Effect of Polyclonal and Monoclonal Antibodies on Surface Properties of *Streptococcus sobrinus*

M. VAN RAAMSDONK,1 H. C. VAN DER MEI,2 J. J. DE SOET,1 H. J. BUSSCHER,2 AND J. DE GRAAFF1*

Department of Oral Microbiology, Academic Centre for Dentistry Amsterdam, 1081 BT Amsterdam,1 and Laboratory for Materia Technica, University of Groningen, 9712 KZ Groningen,2 The Netherlands

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In this study, the effect of antibody adsorption on physicochemical properties of *Streptococcus sobrinus* was studied. Bacteria were preincubated with polyclonal antibodies or with OMVU10, a monoclonal antibody (MAb) reactive with *S. sobrinus*. The zeta potentials and the hydrophobicity as determined by microbial adhesion to hydrocarbons were measured in potassium phosphate buffer with a pH ranging from 2 to 9. *S. sobrinus* preincubated with polyclonal antibodies was positively charged at pH 2, 3, and 4 and had an isoelectric point at pH 4.8. Untreated *S. sobrinus* cells or cells preincubated with MAbs were negatively charged over the whole pH range. X-ray photoelectron spectroscopy showed a decrease in O/C and P/C ratios for bacteria preincubated with polyclonal antibodies. A combination of the pH-dependent zeta potential and the X-ray photoelectron spectroscopy data of the overall chemical composition of the cell surface suggests that polyclonal antibody adsorption occurs through blocking of surface phosphate. The measurement of hydrophobicity by microbial adhesion to hydrocarbons revealed that *S. sobrinus* preincubated with polyclonal antibodies was hydrophobic (90% of the bacteria bound to hexadecane), whereas the controls were relatively hydrophilic. *S. sobrinus* preincubated with OMVU10 was found to be more hydrophobic than the controls at pH 5 and 7. Hydrophobicity as measured by water contact angles showed an increase in hydrophobicity when *S. sobrinus* was preincubated with polyclonal antibodies. The epitopes to which the antibodies are directed were visualized by immunogold labeling and electron microscopy. The results suggested that OMVU10 is reactive with only a few epitopes of the cell surface, whereas polyclonal antibodies were found to be reactive with many epitopes. In conclusion, adsorption of polyclonal antibodies was found to influence the overall physicochemical surface properties of the organism, probably by forming a coating over the whole cell surface. Adsorption of MAbs was more localized, which could explain their lesser influence on these surface properties.

Surface properties such as charge, surface free energy, and hydrophobicity play an important role in the nonspecific adhesion of microorganisms, which occurs predominantly in the initial adhesion phase (4). Nonspecific interactions originate from the entire bacterium, are established immediately upon approach of the interacting surfaces, and operate over considerable distances. Adhesion through nonspecific interactions does not yield immobilization of the bacterium. In the second phase of adhesion, specific interactions mediated by stereochemical structures on the interacting surfaces are thought to occur, resulting in the immobilization of the adhering bacteria (8, 12). Therefore, microbial adhesion is considered to be the result of both nonspecific and specific interactions (3). Adhesion to the tooth surface is an important step in the pathogenesis of dental caries. Therefore, intervention in the colonization of mutans streptococci can lead to prevention of caries. Several studies describe the use of specific antibodies, both polyclonal antibodies and monoclonal antibodies (MAbs), against mutans streptococci to prevent colonization and dental caries (9, 16, 17, 19, 20). We found recently that local application of MAbs reactive with antigen B reduced the colonization of rats by *Streptococcus sobrinus*. Although the results of these studies suggest that colonization and caries scores can be reduced after passive immunization, the mechanisms of this influence are not known. Hydrophobicity and zeta potentials are important features in the adhesion (1, 21). Both adhesion to tooth surfaces and adhesion to other bacteria or cells of the host, i.e., epithelial cells or polymorphonuclear leukocytes, are generally considered to be influenced by hydrophobicity and zeta potentials as a corollary of the surface chemistry of the bacteria. The effect of antibodies on these properties is unknown for *S. sobrinus*. When these properties are influenced, they can affect the adhesion of *S. sobrinus*.

