Multiple Changes in Cell Wall Antigens of Isogenic Mutants of *Streptococcus mutans*

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Isogenic mutants of *Streptococcus mutans* LT11, deficient in the production of the wall-associated protein antigens A and B, were generated by recombinant DNA technology. The hydrophobicity, adherence, and aggregation of the mutants were compared with those of the parent strain. These studies indicated that hydrophobicity, adherence, and salivary- or sucrose-induced aggregation were unaltered in the A− mutant but that hydrophobicity and adherence to saliva-coated hydroxyapatite were greatly reduced in the B− mutant whilst sucrose-dependent adherence and aggregation were increased. To determine whether these changes correlated with changes in the mutated gene product alone, the levels of a number of cell wall antigens were determined in each of the mutants. The loss of antigen A resulted in significantly reduced levels of wall-associated lipoteichoic acid, and loss of antigen B resulted in reductions in both antigen A and lipoteichoic acid. Data presented here thus suggest that changes in the expression of one wall antigen can have a dramatic effect on the levels of others.

It is now widely recognized that *Streptococcus mutans* is the major aetiological agent in dental caries (24). This has led to strenuous efforts to characterize the factors which contribute to the pathogenesis of this organism. Molecular biology techniques have enabled the genes encoding a number of putative virulence factors to be isolated, cloned, and sequenced. Among these are the genes which code for the wall-associated proteins A and B (antigens A and B) (39). The gene encoding antigen A is referred to as *wapA* (10). The *wapA* gene of *S. mutans* encodes a mature protein with a predicted molecular mass of approximately 45 kDa. However, when antigen A is extracted from whole cells and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), it migrates with a molecular mass of approximately 72 kDa, prompting the suggestion that it remains covalently linked to other wall components even after SDS extraction (10). A 29-kDa protein, arising as a result of proteolytic cleavage of the wall-associated antigen, has been identified and purified from culture supernatants (10). To date, the functional role of antigen A has not been established. The wall-associated protein originally described as antigen B (39) is also referred to as antigen I/II (38), antigen P1 (11), and PAc (34, 35). Antigen B is a highly immunogenic 185-kDa protein which also exists as a wall-associated and free form, but the mechanism of its release remains unknown (22). Several studies have suggested that antigen B is responsible for the surface hydrophobicity of *S. mutans* (18–20, 23, 27, 32), and since hydrophobic bonding is thought to be important in initial attachment of bacteria to the tooth surface, antigen B has been implicated as an adhesin molecule which interacts with components of the salivary pellicle (8, 20, 23).

Insertion duplication mutagenesis has been widely used to inactivate specific target genes in order to identify the functions of the proteins which they encode (1, 14, 23, 30, 36, 37, 42). An implicit assumption of this type of strategy is that phenotypic changes observed in mutant cells are due to lack of expression of a single protein as a consequence of the targeted genotypic alteration. However, it has previously been shown that changes in single surface properties such as hydrophobicity may be correlated with differences in multiple wall components rather than changes in an individual surface antigen (18, 27, 44), and recently Jenkinson and Easingwood (17) showed that insertional inactivation of the gene encoding a 76-kDa cell surface protein in *Streptococcus gordonii* had a pleiotropic effect on a number of other cell surface polypeptides. The work described here was therefore aimed at identifying the function of wall-associated protein A by constructing an isogenic mutant of *S. mutans* deficient in the production of this antigen and also at determining whether phenotypic changes in isogenic mutants deficient in either antigen A or B were consistent with the loss of a single surface component or changes in multiple components.

MATERIALS AND METHODS

**Bacteria and growth conditions.** *S. mutans* LT11, a highly transformable serotype c mutants streptococcus, was a kind gift from L. Tao (Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Okla.). *Escherichia coli* JM83 has been previously described (52). Streptococci were grown anaerobically at 37°C without agitation in either brain heart infusion broth, Todd-Hewitt broth, or CasMM medium supplemented with 0.5% (wt/vol) glucose (40). *E. coli* JM83 was grown in Luria-Bertani medium at 37°C with agitation.

**Plasmids.** The plasmids used in this study were the cloning vector pUC18 (31), pSPRA6, a recombinant plasmid containing a cloned copy of the entire *wapA* gene (10), and the *E. coli*-streptococcus shuttle vector pVA891 (25).

