Association of Fimbriae with the Hydrophobicity of Streptococcus sanguis FC-1 and Adherence to Salivary Pellicles

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A nonhydrophobic mutant of Streptococcus sanguis FC-1 was isolated which has a greatly diminished capacity for attaching to experimental salivary pellicles on hydroxyapatite surfaces and for aggregating with salivary components. The mutant appears to be defective in the synthesis of fimbriae, as judged by electron microscopic observations and by its inability to exhibit twitching motility.

Hydrophobic forces have been suggested to be involved in the adherence of a variety of bacteria to host tissues (8, 11, 15, 17). Recently, Nesbitt and co-workers (10) reported that strains of Streptococcus sanguis have hydrophobic surface properties as judged from their ability to adsorb to hydrocarbons such as hexadecane. They also showed that agents which disrupt hydrophobic bonds inhibit the attachment of S. sanguis cells to saliva-treated hydroxyapatite (S-HA) surfaces which mimic those of the teeth. They suggested, therefore, that the adherence of S. sanguis cells to experimental salivary pellicles was at least partially dependent upon the formation of hydrophobic bonds between the streptococci and the adsorbed salivary proteins comprising the pellicle. We now report the isolation a nonhydrophobic mutant of S. sanguis FC-1 which is defective in the synthesis of surface fimbriae and which exhibits a greatly diminished capacity for attaching to experimental pellicles.

S. sanguis FC-1 was originally isolated from a sample of human dental plaque. Cultures of the organism were stored in 50% glycerol at −20°C. The streptococci were propagated in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) and on Trypticase soy agar plates containing 5% sheep blood (Scott Laboratories, Fiskeville, R.I.). A nonhydrophobic mutant of strain FC-1 was selected by procedures described previously by Rosenberg and Rosenberg (16). Briefly, cells of S. sanguis FC-1 were harvested from overnight Todd-Hewitt broth cultures. The organisms were suspended in 2.0 ml of sterile PUM buffer which contained (per liter): K₂HPO₄, 3H₂O, 22.2 g; KH₂PO₄, 7.3 g; urea, 1.8 g; and MgSO₄, 7H₂O, 0.2 g. Sterile hexadecane (1 ml) was added, and the suspension was mixed on a Vortex mixer for 1 min at room temperature. Strongly hydrophobic organisms attach to hexadecane droplets, whereas less hydrophobic cells remain in the aqueous phase. Samples of the lower aqueous phase were then removed and inoculated into fresh tubes of Todd-Hewitt broth. This enrichment procedure was repeated six times. Samples of the enriched Todd-Hewitt broth culture were then spread on Trypticase soy agar (BBL) plates, and after incubation, approximately 50 colonies were picked and transferred as spots onto fresh plates. Colonies of nonhydrophobic mutants were detected by their inability to adhere to polystyrene petri dishes as described previously by Rosenberg (12).

S. sanguis nhm was isolated in this manner. It formed firm hard colonies on mitis salivarius agar which were indistinguishable from those of parental strain FC-1. In addition, strain nhm had fermentation characteristics similar to those of the parent strain FC-1, and both formed alcohol-precipitable polysaccharide when grown in 5% sucrose broth. The relative hydrophobicity of strains FC-1 and nhm was determined by comparing their ability to adsorb to hexadecane as previously described (14). The parent strain FC-1 adsorbed avidly to hexadecane, whereas only a small percentage of nhm cells attached to this hydrocarbon (Fig. 1). Thus, the mutant strain nhm is much less hydrophobic than the parent strain FC-1. The mutant strain nhm also attached in much lower numbers to S-HA surfaces than did strain FC-1 when assayed as previously described (1, 2) (Table 1). Nesbitt et al. (10) previously showed that hydrophobic bond-disrupting agents such as NaSCN strongly inhibited the adsorption of S. sanguis to S-HA surfaces whereas equal molar solutions of NaCl had little effect. Attachment of parental strain FC-1 to S-HA was also found to be strongly inhibited by 0.1 or 0.5 M NaSCN, whereas the weak adher-
The fibrils observed appear to be comparable to the fimbrae described previously on *S. sanguis* cells which are associated with "twitching motility" (6). Therefore, to substantiate the lack of fimbrae on mutant nmh cells, colonies of strain nmh and its parent were examined for twitching motility as previously described (6). The organisms were streaked on heart infusion blood agar plates which were incubated in Brewer jars containing a dish of water to provide a moist atmosphere. Under these conditions, cells which possess polar fimbrae tend to collect at the water-air interface and spread to give rise to irregular fried egg-shaped colonies, whereas nonfimbriated cells do not (5, 6). Colonies of parental strain FC-1 were large and exhibited a fried egg appearance after 5 to 8 days typical of organisms exhibiting twitching motility (Fig. 3A). In contrast, colonies of mutant strain nmh were smaller and much more regular (Fig. 3B). Thus, the apparent absence of twitching motility in mutant strain nmh and also the electron microscopic observations indicate that the defect in nmh cells results in an impaired synthesis of fimbrae. These findings are comparable with those of earlier studies which reported nonhydrophobic mutants of *Acinetobacter calcoaceticus* also have an impaired synthesis of thin fimbrae (13).