The aim of this study was to determine the effect of polyclonal antibodies and MAbs on the physicochemical surface properties of *S. sobrinus* by determining the zeta potential (which is pH dependent), the hydrophobicity by a pH-dependent test of microbial adhesion to hydrocarbons (MATH), and contact-angle measurements and the elemental surface composition by X-ray photoelectron spectroscopy (XPS). By immunogold labeling and transmission electron microscopy, the epitopes to which the antibodies are directed were visualized.

**MATERIALS AND METHODS**

**Polyclonal antibodies and MAbs.** For the production of polyclonal antiserum, whole cells of *S. sobrinus* HGO77 were used. Increasing amounts of the cell suspensions were injected intravenously into a male rabbit (Chinchilla; Harlan CPB, Zeist, The Netherlands) with intervals of 2 or 3 days. Prior to immunization, preimmune serum (PI), which served as a control, was collected. After 4 weeks the rabbit was bled by cardiac puncture. Serum was separated from the blood cells by centrifugation for 20 min at 3,300 × g and stored at −80°C. The MAbs used in this study were OMVU10 (immunoglobulin G2b) and clone 24 (immunoglobulin G2b). OMVU10 is reactive with purified antigen B of *Streptococcus downei* and is specific for *S. sobrinus* and *S. downei* (6, 7). Clone 24, which is specific for lipid A of *Escherichia coli* (14), was used as a control. Clone 24 does not react with *S. sobrinus*. The MAbs were prepared as described previously (26) by means of a continuous-dialysis-tubing growth system in culture medium (1 × RPMI 1640 medium; Flow Laboratories, Irvine, Scotland) supple-
mented with 10% fetal calf serum (Flow Laboratories), penicillin-streptomycin solution (50 IU of penicillin per ml, 50 µg of streptomycin per ml; Flow Laboratories), and 2 mM glutamine (Flow Laboratories). Subsequently, the MAbs were dialyzed extensively against phosphate-buffered saline (PBS) for 24 h and stored for further use at −20°C.

The concentration of antibodies needed to provide an excess of antibodies in the assays was determined. The change in antibody titer of the different antibody dilutions before and after incubation with bacteria was determined. A culture grown for 16 h was incubated with dilutions of antibodies of 1:100, 1:500, 1:1,000, 1:5,000, and 1:10,000 in a final concentration for 1 h at 37°C. Antibody titers were determined by an enzyme-linked immunosorbent assay (ELISA) as described by de Soet et al. (6). In short, ELISA plates (Greiner, Frickenhausen, Germany) were coated with *S. sobrinus* HG977. The antibodies, before and after incubation with bacteria, were titrated in twofold steps. As secondary antibodies, goat anti-mouse or goat anti-rabbit (horseradish peroxidase-conjugated; American Qualex, La Mirada, Calif.) antibodies were used; staining with α-phenylenediamine followed. The titer of the antibodies was defined as the dilution at which the optical density at 490 nm (OD490) of the ELISA is twice the OD490 of the negative control.

**Bacteria and growth conditions.** *S. sobrinus* HG977 was grown in Todd-Hewitt broth at 37°C anaerobically (80% N₂, 10% H₂, 10% CO₂). After 16 h of growth, the late-log-phase cultures were incubated with polyclonal antibodies, PI, OMVU10, or clone 24 for 1 h at 37°C, if appropriate. All antibodies were added in a final concentration of 1:500. The bacterial cells were harvested by centrifugation at 7,000 × g, washed twice with demineralized water, and sonicated, to get a single-cell suspension, for 30 s at 70 W (Labsonic 1510; Braun, Melsungen, Germany).

