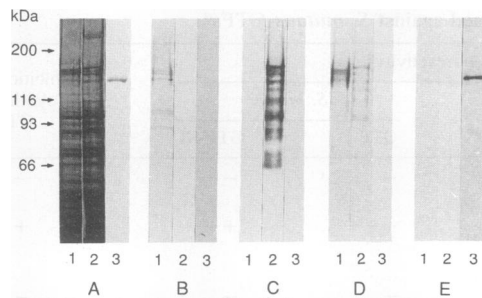
FIG. 1. Immunospecificities of MAbs P4, P13, P32, and P72 by Western blot analysis. (A) Coomassie blue staining; (B to E) immunoblotting with P72, P32, P13, and P4, respectively. Lanes: 1, cell extract of *E. coli* SU20 (expressing *S. mutans* GTF-I); 2, cell extract of *E. coli* NH3 (expressing *S. mutans* GTF-SI); 3, pure GTF-S enzyme.

TABLE 2. Characterization of *S. mutans* clinical isolates

Strain or isolate	Biotype[a]	Serotype[b]	GTF-SI level[c]	Adherence (%)[d]
Strain PS14[e]	I	c	High	78.8 ± 6.3
Isolates				
b	I	c	Low	73.2 ± 3.9
c	I	c	Low	80.2 ± 4.6
d	I	c	Low	83.3 ± 5.1
e	I	c	Low	70.4 ± 4.3
f	I	c	Low	60.7 ± 3.8
g	I	c	Low	78.1 ± 4.4
h	I	c	High	71.4 ± 4.0
i	I	c	High	57.4 ± 8.2
j	I	c	High	61.2 ± 3.9
k	I	c	High	68.1 ± 4.5
l	I	c	High	70.5 ± 5.4
m	I	c	Low	61.5 ± 3.7
n	V	e[f]	High	77.5 ± 4.8

[a] Determined by the method of Shklair and Keene (24).
[b] Determined by a double-immunodiffusion test with serotype c-specific rabbit antiserum.
[c] From the data in Fig. 3.
[d] Calculated from the turbidity at 550 nm. Values are means ± standard deviations from triplicate cultures.
[e] Reference strain.
[f] Determined from the biotype and lack of reactivity to the serotype c-specific antiserum.

that specifically recognize the *S. mutans* GTF-I, GTF-SI, and GTF-S enzymes, respectively, the GTF patterns of *S. mutans* clinical isolates were compared. Table 2 shows the results of the identification and in vitro sucrose-dependent colonization of 13 *S. mutans* strains (b to n) isolated from nine subjects. All of the clinical isolates examined exhibited strong sucrose-dependent colonization of smooth surfaces in vitro comparable to that of the reference laboratory strain PS14. Furthermore, Fig. 3 summarizes the results of Western blot analysis of the concentrated culture fluids and cell extracts from the isolates and reference strain PS14. In all isolates, the majority of the GTF-SI enzyme, as well as the GTF-S and GTF-I enzymes, was detected in the culture fluids. No cell-associated GTF-S enzyme could be detected for any isolate under these conditions. However, the relative amount of GTF-SI produced differed markedly with each strain (Fig. 3B), whereas the relative amounts of the other two enzymes were not as variable (Fig. 3A and C). As with strain PS14, six clinical strains of isolates h, i, j, k, l, and n showed high levels of GTF-SI production. By contrast, GTF-SI expression in seven strains (isolates b, c, d, e, f, g, and m) was extremely low.

