**Functional Variation of the Antigen I/II Surface Protein in* Streptococcus mutans* and* Streptococcus intermedius**

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Although *Streptococcus intermedius* and *Streptococcus mutans* are regarded as members of the commensal microflora of the body, *S. intermedius* is often associated with deep seated purulent infections, whereas *S. mutans* is frequently associated with dental caries. In this study, we investigated the roles of the *S. mutans* and *S. intermedius* antigen I/II proteins in adhesion and modulation of cell surface characteristics. By using isogenic mutants, we show that the antigen I/II in *S. mutans*, but not in *S. intermedius*, was involved in adhesion to a salivary film under flowing conditions, as well as in binding to rat collagen type I. Binding to human fibronectin was a common function associated with the *S. mutans* and *S. intermedius* antigen I/II. Adhesion of *S. mutans* or *S. intermedius* to human collagen types I or IV was negligible. Hydrophobicity, as measured by water contact angles, and zeta potentials were unaltered in the antigen I/II mutant. The *S. mutans* isogenic mutants, on the other hand, exhibited more positive zeta potentials at physiological pH values than did the wild type. The results indicate common and species-specific roles for the antigen I/II in mediating the attachment of *S. mutans* and *S. intermedius* to host components and in determining cell surface properties.

*Streptococcus mutans* and *Streptococcus intermedius* are commensal organisms found in the human oral cavity. *S. mutans* is a late colonizer mainly found in dental plaque (34) and is associated with dental caries (23). *S. intermedius* is found particularly in the gingival crevice around the margins of the teeth (30). *S. intermedius* also is a resident of the gastrointestinal and urogenital tracts and is associated with abscess formation and with tropism for infections of the brain and liver (51). Like other oral streptococci, *S. mutans* and *S. intermedius* are implicated as causative agents of infective endocarditis (9, 10, 12, 40, 51).

Most oral streptococci, including *S. mutans* and *S. intermedius*, express one or more members of a family of structurally and antigenically related surface proteins termed antigen I/II. These proteins have received a variety of names according to the strains or species in which they were identified, such as antigen B (42), Sr (35), I/II (14), and PAc (36) from *S. mutans*, Spa A (18) from *Streptococcus sobrinus*; PAA from *Streptococcus cricetus* (44); and SpA and SspB from two tandemly arranged genes in *Streptococcus gordonii* (8). In *S. intermedius* a 160-kDa protein is observed (38), whereas the antigen I/II molecular mass in other oral streptococci ranges from 170 to 215 kDa (8, 14, 18, 35, 36, 42).

Investigation of the antigen I/II adhesive properties, particularly in *S. mutans* and *S. gordonii,* has been the focus of numerous studies, since attachment to host components represents a crucial event during bacterial colonization and infection (13). Despite its importance as a commensal organism and as a causative agent of purulent lesions, functional investigations on the role of *S. intermedius* surface molecules are limited. Recent studies have shown, however, that the antigen I/II in *S. intermedius* exhibit adhesive and stimulatory properties associated with virulence mechanisms (38).

In the oral cavity, bacterial adhesion occurs on surfaces coated with salivary films. Static binding assays have demonstrated that antigen I/II is essential for *S. mutans* (16) but not for *S. gordonii* adhesion to salivary films (8). Such binding has not been investigated for *S. intermedius*. In the oral cavity, bacterial adhesion to salivary conditioned surfaces occurs under the influence of hydrostatic pressure and shear forces during salivary flow. These dynamic conditions may influence bacterial adhesion. For instance, in *Pseudomonas aeruginosa*, surface structures associated with adhesion under static conditions were not involved in adhesion to the same substrata under flowing conditions (7).