**Electron microscopy.** *S. sobrinus* was grown and preincubated with antibodies as described above. Subsequently, the bacteria were harvested by centrifugation at 7,000 × g, washed twice in PBS, and diluted to an OD₆₅₀ (path length, 1 cm) of 1.0. The bacteria were applied to Formvar-coated grids and incubated with colloidal gold-conjugated goat anti-rabbit polyclonal goldserum (particle size, 6 nm; Aurion, Wageningen, The Netherlands) when preincubated with polyclonal antibodies or PI. When the bacteria were preincubated with OMVU10 or clone 24, colloidal gold-conjugated goat anti-mouse immunoglobulin G (particle size, 6 nm) was used. After 30 min of incubation, the grids were washed with PBS (supplemented with 0.15 M glycine), rinsed with water, and examined with an electron microscope (model EM 301; Philips, Eindhoven, The Netherlands).

**Measurement of zeta potential, hydrophobicity, and elemental surface composition.**

(i) Zeta potential. The zeta potential was measured in the absence and presence of antibodies adsorbed to the bacterial cell surface in 10 mM KP, with pH values ranging from 2 to 9. The bacteria were preincubated as described above. The bacteria were then washed, sonicated, and suspended at an OD₆₅₀ of 0.5, which corresponds to approximately 10⁶ CFU/ml. Zeta potentials were measured in triplicate with separately grown bacterial cultures as described by van der Mei et al. (24).

(ii) Hydrophobicity by MATH test. The adhesion to hexadecane of *S. sobrinus* HG977 was measured in the absence and presence of antibodies adsorbed to the bacterial cell surface in 10 mM KP, with pH values ranging from 2 to 9 as described by van der Mei et al. (22). After preincubation of the bacteria with antibodies, the bacteria were diluted to an OD₆₅₀ of 0.5 in KP. To 3 ml of bacterial suspension, 150 µl of hexadecane (Sigma Chemical Co., St. Louis, Mo.) was added, and the suspension was vortexed for 30 s. The vortexing was stopped for 5 s, and the suspension was vortexed again for 30 s. The aqueous phase and hexadecane phase were allowed to separate for 10 min, after which the aqueous phase was removed. The OD₆₅₀ of the aqueous phase was measured and compared with that of an untreated sample to calculate the percentage of bacteria bound to hexadecane. Hydrophobicity was thus measured four times with separately grown bacterial cultures.

(iii) Hydrophobicity by contact angles. Microbial cell surfaces were characterized before and after antibody adsorption. After incubation with antibodies, the bacterial cells were harvested by centrifugation at 7,000 × g and washed twice with demineralized water. Bacterial laws were then prepared on membrane filters (pore size, 0.45 µm; Millipore, Cork, Ireland) and dried for 30 min at 25°C as described by Busscher et al. (5). Subsequently, plateau contact angles were measured with sessile droplets of water as described by van der Mei et al. (25). Two contact angles were measured on one bacterial lawn, whereas two laws were made out of separately grown bacterial cultures.

(iv) XPS. XPS was performed as described by van der Mei et al. (23). In short, freeze-dried bacteria preincubated with antibodies or untreated bacteria were placed into a spectrometer (S-Probe; Surface Science Instruments, East Sussex, England). A magnesium anode was used for X-ray production (10 kV, 22 mA) of a spot size of 250 by 1,000 µm. After scans of the overall spectrum in the binding energy range of 1 to 1.200 eV at low resolution (150 eV), peaks over a 20-eV binding energy range were recorded at high resolution (50 eV) in the order C₁s, O₁s, P₂p, and again C₁s to account for contamination or deterioration of the samples under X-rays. The area under each peak was used to calculate peak intensities, yielding elemental surface concentration ratios for oxygen/carbon, nitrogen/carbon, and phosphorus/carbon after correction for instrument sensitivity factors as supplied by the manufacturer. XPS results were obtained from duplicate runs with separately grown cells.

**RESULTS**

After incubation of the bacteria with antibodies, the titers were determined by an ELISA. The titers of antibody dilutions of 1:100 to 1:1,000 did not change compared with those of the antibody dilutions before incubation. The titers of antibody dilutions of 1:5,000 and 1:10,000 were lower after incubation with bacteria than before (data not shown). Therefore, we concluded that an antibody dilution of 1:500 provided an excess of antibodies. This antibody dilution was used in all assays.