DISCUSSION

In order to prepare MAbs which can differentiate the three GTFs of *S. mutans*, we established 33 murine hybridomas producing anti-GTF antibody, using homogeneous GTF-S and partially purified GTF-I preparations from strain PS14 as
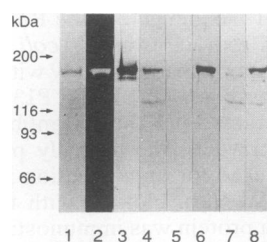

FIG. 2. SDS-PAGE and Western blot analyses of the GTF-I enzyme from *S. mutans* PS14. Lanes: 1, Coomassie blue staining; 2, activity displayed after incubation with sucrose; 3, activity displayed after the incubation and subsequent PAS staining; 4 to 8, respectively, immunoblotting with a PAb-I antiserum; a PAb-S antiserum; a GTF-I-specific MAb, P72; a GTF-SI-specific MAb, P32; and a MAb, P13, recognizing GTF-I and -SI.

antigens. The results of immunoreactivity tests using the three GTF antigens, which were not contaminated with each other, clearly indicated that five antibodies reacted specifically with the GTF-S enzyme, eight antibodies reacted specifically with the GTF-I enzyme, and four antibodies reacted specifically with the GTF-SI enzyme. On the other hand, the remaining 16 antibodies reacted with both the GTF-I and GTF-SI enzymes but not with the GTF-S enzyme and *S. sobrinus* GTFs. These results suggest that both GTF-I and GTF-SI possess many common epitopes. Recent genetic approaches regarding the *S. mutans* GTFs (21, 23, 26) have indicated that the *gtfB* and *gtfC* genes have extremely homologous sequences in their internal regions. For example, a stretch of approximately 100 amino acids in the interior of each gene product is approximately 98% homologous, while both enzymes are 75% homologous for the entire sequence. Most of the common epitopes, therefore, may be coded in the internal homologous regions of GTF-I and GTF-SI. Although Russell et al. have pointed out a high degree of homology (57.7%) between the *S. sobrinus gtfI* and *S. mutans gtfB* genes (22), none of the MAbs obtained in this study recognized any GTFs from *S. sobrinus* B13N because of the strict specificity of antigen-antibody interaction. Recently, we isolated a MAb (B86) raised with *S. sobrinus* GTF-S$_1$ that recognized all GTFs from *Streptococcus* and *Leuconostoc* species, suggesting the existence of a common epitope in all GTF molecules (7). In the present study, however, no MAb against such a common epitope from mice immunized with *S. mutans* GTFs was identified. Of the 10 MAbs which inhibited WIG synthesis by purified GTF-I from PS14, 3 reacted specifically with the *gtfB* gene product but not the *gtfC* gene product (Table 1). This observation suggests that at least one catalytic site of the GTF-I enzyme may not exist in the internal highly homologous region encoded by the HindIII-HindIII fragment (23, 26). To clarify the structural and functional relationships of the GTF enzymes, further characterization of these MAbs and identification of their respective epitopes are in progress.
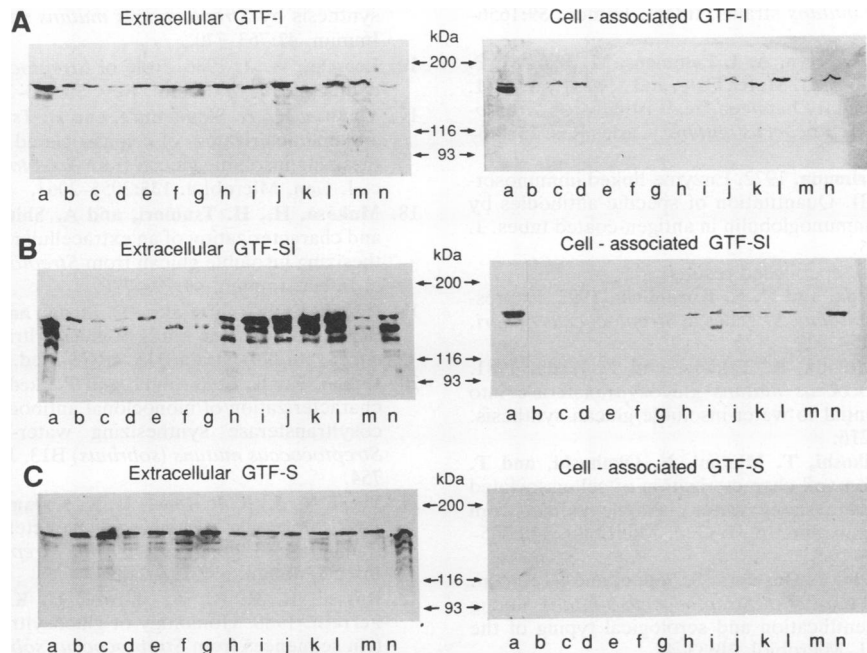
FIG. 3. Western blot analysis of the three GTFs produced by *S. mutans* clinical isolates. Extracellular GTFs and cell-associated GTF samples were analyzed by Western blotting with a GTF-I-specific MAb, P72 (A), a GTF-SI-specific MAb, P32 (B), and a GTF-S-specific MAb, P4 (C). Lanes a, samples from strain PS14; lanes b to n, samples from 13 clinical isolates (b to n).