The ability of bacteria to bind extracellular matrix (ECM) components is regarded as a significant factor in the development of abscesses and infective endocarditis, since tissue damage often precedes streptococcal colonization (2, 52). Immunobilized antigen I/II fragments from several oral streptococci are shown to bind soluble ECM, such as fibronectin, laminin, and collagen type I (43). Moreover, isogenic mutants of *S. mutans* and *S. gordonii* deficient in antigen I/II exhibit diminished adhesive capacity to immobilized collagen type I (24). Collagen binding, suggestively, contributes to the ability of streptococci to invade dentinal tubules (24). Streptococci may, however, interact differently with soluble and immobilized ECM (5, 25, 29).

Cell surface properties associated with adhesion, such as hydrophobicity and surface charge are influenced by bacterial surface molecules, such as those present in fimbiae and fibrils (28, 48). Although *S. mutans* does not exhibit such structures, a “fuzzy layer” on its surface has been associated with the antigen I/II (21). Hydrophobicity is reported to be reduced in *S. mutans* isogenic mutants deficient in antigen I/II (11, 27, 36).
The aim of the present study was to investigate the role of the *S. mutans* and *S. intermedius* antigen I/II in bacterial binding to and detachment from a salivary film under flowing conditions and its role in adhesion to immobilized extracellular matrix proteins. We also examined the contribution of antigen I/II to hydrophobicity and surface charge.

**MATERIALS AND METHODS**

**Bacterial strains and culture media.** The *S. intermedius* strains used in this study included the type strain NCTC 11324 and its antigen II isogenic mutant IB08981 (38). The *S. mutans* strains included *S. mutans* LT1, a highly transformable variant of UA159 (46), and two *S. mutans* I/II isogenic mutants inactivated at different sites, IB10991 and IB03987 (described below). The strains were stored at -70°C in brain heart infusion broth (BHH; Difco Laboratories, Detroit, Mich.) supplemented with 15% (vol/vol) glycerol. Streptococcal cultures in the exponential phase. The pellets were washed twice with BHH at 37°C under microaerophilic conditions. For streptococcal transformation, Todd-Hewitt broth (THB; Difco Laboratories) containing 5% heat-inactivated horse serum (HS) was used as the growth medium, and incubation was at 37°C under microaerophilic conditions. Escherichia coli carrying the plasmid psF151 was grown in Luria-Bertani broth (THB; Difco Laboratories) supplemented with kanamycin (Sigma-Aldrich AS, Oslo, Norway) at a final concentration of 50 μg/ml. For the selection of isogenic mutants, kanamycin was used at a final concentration of 500 μg/ml.

**DNA isolation.** The streptococcal integration vector pSF151 was isolated from E. coli with the Plasmid Maxi kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer’s recommendations for high-copy-number plasmids. psF151 replicates in E. coli but not in streptococci and expresses kanamycin resistance in both organisms (45).

**PCR amplification of target insert.** The forward primer (5'-GTCAGTGCGCA ACAGATTTATCACA) and the reverse primer (5'-AATATTTTCGTGAGGCAACGCG CCAAGA) were used to amplify a homologous region in *S. mutans* NCTC 11324. The amplified fragments were extracted from 0.7% agarose gel by high-speed centrifugation (53), purified with glass milk according to the recommended protocol (Geneclean; Bio 1 Inc., Carlisle, Calif.), and digested with the restriction enzyme *Tsp*509I. The plasmid psF151 was digested with EcoRI and ligated to the *Tsp*509I-restricted fragments. The ligation mixtures (10 μg of psF151 and 0.75 μg of the targeting insert) were used to transform *S. mutans* LT1 and *S. intermedius* NCTC 11324. Transformation of *S. intermedius* by the addition of synthetic competence factor resulted in the isogenic mutant IB08981, previously described (38). *S. mutans* transformation was performed as described by Perry and Kuramitsu (37) with modifications (39). Briefly, *S. mutans* overnight culture was transferred to a fresh THB-HS medium in a 1:40 dilution and incubated at 37°C for 3 h. At this point, the psF151-targeting insert ligation mixture was added to 1 ml of the bacterial culture and incubated for an additional 2 h. The transformants were selected by plating the cells on THB-HS agar plates containing kanamycin. An isogenic mutant was randomly selected for further characterization and named IB10991. For the inactivation of the *S. mutans* antigen I/II gene at the 5'-terminal end, a similar strategy was used, except that the target insert was derived from *Sac*3AI-digested psAD7B ligated to *Bam*HI-restricted psF151, psAD7B carries the N-terminal encoding sequence of the *S. mutans* OMZ 175 antigen I/II (43). An isogenic mutant that did not react with the antibody for the antigen I/II was randomly selected for further characterization and named IB03987.