**Construction of recombinant plasmids for insertional inactivation.** A 0.7-kb *HindIII*-NruI fragment from within the *wapA* gene of the recombinant plasmid pSPRA6 (10) was subcloned into the shuttle vector pVA891 to give the plasmid pVHA7 (Fig. 1a). A 10-kb partial *HindIII*-digested fragment of a recombinant lambda phage expressing antigen B (29)

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FIG. 1. Construction of recombinant plasmids pVHA7 (a) and pVHB6 (b) for insertional inactivation. Shaded areas denote lambda DNA sequences and hatched areas denote antigen A- or antigen B-coding sequences. Abbreviations: B, BamHI; Bg, BglII; C, ClaI; Ei, EcoRI; EV, EcoRV; H, HindIII; N, NruI; P, PvuII; S, SphI; X, XbaI; Amp', ampicillin resistance; Cm', chloramphenicol resistance; Em', erythromycin resistance.

was subcloned into pUC18 to give the recombinant plasmid pSMB16. A 2.5-kb EcoRV fragment from within the antigen B gene was subsequently subcloned from this recombinant plasmid into pVA91 to generate the plasmid pVHB6 (Fig. 1b). All plasmids were purified with Qiagen ion-exchange columns (Hybaid Ltd., Twickenham, England).

Bacterial transformation. Transformation of E. coli JM83 was performed by the calcium chloride method of Cohen et al. (5). Transformation of S. mutans LT11 was achieved by a modification of the method of Lee et al. (23). Late-exponential-phase cultures of LT11 were diluted 1/40 into fresh culture medium (brain heart infusion broth) and grown to early-exponential phase. Bacterial cells were then harvested by centrifugation, washed three times with 1 ml of ice-cold 10% (vol/vol) glycerol, and resuspended in 0.3 ml of cold 10% glycerol. Aliquots (0.5 to 2.0 µg) of the recombinant plasmids pVHA7 and pVHB6 were added to 50 µl of the cell suspension, and the mixture was electroporated in a 0.2-cm-diameter cuvette (200 ohms, 2.0 kV, 4 to 5 ms). Electroporated cells were immediately placed on ice for 1 to 2 min, 200 µl of prewarmed brain heart infusion broth containing 0.5 M sucrose was added, and the cells were then incubated at 37°C for 2 to 3 h to allow expression of the erythromycin resistance determinant of the transformed plasmids. After the expression period, cells were plated on selective agar and erythromycin-resistant (Em') colonies were picked for further analysis.

Electrophoretic separation of proteins and Western immunoblotting. Proteins were separated by SDS-PAGE on 8 or 12% gels by the discontinuous system of Laemmli (21) and either stained with Coomassie brilliant blue or transferred onto nitrocellulose membranes (Schleicher & Schuell BAS85; Anderman and Co., Twickenham, England) at 0.8 mA/cm² for 1 h as described by Towbin et al. (50). Membranes were immunoblotted with rabbit antiserum raised against antigen A or B as the primary antibody and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Sigma Chemical Co. Ltd., Poole, England) as the secondary antibody. Alkaline phosphatase activity was detected by incubating the filters in the presence of 5-bromo-4-chloro-3-indolyl phosphate–nitroblue tetrazolium salt substrate (Zymed Laboratories, Inc., South San Francisco, Calif.).

Isolation of chromosomal DNA. Chromosomal DNA was extracted from S. mutans strains as described by Ushiro et al. (51). Approximately 2 to 3 µg of each DNA was digested to completion with EcoRI and separated by electrophoresis on a 0.8% (wt/vol) agarose gel. After electrophoresis, the gel was treated as described by Maniatis et al. (26) to denature the DNA and the DNA fragments were transferred onto nitrocellulose membranes (BAS85; Schleicher & Schuell)
with a Posiblot transfer apparatus (Stratagene Ltd., Cambridge, England).

**Labelling of DNA probes and Southern hybridization.** Linearized pVA891, the cloned HindIII-NruI fragment of pVHA7, and the cloned EcoRV fragment of pVHB6 were used as probes for Southern hybridization. Probes were labelled with the Nonradioactive (digoxigenin) DNA Labeling and Detection Kit (Boehringer Mannheim Ltd., Lewes, England) and used in hybridizations under high-stringency conditions according to the manufacturer’s instructions.

**Measurement of bacterial hydrophobicity.** The hydrophobicity of *S. mutans* strains was determined as previously described by Lee et al. (23).