The MAbs which can distinguish between the GTF-I and GTF-SI enzymes are important for identifying the expression of the GTF-I and GTF-SI enzymes in *S. mutans* because of the limited utility of the PAb in this type of analysis. Immunoblotting with a GTF-SI-specific MAb, P32, suggests that the final preparation of GTF-I purified from the culture fluid of strain PS14 was contaminated with a significant amount of the GTF-SI enzyme (Fig. 2). Polyclonal anti-GTF-I (PAb-I) can distinguish between the GTF-I and GTF-SI enzymes on the basis of their relative sizes (Fig. 2) (27). However, such an analysis must be made with caution, since these GTFs can be processed into multiple lower-molecular-weight species during culture, storage, and purification (8, 12, 18) (Fig. 1 and 3). Therefore, these monospecific MAbs should be useful in further determining the role of each GTF in cariogenesis.

The GTFs produced by *S. mutans* clinical isolates were analyzed by using the three monospecific MAbs (P4, P32, and P72). The results (Fig. 3) suggest that the *gtfC* gene is expressed in most, if not all, strains of *S. mutans* but the relative amount of the GTF-SI enzyme produced varies markedly among strains. Genetic polymorphism in the genes coding for the GTFs has been recently detected among *S. mutans* serotype c strains (4). Therefore, it is possible that the GTF-SI enzymes of some strains may have reduced avidity for the P32 antibody. To further confirm the GTF-SI levels determined in this investigation, immunoblot analysis with other MAbs and PAbs is in progress.

Hanada and Kuramitsu (12) have demonstrated that *S. mutans* GS-5 *gtfC* deletion mutants do not display sucrose-dependent adherence to smooth surfaces in vitro. In addition, we have found that *S. milleri* KSC43 cells expressing GTF-SI activity display in vitro sucrose-dependent adherence while *S. milleri* KSB8 cells expressing GTF-I activity do not (8). From these observations, it might be predicted that low-GTF-SI-producing strains of *S. mutans* may exhibit

cariogenicities lower than those of the high-GTF-SI-producing strains. However, the results of in vitro colonization (Table 2) indicated no correlation between the GTF-SI levels and the abilities of the strains to adhere to glass surfaces. These results suggest that the GTF-SI enzyme is required for sucrose-dependent colonization of hard surfaces but it is not the limiting factor in this process. Furthermore, it is likely that the relative proportions of the GTF-I, GTF-SI, and GTF-S enzymes are important for maximal sucrose-dependent adherence of *S. mutans*, as suggested for *S. sobrinus* (14). In this regard, de Soet et al. have reported that the cariogenicities of clinical isolates of *S. mutans* and *S. sobrinus* differ among strains (5). It will be of interest, therefore, to compare the in vivo cariogenicities of clinical isolates from the present study relative to their differential expression of the GTF-SI enzymes.

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