**Immunoblotting.** Bacterial pellets and supernatants were obtained from streptococcal cultures in the exponential phase. The pellets were washed twice with phosphate-buffered saline (PBS) resuspended in extraction buffer (0.05 M Tris pH 6.8; 2% sodium dodecyl sulfate [SDS], 10% glycerol). The proteins were boiled for 5 min. The culture supernatants were lyophilized and resuspended in loading buffer. Polypeptides derived from equivalent amounts of bacterial pellets and supernatants were separated by SDS-polyacrylamide gel electrophoresis (PAGE) on 7.5% gels with the discontinuous buffer system of Laemmli (17) and then electroblotted onto nitrocellulose membranes. The blots were incubated with rabbit anti-I/II immunoglobulin G raised against purified Sr (anti-SR I/II) from *S. mutans* OMZ 175. Antibody binding was revealed with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G and enzyme substrate (BCIP [5-bromo-4-chloro-3-indolylphosphate] and nitroblue tetrazolium [both from Sigma]).

**Southern blotting and sequencing.** Chromosomal DNA was digested with EcoRI, electrophoresed on 0.7% agarose gel, and transferred to a positively charged nylon membrane (Boehringer Mannheim GmbH, Mannheim, Germany). The random primed probe was used for *digoxigenin*-labeled (Boehringer Mannheim) labeling of the psF151 probe, as recommended by the manufacturer. Prehybridization and hybridization were performed with DIG Easy Hyb (Boehringer Mannheim) at 45°C. Chemiluminescent substrate was used for the detection of hybridization signals on X-ray films.

**Insertion inactivation at homologous sites in the selected isogenic mutants.** *S. mutans* IB10991 and *S. intermedius* IB08981 was confirmed by sequencing (Fig. 1). The forward primer described above for gene inactivation, 5'-GTCAGTGCGCAACAGATTTATCACA, and the reverse primer annealing to psF151, 5'-AGCGGATAACATCTTGACACGAGA, were used. The parameters for PCR amplification were the same as described above for amplification of the target insert.
The bacterial suspension were added to each well. The plates were incubated with aspirated, and 50 were blocked with 200 acetic acid. Wells coated with TBS or acetic acid were used as the respective fi.

At least two experiments, with parallels each, were conducted. Fibronectin conditions likely to be found on the tooth surface (6). Twenty-fi

Collaborative Biomedical Products, Bedford, Mass.) in a volume of 100 /H9262 Sigma), human collagen IV (2.5 /H9262 g/well; Sigma), or rat collagen I (5

The bacterial adhesion to the hydrocarbon hexadecane was measured as described by Westergren and Olsson (50). Cells at early, mid-, late, and stationary phases were washed twice with PBS and resuspended to an OD600 of 1.0. Volumes of 1.2-ml bacterial suspensions were mixed with 75, 150, or 200 μl of hexadecane. The OD in the aqueous phase was then measured (at 450 nm), and the reduction in binding to hexadecane was calculated.

Statistical analysis. Student’s t test or the paired t test was used for two group comparisons. One-way analysis of variance followed by the Student-Newman-Keuls test was used for multiple comparisons.