**Measurement of bacterial adherence to saliva-coated hydroxyapatite.** Adherence of *S. mutans* strains to saliva-coated hydroxyapatite (SHA) was assayed by a modification of the method of Lee et al. (23). *S. mutans* was grown to stationary phase of growth in Todd-Hewitt broth supplemented with 0.5% (wt/vol) yeast extract and 50 μCi of [methyl-3H]thymidine (Amersham International, Aylesbury, England) per ml (specific activity, 50 Ci/mmol). Cells were harvested, washed three times with adherence buffer (50 mM KCl, 1 mM CaCl₂, 38.3 mM MgCl₂, 6 mM glucose, 0.78 mM KH₂PO₄, 1.22 mM K₂HPO₄ [pH 7.2]), and resuspended in 3 ml of adherence buffer. Cell suspensions were sonicated for 10 s at a power output of 20% with an XL2010 sonicator (Heat Systems Incorporated, Farmingdale, N.Y.), pelleted by centrifugation, and resuspended in adherence buffer to give an optical density of 0.1 at 550 nm (approximately 10⁷ cells per ml). Microscopic examination confirmed that the majority of bacteria were present as single cells as opposed to chains.

Hydroxyapatite beads (8 mg; BDH Ltd., Poole, England) were rehydrated overnight in 200 μl of adherence buffer. The buffer was removed by aspiration, and 200 μl of clarified whole saliva was added to the beads. The suspension was incubated at room temperature, and mixing, for 1 h. The beads were washed once with adherence buffer and incubated with 200 μl of radiolabelled cell suspension, with mixing, at room temperature. After 1 h, the beads were allowed to settle under gravity and 100 μl of liquid were removed and counted in a liquid scintillation counter. Percent adherence of cells to the hydroxyapatite beads was calculated by the following formula: [(control counts − test counts)/control counts] × 100, where control counts were counts obtained from tubes in which hydroxyapatite beads were omitted.

Sucrose-dependent adherence to SHA was determined as described by Schilling and Bowen (45), by using radiolabelled bacterial cultures resuspended to a concentration of 10⁶ cells per ml. Percent adherence was calculated as described above.

The saliva used in these studies was collected and pooled from healthy male and female volunteers in the Department of Oral Biology, The Dental School, University of Newcastle upon Tyne.

**Measurement of bacterial aggregation.** Sucrose- and dextransucrose-induced aggregations were measured as described by Douglas and Russell (7). Saliva-induced aggregation was measured as described by Koga et al. (20).

**Preparation of cell wall antigens.** Cultures of *S. mutans* LT11 and the isogenic mutants were incubated overnight in CasMM supplemented with 0.5% (wt/vol) glucose at 37°C, and the cell density of each culture (optical density at 550 nm) was determined. Bacterial cells were pelleted by centrifugation, freeze-dried, and resuspended at 20 mg/ml in 50 mM Tris-HCl, pH 7.5. One hundred units of mutanolysin (Sigma Chemical Co. Ltd.) per milliliter was added to each suspension and incubated at 37°C for 5 h with mixing. Insoluble cell material was removed by centrifugation, and the resulting soluble wall extracts, which are essentially free of membrane and cytoplasmic components (13, 47), were recovered and stored at −20°C until required.

Culture supernatants were brought to 65% saturation with ammonium sulfate, and the precipitates which formed at 4°C overnight were pelleted by centrifugation; resuspended in 1/100 of the original volume in 50 mM Tris-HCl, pH 7.5, and dialyzed against several changes of the same buffer. The final volume of each dialysate was adjusted to account for the cell density of the original culture.