Figure 1 shows the result of the immunogold labeling. OMVU10 and polyclonal antibodies were reactive with

![FIG. 1. Whole-mount immunoelectron microscopy of *S. sobrinus* preincubated with polyclonal antibodies (A) or MAb OMVU10 (B). Antibody binding was detected with colloidal gold-conjugated antibodies. Magnifications, ×40,000 (A) and ×52,000 (B).](image)
epitopes of the fuzzy coat, although polyclonal antibodies were found to be reactive with a larger number of epitopes than OMVU10.

The effect of preincubation of *S. sobrinus* with polyclonal antibodies and MAbs on the zeta potential is shown in Fig. 2. At pH 2, 3, and 4, bacteria preincubated with polyclonal antibodies were found to be positively charged, whereas the controls were negatively charged (Fig. 2A). Bacteria preincubated with polyclonal antibodies had an isoelectric point at pH 4.8. No differences were found between the zeta potentials of OMVU10- and clone 24-preincubated bacteria (Fig. 2B).

In Fig. 3, the effect of the antibodies on adhesion to hexadecane is shown. At pH 2 and 3, untreated bacteria and bacteria preincubated with antibodies or controls were very hydrophobic, with binding to hexadecane ranging between 84 and 96%. From pH 4 to 9, *S. sobrinus* preincubated with polyclonal antibodies remained hydrophobic, whereas the controls were hydrophilic (Fig. 3A). Marginally significant differences in hydrophobicity were found at pH 5 and 7, when *S. sobrinus* preincubated with OMVU10 was compared with bacteria preincubated with clone 24 (Fig. 3B; *P* < 0.03 and *P* < 0.09, respectively, by analysis of variance).

In Table 1, the results of the water contact angle measurements and XPS are shown. Preincubation of *S. sobrinus* with polyclonal antiserum resulted in an increase in the water contact angle compared with that of untreated bacteria. Bacteria preincubated with OMVU10, clone 24, or PI had similar water contact angles.

![Figure 2](https://example.com/fig2.png)

![Figure 3](https://example.com/fig3.png)

**Table 1.** Hydrophobicity measured by water contact angles (θ<sub>WCA</sub>) and elemental surface composition ratios determined by XPS of *S. sobrinus* alone and *S. sobrinus* preincubated with antibodies

<table>
<thead>
<tr>
<th><em>S. sobrinus</em> preincubated with:</th>
<th>θ&lt;sub&gt;WCA&lt;/sub&gt; (degrees)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N/C</td>
<td>O/C</td>
</tr>
<tr>
<td>—</td>
<td>30 ± 6</td>
<td>0.090</td>
</tr>
<tr>
<td>Polyclonal antibodies</td>
<td>88 ± 12</td>
<td>0.095</td>
</tr>
<tr>
<td>PI</td>
<td>33 ± 8</td>
<td>0.083</td>
</tr>
<tr>
<td>OMVU10</td>
<td>32 ± 8</td>
<td>0.087</td>
</tr>
<tr>
<td>Clone 24</td>
<td>32 ± 7</td>
<td>0.083</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results of duplicate water contact angles on lawns of two separately grown bacterial cultures. Values are means ± standard deviations.

<sup>b</sup> Results from duplicate runs with two separately grown bacterial cultures, coinciding within 20% on average.

<sup>c</sup> No antibodies.
contact angles. Preincubation of S. sobrinus with polyclonal antiserum resulted in a decrease in the O/C and P/C ratios compared with those of untreated bacteria but did not influence the N/C ratio. Untreated bacteria or bacteria preincubated with OMVU10, clone 24, or P1 had similar N/C, O/C, and P/C ratios.

**DISCUSSION**

Passive immunization with specific MAbs against antigen B has led to reductions in the colonization of mutants streptococci and in caries scores (16, 17, 26). These results are also found after passive immunization with polyclonal antibodies (9, 19, 20). However, the mechanism of influence of these antibodies after passive immunization is not known. It has been suggested that overall physicochemical properties such as hydrophobicity and charge of the bacterial cell surface influence the deposition kinetics of bacteria during the first step of adhesion. Also, zeta potentials and hydrophobicity are thought to play a role in the phagocytosis of bacteria by polymophonuclear leukocytes (1). Therefore, we studied the effect of antibodies on the zeta potential, hydrophobicity, and chemical surface composition of S. sobrinus.