RESULTS

Construction of antigen I/II isogenic mutants. Disruption of the antigen I/II gene at homologous sites in the isogenic mutans S. intermedius IB08981 and S. mutans IB10991 was verified by sequencing (Fig. 1). These isogenic mutants expressed a truncated antigen I/II gene product of ca. 125 kDa in S. intermedius IB08981 and of 150 kDa in S. mutans IB10991, which was found predominantly in the supernatants (Fig. 2). This was in contrast to the wild types S. intermedius NCTC 11324 and S. mutans LT11, in which antigen I/II was found primarily in the whole-cell extracts. The antigen I/II molecular masses in S. intermedius NCTC 11324 and S. mutans LT11 were ca. 160 and 185 kDa, respectively (Fig. 2). Neither the cell extract nor the supernatant of the isogenic mutant S. mutans IB03987 reacted with anti-SR (Fig. 2). The insertion of pSF151 in the chromosome of the isogenic mutants was confirmed by Southern hybridization.

Binding to salivary film. The role of the S. mutans and S. intermedius antigen I/II in adhesion to a salivary film under flowing conditions was investigated by comparing isogenic mu.

FIG. 2. Immunoblot analysis of antigen I/II expression in the cell extract (lanes 1 to 5) and supernatant (lanes 6 to 10) of S. intermedius NCTC 11324 (lanes 1 and 6) and the isogenic mutant IB08981 (lanes 2 and 7) and of S. mutans LT11 (lanes 3 and 8) and the isogenic mutants IB0991 (lanes 4 and 9) and IB03987 (lanes 5 and 10). The positions of the molecular mass markers are indicated.

The PCR products were prepared for sequencing by purification with shrimp alkaline phosphatase and Exonuclease I (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer’s instructions. Sequencing was undertaken by using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Stockholm, Sweden), and the final products were run on the ABI Prism 377 (PE Applied Biosystems) by using 5% Long Ranger Gels (FMC Bioproducts, Rockland, Maine). The DNA sequences were determined on both strands.

Adhesion to a salivary film in a parallel plate flow chamber. Bacterial adhesion to the bottom glass plate of a parallel plate flow chamber was determined with a phase-contrast microscope coupled to a CCD-MXR camera and an image analyzer. Bacterial overnight cultures collected by centrifugation were washed with a phase-contrast microscope coupled to a CCD-MXR camera and an image analyzer.

antigen I/II expression in the cell extract (lanes 1 to 5) and supernatant (lanes 6 to 10) of S. mutans and S. intermedius isogenic mutants. The positions of the molecular mass markers are indicated.

The bacterial flow rate was adjusted to 1.4 ml/min under the in

The bacterial adhesion rate was calculated as the number of microorganisms that adhered during the first 30 min per unit of time and area. An air bubble was then passed through the chamber, and the percentage of detached cells was calculated.

Adhesion to matrix proteins. S. mutans LT11 and the isogenic mutant IB03987 were assayed for adhesion to immobilized matrix proteins according to the protocol previously described for S. intermedius NCTC 11324 and its isogenic mutant IB08981 (38). Bacterial cells were radioactively labeled by overnight growth in the presence of [methyl-3H]thymidine (6 μCi/ml, 85 Ci/mmol; Amer-

TABLE 1. Bacterial adhesion to saliva-coated glass

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean no. of attached cells/cm² (SD)</th>
<th>% Detachment (SD) after air bubble passage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial bacterial adhesion rate (s⁻¹)</td>
<td>After 4 h of flowing (10%)</td>
</tr>
<tr>
<td>S. mutans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT11</td>
<td>962 (314)</td>
<td>8.4 (1.5)</td>
</tr>
<tr>
<td>IB08981</td>
<td>103 (119)</td>
<td>0.4 (0.2)</td>
</tr>
<tr>
<td>IB03987</td>
<td>26 (12)</td>
<td>0.3 (0.2)</td>
</tr>
<tr>
<td>S. intermedius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCTC 11324</td>
<td>1,558 (536)</td>
<td>9.9 (1.0)</td>
</tr>
<tr>
<td>IB08981</td>
<td>1,370 (840)</td>
<td>11.0 (0.9)</td>
</tr>
</tbody>
</table>

’ Mean and standard deviation values were derived from triplicate experiments.