**Quantitation of antigen A and LTA by competitive enzyme-linked immunosorbent assay (ELISA).** Each well of polystyrene 96-well microtiter plates (Immulon 1; Dynatech Laboratories Ltd., Billinghamurst, England) was coated with 75 μl of a solution of antigen in either carbonate-bicarbonate buffer, pH 9.6 (antigen A), or phosphate-buffered saline (PBS) (lipoteichoic acid [LTA]) for 1 h at 4°C. Plates were blocked with 150 μl of PBST (PBS plus 0.05% Tween 20) containing 10% (vol/vol) fetal calf serum for 1 h at 37°C. After blocking, dilutions of either cell wall extracts or concentrated culture supernatants of *S. mutans* LT11, LT11/ A−, or LT11/B− (75 μl) were added in triplicate to the coated wells, followed by 75 μl of an empirically determined dilution of the appropriate antiserum. The plates were tapped gently to allow mixing and incubated at 37°C for 2.5 h. After this time the wells were aspirated and washed extensively with distilled water and PBST, and 75 μl of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Sigma Chemical Co. Ltd.) (1/15,000 dilution) was added to each well. After 1 h of incubation at 37°C, unbound conjugate was removed by extensive washing with distilled water and PBST. Alkaline phosphatase substrate (6 mM p-nitrophenyl phosphate [Sigma Chemical Co. Ltd.] in diethanolamine buffer, pH 9.8, containing 0.5 mM MgCl₂) was then added to each well, and color was allowed to develop for 30 to 90 min at 37°C. Absorbance was measured with a Titertek Multiskan plate reader, and the amount of antigen in each sample was calculated by using a standard curve generated from the antigen standards included on each plate. Antigen levels in the mutant strains were compared with those in LT11 by using Student’s t test.

**Quantitation of antigen B by direct ELISA.** Antigen B was quantitated as described above except that wells were coated with 75 μl of appropriate dilutions (in carbonate-bicarbonate buffer, pH 9.6) of cell wall extracts or culture supernatants of *S. mutans* LT11, LT11/A−, or LT11/B− and no competing antigen was added upon addition of the anti-B antiserum.

**Labeling of lipoproteins.** Total lipoproteins of *S. mutans* LT11 and its mutants were labelled with [¹⁴C]palmitate and detected by autoradiography as described by Sutcliffe et al. (49).

**RESULTS**

**Construction of isogenic mutants.** Isogenic mutants of the parent *S. mutans* LT11 were generated by the technique of insertional duplication mutagenesis. Electroporation of *S. mutans* LT11 with the recombinant plasmid pVHA7 or pVHB6 resulted in approximately 100 Em' colonies per μg of DNA. Since the vector pVA891 cannot replicate autonomously, these Em' colonies should contain a chromosomally

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integrated copy of the recombinant plasmid, a consequence of a homologous recombination event between the cloned gene fragment and the endogenous gene of the transformed strain. A number of putative A<sup>-</sup> and B<sup>-</sup> mutants were picked and subjected to SDS-PAGE analysis of their cell wall proteins. Although it was not possible to demonstrate the loss of a stained band corresponding to wall-associated protein A in Coomassie blue-stained cell wall extracts of putative A<sup>-</sup> mutants, because of the presence of multiple staining bands in the 72-kDa region of the gel (Fig. 2a, lanes 2 to 6), the absence of this antigen in the mutants was confirmed by Western immunoblotting with a polyclonal anti-A antiserum (Fig. 2b, lanes 2 to 6). In contrast, the cell wall extracts of all of the five putative B<sup>-</sup> mutants tested were shown to be deficient in a 185-kDa protein corresponding to antigen B upon Coomassie blue staining (Fig. 3a, lanes 2 to 6). Western immunoblotting with a polyclonal anti-B antiserum did, however, reveal that the antiserum reacted weakly with a protein of approximately 162 kDa in all of the putative B<sup>-</sup> mutants (Fig. 3b, lanes 2 to 6). This is believed to be a truncated product of the insertionally inactivated antigen B gene, though since such a protein would lack its C terminus, it would not be expected to remain anchored in the cell wall.

The cloned fragments of plasmids pVHA7 and pVHB6 and the vector pVA891 were digoxigenin labelled and used as probes for Southern hybridization of EcoRI-digested DNA from representative A<sup>-</sup> and B<sup>-</sup> mutants. Labelled pVA891 was shown to hybridize to DNA fragments of approximately 5.5 and 2.6 kb in both the A<sup>-</sup> and the B<sup>-</sup> mutants but not to DNA isolated from LT11 (Fig. 4a). The presence of vector sequences in the DNA isolated from each of the mutants confirmed the integration of the respective recombinant plasmids in these strains. Furthermore, probing of DNA isolated from LT11 and the A<sup>-</sup> mutant with the cloned 0.7-kb HindIII-NruI fragment (probe A) and DNA from LT11 and the B<sup>-</sup> mutant with the cloned 2.5-kb EcoRV fragment (probe B) indicated that each of the mutant DNAs contained one extra hybridizing fragment compared with the parent strain (Fig. 4b). This was consistent with duplication of the cloned (target) sequence in the genome of the mutants. For both mutants, the sizes of these extra bands and those observed when probing with pVA891 suggested that insertion of the recombinant plasmids into the genes encoding