The measurement of the zeta potential reveals information about the nature and number of ionogenic groups on the bacterial cell surfaces (22). By use of buffers with different pH values, the nature of the ionogenic groups can be studied in detail. Zeta potentials were measured in this pH range and not only at salivary and plaque pH values to obtain a sort of titration profile of the bacterial cell surface, yielding indications of the chemistry of the cell surface (24). In particular, the observations that the untreated and OMVU10-preincubated cells remain negatively charged over the entire pH range and that their O/C and P/C elemental surface concentration ratios are relatively high compared with those of bacteria preincubated with polyclonal antibodies suggest the presence of high numbers of phosphate groups. Since preincubation of S. sobrinus with polyclonal antibodies yields a positive zeta potential at low pH values and decreases in O/C and P/C ratios, it is suggested that polyclonal antibody adsorption occurs through blocking of surface phosphate groups.

On the basis of water contact angles, untreated bacteria and bacteria preincubated with OMVU10 can be classified as hydrophilic, and bacteria preincubated with polyclonal antibodies can be classified as hydrophobic. This is in line with the results of the MATH test when carried out at the higher pH values. The MATH test was also done over a wide pH range to determine whether an involvement of electrostatic interactions existed for the bacteria studied presently, as was observed previously for various other strains (10, 23). At low pH values, all bacteria bind to hexadecane, presumably as a result of electrostatic attraction between the slightly positively charged hexadecane at these pH values (10) and the negatively charged bacteria. The intrinsic hydrophobicity of S. sobrinus preincubated with polyclonal antibodies, as indicated by the water contact angles, is very high. Therefore, electrostatic interactions do not influence the adhesion to hexadecane, resulting in a high percentage of adhesion to hexadecane throughout the pH range tested.

Fibrillar structures on the cell surface are thought to play a role in the adhesion to mucosa, teeth, and other sites of oral bacteria (11). For mutants streptococci, these structures are associated with a fuzzy coat around the bacterial cell (2, 15). Antigen B, a surface protein which is covalently linked to the cell wall, is associated with the fuzzy coat and contributes to the hydrophobicity of these bacteria (2). Loss of antigen B, due to subcellular or induction of mutants, results in a decrease in hydrophobicity (13, 15, 18, 27). A change in hydrophobicity after preincubation of S. sobrinus with OMVU10 could be expected since this MAb is reactive with antigen B. However, a change in hydrophobicity was not found for OMVU10. The electron microscopy studies showed that after preincubation with OMVU10, fewer immunoglobulins were present on the cell surface than there were after preincubation with polyclonal antibodies. Since all experiments were performed with an excess of antibodies, this can explain the differences found in zeta potential, hydrophobicity, and XPS measurements.

Although it is difficult to associate physicochemical surface properties of bacterial cells with adhesion to the tooth surface, it was suggested by van der Mei et al. (22) that the more negatively charged bacteria adhere in lower numbers than the less negatively charged bacteria do. We found a change in zeta potential at certain pH values after preincubation of S. sobrinus with polyclonal antibodies, which can play a role in adhesion and phagocytosis. Possibly, the bacteria preincubated with polyclonal antibodies are more readily phagocytosed. Also, a change in hydrophobicity can play a role in adhesion and phagocytosis. Opsonization with immunoglobulin G leads to an increased hydrophobicity of the bacterial surface and causes increased phagocytic ingestion in vitro (1). Therefore, the increase in hydrophobicity after preincubation of S. sobrinus with polyclonal antibodies suggests that if an increased hydrophobicity plays a role in the recognition by polymophonuclear leukocytes and adhesion to the tooth surface, polyclonal antibodies may contribute to this phenomenon.

The data of the present study demonstrate that specific antibodies to antigen B of S. sobrinus do influence physicochemical surface properties of S. sobrinus to an extent which makes it likely that this action constitutes one of the possible mechanisms by which antibodies intervene in the formation of dental plaque.

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**REFERENCES**