’ Significantly different from the wild type (P < 0.05) as calculated by one-way analysis of variance, followed by the Student-Newman-Keuls test.

The number of adhering cells was too low for accurate analysis (<0.15× 10⁶/cm²).

The bacterial cells were radioactively labeled by overnight growth in the presence of [methyl-3H]thymidine (6 μCi/ml, 85 Ci/mmol; Amer-

The bacterial flow rate was adjusted to 1.4 ml/min under the influence of a hydrostatic pressure at a shear rate of 15 s⁻¹, which corresponds to physiological conditions likely to be found on the tooth surface (6). Twenty-five live images were taken every 1 to 2 min for the first 30 min and thereafter at 10 to 30 min intervals for up to 4 h, when the flow was stopped. The images obtained were subtracted from the out-of-focus image, after which the number of attached cells was determined. The initial deposition rate was calculated as the number of microorganisms that adhered during the first 30 min per unit of time and area. An air bubble was then passed through the chamber, and the percentage of detached cells was calculated.

Detachable microtiter plates (MastiSorp; Nunc A/S, Roskilde, Denmark) were coated with human fibronectin (2 μg/well; Sigma), human collagen I (2.5 μg/well; Sigma), human collagen IV (2.5 μg/well; Sigma), or rat collagen I (5 μg/well; Collaborative Biomedical Products, Bedford, Mass.) in a volume of 100 μl/well. At least two experiments, with five parallels each, were conducted. Fibronectin was diluted in 0.05 M Tris-buffered saline (pH 7.5; TBS) and collagen in 0.01 M acetic acid. Wells coated with TBS or acetic acid were used as the respective controls. The plates were incubated at room temperature for 2 h. After incubation the wells were washed four times with PBS. Nonspecific protein-binding sites were blocked with 200 μl of 1% bovine serum albumin (Sigma) in PBS by incubation at room temperature for 30 min. The content of the wells was then aspirated, and 50 μl of 0.2% PBS-bovine serum albumin and 50 μl of the bacterial suspension were added to each well. The plates were incubated with gentle shaking at 37°C for 2 h. Unbound cells were removed by washing the
tants defective in production of antigen I/II with the respective wild types (Table 1). Phase-contrast microscope images of adherent cells are shown in Fig. 3. Compared to *S. mutans* LT11, the *S. mutans* IB10991 initial adhesion rate and final number of attached cells (4 h) to the salivary coated glass was reduced by 90%. No significant difference was found between the *S. mutans* isogenic mutants IB10991 and IB03987. *S. intermedius* NCTC 11324 and the isogenic mutant IB08981 adhered similarly and in significant numbers to the salivary film.

Bacterial detachment after 4 h of flowing was calculated as the percentage of detached cells by air bubble passage (Table 1). Detachment of *S. mutans* LT11 was ca. 45%, while for the isogenic mutants the remaining adherent bacteria were in too low numbers to allow accurate analysis. No significant difference in detachment was observed between *S. intermedius* NCTC 11324 and its isogenic mutant IB08981.

**Binding to immobilized matrix proteins.** The isogenic mutant IB03987 was compared to the wild-type *S. mutans* LT11 for its ability to adhere to fibronectin and collagen immobilized onto microtiter plate wells (Table 2). Adhesion of the isogenic mutant to fibronectin and rat collagen I was reduced by 19 and 65%, respectively. Neither the wild type nor the mutant exhibited significant binding to human collagen types I and IV. In *S. intermedius* IB08981, adhesion to fibronectin was reduced by
75%, whereas neither the wild type nor the mutant adhered significantly to rat collagen I or human collagen types I and IV.