![FIG. 2. SDS-PAGE analysis of putative A<sup>-</sup> mutants. SDS-extractable proteins from S. mutans LT11 (lanes 1) and putative A<sup>-</sup> mutants (lanes 2 to 6) were separated by electrophoresis through 12% polyacrylamide gels and either stained with Coomassie brilliant blue (a) or transferred onto a nitrocellulose membrane and reacted with antiserum raised against antigen B (b).](http://jb.asm.org/)

![FIG. 3. SDS-PAGE analysis of putative B<sup>-</sup> mutants. SDS-extractable proteins from S. mutans LT11 (lanes 1) and putative B<sup>-</sup> mutants (lanes 2 to 6) were separated by electrophoresis through 7.5% polyacrylamide gels and either stained with Coomassie brilliant blue (a) or transferred onto a nitrocellulose membrane and reacted with antiserum raised against antigen B (b). The position of the 185-kDa antigen B is indicated by arrowheads.](http://jb.asm.org/)
antigens A and B had occurred immediately downstream of the DNA sequences homologous to those of the cloned fragments in the recombinant plasmids. Such insertions would give rise to the expression of truncated forms of antigen A and antigen B with predicted molecular masses of 27 and 162 kDa, respectively, both lacking the C terminus of the molecule which includes the proposed wall anchor region. This predicted size for the truncated antigen B produced by the B- mutant is consistent with the Western immunoblotting results described above (Fig. 3b).

Adherence, aggregation, and hydrophobicity. S. mutans LT11 and its isogenic mutants were assayed for their ability to adhere to SHA in the presence and absence of glucan synthesized in situ. Adherence of the A- strain was unaffected by the presence or absence of glucan when compared with the parent strain LT11 (Table 1). The B- mutant, however, showed reduced adherence to SHA compared with the parent strain, but in the presence of glucan the adherence of this strain was increased relative to LT11 (Table 1).

Hydrophobicity of the mutants as determined by their adsorption to hexadecane indicated that the A- strain exhibited hydrophobicity similar to that of the parent strain but that the B- mutant was significantly less hydrophobic (Table 1).

When the strains were tested for their ability to aggregate in the presence of sucrose, there was little difference in the timing and extent of aggregation between the parent strain and the A- mutant (Fig. 5). The B- mutant, on the other hand, was shown to aggregate more readily and to a greater extent than LT11 (Fig. 5). It was also observed that whilst neither LT11 or the A- mutant aggregated in the presence of dextran 2000, dextran-induced aggregation of the B- mutant followed much the same course as its sucrose-induced aggregation (data not shown). Aggregation of the three strains in the presence of clarified, whole saliva indicated that there was again little difference between the parent strain and the A- mutant (data not shown) but that the B- strain self-aggregated in the absence of saliva, even in low-salt-concentration (10 mM Tris-HCl) buffer (data not shown).

Quantitation of cell wall antigen levels. In order to determine whether the changes in adherence and hydrophobicity observed for the mutants correlated solely with the loss of the individual wall antigens, S. mutans LT11 and the isogenic mutants were quantitatively analyzed for a number of cell wall components by ELISA. These studies showed that there was a marked decrease in the amount of extractable wall LTA in the A- mutant compared with that in LT11 ($P < 0.001$) and that loss of antigen B also resulted in a reduction in the level of wall-associated LTA ($P < 0.1$). These results were mirrored by those which indicated that both these mutants had increased levels of supernatant LTA when compared with LT11 (Fig. 6a). Since the total amounts of LTA were approximately the same in all three strains, this suggested a difference in the location or release of this antigen rather than a change in the level of expression of LTA. These results were confirmed when an independent method, rocket immunoelectrophoresis, was used to assay the same wall extracts (data not shown). In addition, the B- mutant was also shown to have a reduced level of antigen A when compared with the parent strain ($P < 0.001$), which was again accounted for by increased release of this antigen into the culture supernatant (Fig. 6b).