If the adherence of *S. sanguis* to S-HA is dependent upon specific interactions between the fimbrae of the organism and immobilized salivary macromolecules in the pellicle which serve as specific receptors, one would expect that there would be fewer potential binding sites present in experimental pellicles for mutant strain nmh. Therefore, we carried out adsorption isotherms to estimate the numbers of binding sites and the affinities of strain FC-1 and its mutant on S-HA surfaces as previously described (1, 2). The streptococcal concentrations used for the isotherms ranged between 1 \times 10^7 and 100 \times 10^7 cells per ml. Analyses of the isotherms indicated that the experimental pellicles contained much lower numbers of binding sites for the mutant strain nmh than for the parental strain FC-1.

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**TABLE 1.** Attachment of *S. sanguis* FC-1 and mutant strain nmh to S-HA in the presence or absence of thiocyanate

<table>
<thead>
<tr>
<th>Addition to reaction mixture</th>
<th><em>S. sanguis</em> FC-1</th>
<th><em>S. sanguis</em> nmh</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cells (\pm) SE ((\times 10^7)) attached per 5 mg of S-HA*</td>
<td>% of control</td>
<td>No. of cells (\pm) SE ((\times 10^7)) attached per 5 mg of S-HA*</td>
</tr>
<tr>
<td>None</td>
<td>94.1 ± 3.3</td>
<td>100</td>
</tr>
<tr>
<td>0.1 M NaSCN</td>
<td>18.8 ± 1.3</td>
<td>20</td>
</tr>
<tr>
<td>0.5 M NaSCN</td>
<td>2.0 ± 0.2</td>
<td>2</td>
</tr>
</tbody>
</table>

* Reaction mixtures (125 \(\mu\)l) contained 5 mg of S-HA and 2.5 \(\times\) 10\(^7\) \[^{3}H\]thymidine-labeled streptococci in 0.05 M KCl containing 1 mM PO\(_4\) (pH 6.0), 1 mM CaCl\(_2\), and 0.1 mM MgCl\(_2\).
sites for mutant strain nhm than for parental strain FC-1 (Table 2). Also, the sites available for strain nhm were of lower affinity. The data obtained support the suggestion of Nesbitt et al. (10) that hydrophobic interactions play an important role in the adherence of S. sanguis cells to salivary pellicles similar to those on teeth. Nonhydrophobic mutant strain nhm

FIG. 2. Electron micrographs of molybdenum-stained cells of S. sanguis FC-1 (A) and mutant strain nhm (B). Note the presence of short, thin fibrils (fimbriae) on the surface of FC-1 cells which are absent on mutant strain nhm cells.

FIG. 3. Colonies of S. sanguis FC-1 (A) and mutant strain nhm (B) developing on blood agar plates incubated under a moist atmosphere for 5 days. Note the spreading tendency of FC-1 colonies due to twitching motility, and the lack of spreading of nhm colonies.
TABLE 2. Parameters for adsorption of S. sanguis FC-1 and mutant strain nhm to S-HA

<table>
<thead>
<tr>
<th>Strain</th>
<th>Correlation coefficient</th>
<th>No. of binding sites ± SE (x10^6)</th>
<th>Affinity (ml/cell) ± SE</th>
<th>S-HA (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC-1</td>
<td>0.95</td>
<td>35.0 ± 1.6</td>
<td>21.2 ± 1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>nhm</td>
<td>0.86</td>
<td>1.7 ± 0.2</td>
<td>12.3 ± 1.7</td>
<td>1.0</td>
</tr>
</tbody>
</table>

exhibited greatly diminished surface hydrophobicity as judged from its weak adherence to hexadecane, and it had a greatly impaired capacity for attaching to S-HA surfaces; it also no longer aggregated with components present in clarified whole human saliva. These observations support the notion that there is a relationship between salivary aggregating factors and adherence of S. sanguis cells to S-HA (3). The nature of the defect in nhm cells appears to be an impaired synthesis of fimbriae, as determined by electron microscopic observations of negatively-stained preparations and also by the loss of twitching motility. The data, therefore, suggest that the fimbriae of S. sanguis cells are largely responsible for its hydrophobic properties and also for its adsorption to specific salivary receptors in experimental pellicles on hydroxyapatite surfaces. The latter observation is consistent with the report of Fives-Taylor (1a), who previously observed that certain nonadherent mutants of S. sanguis exhibited defects in fimbriation. It seems likely that these fimbriae also contain the protein adhesin (7) or sialic-binding lectin (9) (or both) previously described for this organism.

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