**Cell surface properties associated with antigen I/II.** Inactivation of the *S. mutans* and *S. intermedius* antigen I/II gene had no significant effect on the contact angles with water (Table 3).

The effect of antigen I/II inactivation on bacterial zeta potentials differed between *S. mutans* and *S. intermedius* (Fig. 4). At physiological pH values (pH 5 and 7), *S. mutans* LT11 exhibited a more positive zeta potential than its isogenic mutants IB10991 and IB03987, whereas no difference was found between *S. intermedius* NCTC 11324 and IB08981. In *S. mutans*, disruption of the antigen I/II gene in the mutants resulted in the shift of the isoelectric point toward more acidic values than in the wild type.

The adhesion to hexadecane was calculated as the percent loss in OD relative to that of the initial bacterial suspension (Fig. 5). Maximum *S. mutans* adhesion occurred with 150 μl of hexadecane. Reduction in adhesion of the *S. mutans* isogenic mutants to hexadecane varied from 25% at early growth phase to 68% at the stationary phase. In contrast, binding of the *S. intermedius* mutant to hexadecane was reduced at the early and mid-phases of exponential growth by 18 and 4%, respectively.

**DISCUSSION**

The region C terminal to the antigen I/II proline-rich segment (26) was used as a target to disrupt the gene in *S. mutans* and *S. intermedius* at homologous sites. Disruption at this site resulted in truncated proteins, observed as faint bands on the immunoblotting of whole-cell preparations. The transformants revealed high levels of truncated proteins in the supernatants, in contrast to the wild types, which showed low levels of the native protein. This is in accordance with previous observations that the *S. mutans* antigen I/II surface anchoring region is localized at the C terminus (20, 32, 33). The known antigen I/II sequences show the conserved LPXTG C-terminal motif commonly found in other gram-positive C-terminal anchored proteins (8, 14, 35, 36, 44, 47). The respective secreted truncated proteins were ca. 35 kDa smaller than the antigen I/II expressed by *S. intermedius* and *S. mutans* wild types. This suggests that the difference in size of the *S. intermedius* and *S. mutans* native antigen I/II, which corresponds to ca. 160 and 185 kDa, respectively, is most likely due to differences upstream of the insertion site. As the present study was being finalized, the entire sequence of the *S. intermedius* antigen I/II became available (gi 14571813, deposited by H. Tamura). It reveals that the antigen I/II amino acid sequence in *S. intermedius* exhibits ca. 250 fewer residues than in *S. mutans*, confirming the size difference observed by immunoblotting. The overall degree of sequence identity is ca. 70%, with the *S. intermedius* sequence varying mostly due to fewer residues in the alamine-rich repetitive region and in the conserved proline-rich sequence.

In this study we showed that adhesion to salivary film under conditions of flow was reduced in the *S. mutans* isogenic mutant, whereas both *S. intermedius* wild-type and mutant strains adhered in significant numbers to the salivary film. The results indicate that while antigen I/II is essential for adhesion of *S. mutans* to the salivary film, adhesion of *S. intermedius* was unaffected by the inactivation of the antigen I/II gene. Antigen I/II segments C terminal of the proline-rich region and N-terminal sequences, including the alamine-rich region, have been identified as being involved in *S. mutans* binding to salivary components (14, 15, 31). Protein sequence homology search with BLAST (1) reveals that the *S. mutans* segment C terminal of the proline-rich region (amino acid residues 1025 to 1044) (14) shows homology to one of the sequenced segments in *S. intermedius* (44). Four- to five-amino-acid substitutions are, however, observed. One of the substitutions is in the E1037 residue, being K in *S. intermedius*. Site-directed mutagenesis studies have identified E1037 as one of the two residues important for *S. mutans* binding to salivary compo-