The amount of wall-associated antigen B in the three strains was assayed by direct rather than competitive ELISA because of anomalous results being obtained in a competitive assay. The anomalies were apparently due to interactions between the competing antigen B in the cell wall extracts and a number of the blocking reagents (fetal calf serum, bovine serum albumin, and semi-skimmed milk) used in this system. No significant difference in the level of wall-associated or supernatant antigen B was observed between LT11 and the A- mutant (Fig. 6c). Quantitation of antigen B in cell wall extracts of the B- mutant revealed a detectable but greatly reduced level of this antigen and suggested that the antiserum used in this assay was reacting with the truncated antigen B observed upon Western blotting of SDS-extracted wall proteins from this strain (Fig. 3b). The high levels of antigen B detected in the culture supernatants of the mutant confirmed that the majority of the truncated antigen B produced by this strain was being released into the supernatant as a consequence of the loss of the wall-anchoring region of the protein.

Radiolabelling of cell wall lipoproteins of LT11 revealed the presence of about 10 proteins and indicated that there were no qualitative differences between the parent strain and the mutants (Fig. 7).

![Image](https://example.com/image.png)

**FIG. 4.** Southern blot analysis of EcoRI-digested DNA from S. mutans LT11 and isogenic mutants. Chromosomal DNAs (1 to 2 μg) were electrophoretically separated, transferred to nitrocellulose membranes, and probed with the following digoxigenin-labelled probes: linearized plasmid pVA891 (a), the 0.7-kb HindIII-NrI fragment from the wapA gene (probe A) (b), and the 2.5-kb EcoRV fragment from the antB gene (probe B) (c).

**TABLE 1.** Hydrophobicity and adherence of S. mutans LT11 and isogenic mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hydrophobicity</th>
<th>% Adherence to:</th>
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<tr>
<td></td>
<td></td>
<td>SHA</td>
<td>Glucan-SHA</td>
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<td>81</td>
<td>29.3 ± 8.9</td>
<td>27.2 ± 7.9</td>
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</tr>
<tr>
<td>LT11/A-</td>
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<td>28.7 ± 1.3</td>
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<tr>
<td>LT11/B-</td>
<td>12</td>
<td>4.2 ± 4.2</td>
<td>50.9 ± 2.1</td>
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* Percent adsorption to hexadecane.

* Values are means ± standard deviations for triplicate samples from a single experiment and are representative of two separate experiments.

* Values represent percent adherence to glucan-SHA minus percent adherence to SHA alone.
**DISCUSSION**

The cell wall of *S. mutans* is a complex and dynamic structure which contains peptidoglycan, LTA, and a variety of proteins, lipids, lipoproteins, and carbohydrates (12, 46). It is likely, therefore, that a high degree of ordering and interaction of these components is necessary to maintain the integrity of the wall and to enable the various components to carry out their specific functions. Knowledge of the functions of these components and a greater understanding of their interactions in *S. mutans* would clearly enable us to define more precisely the specific factors which are involved in its colonization of the tooth surface and maintenance in the oral cavity.

The major wall-associated protein known as antigen A has been shown to confer protective immunity against caries in immunized rats and monkeys (4, 43), suggesting that it is a possible virulence factor. To date, however, the role of this antigen has not been identified. In order to investigate possible functions of antigen A, we constructed a mutant strain of *S. mutans* deficient in the production of this protein. Our studies indicate that the A− mutant showed no significant difference in hydrophobicity, saliva-induced adherence, or saliva-induced aggregation when compared with the parent strain. This is consistent with earlier reports of experiments with specific antisera which indicated that antigen A is not involved in adherence (7, 8). Furthermore, since the A− mutant also has a significantly reduced level of wall-associated LTA, it suggests that LTA similarly does not contribute to the hydrophobicity of the cell surface or the adherence of *S. mutans* to SHA. These results are consistent with those obtained by McBride et al. (27), who demonstrated that there was no difference in LTA content between hydrophobic and hydrophilic strains of *S. mutans*, and those of Stashenko et al. (48), who showed that a number of monoclonal antibodies directed against the LTA of *S. mutans* JBP did not inhibit the adherence of a number of oral streptococci to experimental pellicles. In contrast, numerous studies suggest that LTA is involved in both adherence (for a review, see reference 16) and hydrophobicity (28) in group A streptococci.

Neither the parent strain nor the A− mutant showed any significant aggregation in the presence of dextran 2000. Drake et al. (9) have previously shown that a number of the classical *S. mutans* strains fail to aggregate in the presence of exogenously added high-molecular-weight glucans. Moreover, the finding that the A− mutant was unaltered in its ability to adhere to sucrose-derived dextran-SHA or to aggregate in the presence of sucrose-derived dextran suggests that antigen A is not involved in dextran binding as has previously been proposed (6).