**TABLE 2. Adherence to ECM proteins**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Human fibronectin (n = 3)</th>
<th>Rat collagen I (n = 3)</th>
<th>Human collagen I (n = 2)</th>
<th>Human collagen IV (n = 2)</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. mutans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT11</td>
<td>37 (9.0)</td>
<td>21 (12.0)</td>
<td>NS</td>
<td>NS</td>
<td>This study</td>
</tr>
<tr>
<td>IB03987</td>
<td>30 (10.8)*</td>
<td>7 (14.4)*</td>
<td>NS</td>
<td>NS</td>
<td>This study</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCTC 11324</td>
<td>1.6 (0.5)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>39</td>
</tr>
<tr>
<td>IB08981</td>
<td>0.4 (0.2)*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>39</td>
</tr>
<tr>
<td>Control (S. mutans OMZ 175)</td>
<td>–</td>
<td>26 (3.0)b</td>
<td>22 (4.3)</td>
<td>16 (1.2)</td>
<td>39</td>
</tr>
</tbody>
</table>

* a*, Significantly different from the wild type (P < 0.05) as calculated by paired t-test. NS, counts not significantly different from background values. ~, Not tested.

b n = 2.

**TABLE 3. Contact angles with water (θw)****

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean θw (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. mutans</em></td>
<td></td>
</tr>
<tr>
<td>LT11</td>
<td>29 (2.7)</td>
</tr>
<tr>
<td>IB03991</td>
<td>31 (1.0)</td>
</tr>
<tr>
<td>IB03987</td>
<td>33 (1.0)</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td></td>
</tr>
<tr>
<td>NCTC 11324</td>
<td>51 (3.0)</td>
</tr>
<tr>
<td>IB08981</td>
<td>44 (10.0)</td>
</tr>
</tbody>
</table>

* The mean and standard deviation values derived from triplicate experiments are shown.
Since binding of antigen I/II requires sialic acid residues in \( S. \) gordonii, a different receptor to salivary components has been suggested. In \( S. \) mutans binding seems to occur in a sialic-independent manner (15). In \( S. \) gordonii antigen I/II, a K residue substitutes the E1037 residue, as in \( S. \) intermedius. It has been suggested that such a substitution could affect the recognition of salivary receptors by this domain (15). It is also possible that the difference in size of the alanine-rich sequence in \( S. \) intermedius, compared to \( S. \) mutans, may have an effect on the capacity of the \( S. \) intermedius antigen I/II to recognize salivary receptors. A definitive conclusion as to the difference in the role of the antigen I/II observed between \( S. \) mutans and \( S. \) intermedius in binding to salivary components requires, however, further investigation.

Bacterial surface structures associated with adhesion under static conditions may not play a similar role in adhesion under flowing conditions (7, 41). Factors such as shear forces and physical collisions between suspended and adhering microorganisms are part of the dynamic conditions present in a flowing system. In the oral cavity, bacterial adhesion occurs under salivary flowing conditions. The \( S. \) mutans antigen I/II binding to the salivary film under flowing conditions was in accordance with static binding assays showing adhesion of \( S. \) mutans to salivary components by antigen I/II (16, 19). The two \( S. \) mutans isogenic mutants exhibited more than 90% reduction in the initial adhesion rate to the salivary film. This difference was still observed after 4 h of flow. There was no difference between the \( S. \) mutans isogenic mutants IB10991 and IB03987. Accordingly, inactivation of the gene at any site will result in loss of the anchoring motif and release of the truncated protein.

In the oral cavity, the microorganisms are exposed to high detachment forces as, for instance, during eating and swallowing. During such events, air-liquid interfaces frequently pass over the salivary conditioned enamel surface. In our study, the percentage of detached cells after the passage of an air bubble was similar for both the \( S. \) intermedius wild type and the mutant strain, suggesting that the wild type and the isogenic mutant present similar binding affinities to the salivary film. The low number of adhering \( S. \) mutans isogenic mutants after air bubble passage restrained comparison of detachment values in the isogenic mutants and wild type.

Tissue damage may expose ECM macromolecules and allow bacterial adhesion. Streptococcal adhesion to fibronectin is purported, for instance, as a pivotal step in the pathogenesis of endocarditis (2, 52). ECM proteins may expose different microbial binding domains when they are in soluble or immobi-

**FIG. 4.** Effect of antigen I/II inactivation on bacterial zeta potentials measured at various pH values. The means and standard errors (bars) of triplicate assays are shown.
Bacterial hydrophobicity and surface charge are postulated as driving forces for the initial adhesion of microorganisms. These properties may be influenced by the presence of specific cell surface receptor sites (3). Adhesion to hydrocarbons and water contact angles are currently used as measurements of bacterial hydrophobicity. Accordingly, the antigen I/II is related to binding to hexadecane at the stationary phase. Our results indicate therefore that disruption of the antigen I/II affected the S. mutans surface charge rather than the bacterial hydrophobicity.

The inactivation of the antigen I/II-encoding gene provided evidence for the species-specific role of the antigen I/II protein in the ability of S. mutans to bind to a salivary film under flowing conditions, and to influence S. mutans surface charge, compared to S. intermedius. On the other hand, the antigen I/II was associated with binding of both S. mutans and S. intermedius to fibronectin. There has been considerable interest in developing strategies to interfere with S. mutans adhesion by targeting the antigen I/II protein. Since most oral streptococci express proteins belonging to the antigen I/II family, identification of common, as well as species-specific, antigen I/II functions might have future clinical implications.

ACKNOWLEDGMENTS

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FIG. 5. Hydrophobicity of S. mutans LT11 and the isogenic mutants IB10991 and IB03987 (A) and of S. intermedius NCTC 11324 and the isogenic mutant IB08981 (B) during early (EP), mid- (MP), late (LP), and stationary (SP) phases. Hydrophobicity was calculated as the percent adhesion to hexadecane. Error bars indicate the standard errors of triplicate samples. *, Significantly different from the wild type (P < 0.05), as calculated by one-way analysis of variance followed by the Student-Newman-Keuls test (A) or Student’s t test (B).

lized forms (22). Binding of antigen I/II derived polypeptides to soluble fibronectin has previously been reported (43). In this study we show that the S. mutans, as well as the S. intermedius antigen I/II were associated with binding also to immobilized human fibronectin.

Streptococcal adhesion to collagen type I may be a critical factor for invasion of dentinal tubules during dental caries. The antigen I/II produced by S. mutans and S. gordonii are related to both tubule invasion and binding to collagen type I (24). We found that the antigen I/II in S. mutans LT-11 was associated with binding to rat collagen type I, but not to human collagen I. The results stress the importance of the matrix protein source used, since the structure of human ECM proteins may differ from those of other animals (22). Adhesion of S. mutans and S. intermedius to human collagen types I and IV was feeble for both the wild type and the mutants. In contrast to S. mutans, the S. intermedius antigen I/II is not associated with binding to rat collagen type I (38).

Bacterial hydrophobicity and surface charge are postulated as driving forces for the initial adhesion of microorganisms. These properties may be influenced by the presence of specific cell surface receptor sites (3). Adhesion to hydrocarbons and water contact angles are currently used as measurements of bacterial hydrophobicity. The role of the antigen I/II in binding to the hydrocarbon hexadecane varied among S. mutans and S. intermedius. Disruption of the antigen I/II in S. mutans resulted in mutants with progressively reduced adhesion to hexadecane with growth. In S. intermedius, the mutant bound less to the hexadecane at initial growth phases, compared to the wild type, whereas no significant difference was observed between the wild type and its mutant at late log or stationary phase. Our results confirmed previous reports that the S. mutans antigen I/II is related to binding to hexadecane at the stationary phase (11, 27, 36). Measurement of hydrophobicity based on adhe-

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ton forms surface fibrils that confer hydrophobic and