The demonstration that a mutant strain deficient in the production of antigen B exhibited reduced hydrophobicity and adherence to SHA relative to the parent strain LT11 is consistent with the findings of other workers studying mutants deficient in the same antigen (20, 23). The increased adherence of this mutant to SHA in the presence of sucrose-glucosyltransferase confirms a similar finding by Bowen et al. (2) with respect to the P1-deficient mutant 834. Together with the demonstration that this strain also exhibited increased aggregation in the presence of both sucrose and dextran when compared with the parent strain, these results suggest that antigen B may mask glucan chains on the cell surface or may sterically hinder the interaction between glucan chains and their binding sites in the salivary pellicle or on other bacteria. Previous reports (20, 23) have suggested that antigen B-deficient mutants fail to aggregate in the presence of either whole, clarified saliva or salivary agglutinin. More recently, however, it has been shown that the P1-deficient mutant 834 does in fact exhibit some degree of aggregation in the presence of salivary agglutinin (3). Our demonstration and the observation of others (3a) that the B− mutant retains a low but detectable level of a truncated form of the antigen in the cell wall despite the loss of its C-terminal anchor may help to explain such results. It also suggests
that parts of the antigen B molecule other than the C terminus may contribute to its retention at the cell surface.

The use of recombinant DNA technology to create isogenic mutants deficient in a single gene product has been widely used to investigate protein function. However, Jenkinson and Easingwood (17) recently demonstrated that insertional inactivation of the gene encoding a surface polypeptide in *S. gordonii* Challis had a pleiotropic effect on cell surface composition and properties. Furthermore, Russell and Smith (44) had previously suggested that changes in bacterial surface hydrophobicity did not correlate with the loss of just a single wall component. To investigate the possibility that alterations in one wall component can have a significant effect on the levels of other apparently unrelated components, the levels of a number of wall components in mutant strains deficient in the production of antigen A and B were determined. Our results indicate a striking reduction in the level of extractable wall-associated LTA in a mutant strain which was deficient in the wall-associated protein A and significant differences in both LTA and antigen A levels in a mutant strain deficient in antigen B. These differences were accounted for by an increased shedding of the antigens into the culture supernatant. These pleiotropic effects and those described by Jenkinson and Easingwood (17) presumably occur either through the abolition of specific interactions which serve to hold particular components within the wall or a general destabilization of the cell surface. We were able to demonstrate that there were no qualitative differences in the lipoprotein profiles of the three strains and that these profiles were consistent with those recently reported (49). However, it remains possible that there were changes in other components, such as carbohydrates, which were not assayed.

The possibility that mutations in *wagA* could have a polar effect on the expression of antigen B (and vice versa) is unlikely, since the two genes are some distance apart on the *S. mutans* chromosome (15, 33). Furthermore, as they are...
located on either side of the multiple sugar metabolism (msm) operon, any polar effect would also result in disruption of the function of this operon. This operon confers the ability to ferment the sugar melibiose (42). The fact that both A− and B− mutants were shown to be unaltered in their ability to ferment melibiose (data not shown) suggests that this operon was unaffected by mutations in the genes encoding antigens A and B. It is also unlikely that inactivation of wapA has any effect on the gene immediately downstream, which starts 320 bp after the termination codon of wapA (10).

Although the work described here does not enable us to speculate on the function of antigen A, the possible association between this antigen and both antigen B and LTA warrants further investigation. Whether antigen A is somehow involved in anchoring LTA in the cell wall is unclear at this time. It is interesting to note, however, that we have previously observed the binding of LTA to an immunosor- bent column prepared with an anti-A monoclonal antibody (41), possibly indicating an interaction between the two molecules. Furthermore, it has previously been suggested that the anomalous migration of the wall-associated form of antigen A is due to the fact that it remains covalently linked to other wall components even after SDS extraction (10). The nature of this interaction remains unknown, but immunoblotting of wall-extractable antigen A with lectins which recognize N-acetylglucosamine-containing glycoproteins indicated that the linkage did not involve this peptidoglycan component of the cell wall (data not shown). However, the threonine-serine-rich domain of antigen A could potentially be coupled to LTA or some other intermediary molecule and contribute to the overall organization and stability of the cell wall.

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