Mechanism of Adherence of Streptococcus mutans to Smooth Surfaces

I. Roles of Insoluble Dextran-Levan Synthetase Enzymes and Cell Wall Polysaccharide Antigen in Plaque Formation

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The mechanism of adherence of Streptococcus mutans to smooth glass surfaces has been studied. The results with both viable and heat-killed cells showed that the process required (i) the synthesis of a water-insoluble dextran-Levan polymer by cell-bound enzymes and (ii) the participation of a binding site on the surface of the S. mutans cell. Synthesis of the polymer from sucrose in the presence of the cells was required for adherence, and indicates that an "active" form of the polymer was required. Polymer synthesized by cell-free S. mutans enzymes when added to S. mutans cells did not produce adherence. Purified antibody globulin, specific for the a-d site in the polysaccharide S. mutans group a antigen, completely inhibited adherence. Antibody to the second antigen present in the polysaccharide molecule, the a antigen, did not inhibit adherence. The evidence indicates that adherence did not require an antigenic binding site which might be common to all S. mutans strains. The orientation of the synthetase enzyme(s), antigenic binding site, and dextran-Levan polymer on the cell surface is under study.

The synthesis of high-molecular-weight water-insoluble dextran and Levan polymers from sucrose by cariogenic strains of Streptococcus mutans is considered to be primarily responsible for the ability of these microorganisms to colonize and develop plaques on the smooth surfaces of teeth (5, 9, 10, 18; cf. reviews 12, 21). The cohesive character of these insoluble polymers in plaque very likely contributes significantly to the adherence of the cells. The in vitro adherence of growing cells of S. mutans to smooth wire (13) and glass (19) surfaces has been demonstrated. Whole-cell antisera to S. mutans have been shown to inhibit extracellular enzymes present in culture fluids which synthesize ethanol-insoluble polymers from sucrose (4). The adherence of S. mutans to a glass surface is inhibited by whole cell serum (19).

The mechanism of adherence under these conditions has not been described. The nature of a binding site which may hold the insoluble polymer to the surface of the streptococcal cell is of great interest. Various types of surface polymer structures might function in such a capacity. Only recently has knowledge become available on the nature of such surface polymers. The chemical nature and immunological specificity of the principal surface antigen of the group a and b S. mutans cells have been described (15, 16). These are polysaccharides which possess the a or b serological specificity. The a antigen molecule, in addition to the a antigen, also carries an antigenic specificity which is shared by group d strains. This antigen has been termed the a-d antigen (15).

In this report we show that a surface polysaccharide antigen of the S. mutans function is a binding site for the synthesis of a dextran-Levan polymer by cell-bound enzymes and adherence of the cell to a smooth glass surface. Evidence for an "active" form of the polymer is presented.

MATERIALS AND METHODS

Cultures. Representative strains of various serological groups of streptococci were obtained from our collection, S. mitis and S. sanguis were received from the American Type Culture Collection, and the S. mutans strains were received from sources previously indicated (16). All of the results to be reported concerning the mechanism of adherence were performed with S. mutans (strain HS6, group a).

Streptococcus mutans cells. The cells were grown in Todd-Hewitt broth (Difco) with 1.8% glucose and harvested by centrifugation (23). For the adherence
test, viable cells, cells killed by two exposures to 65 C for 1 h each, and those killed by exposure to 100 C for 20 min were used. The optical density (OD) of the suspensions was 6.0 at 550 nm. The purpose of the heat treatments was to render the cells nonviable and to inactivate proteolytic and polysaccharide synthetase enzymes. After the heat treatment, the cells were twice washed with 0.05 M phosphate buffer, pH 6.8, and stored at 0.5% concentration in buffer with 0.02% merthiolate at 5 C. Except where indicated, all cells were treated at 100 C.

Crude enzyme preparations and antisera. Crude enzyme preparation (CEP) for polysaccharide synthesis was prepared as follows. Strain HS6 was grown in 4 liters of Todd-Hewitt broth as described above, and after overnight incubation the culture supernatant obtained by centrifugation was precipitated with (NH₄)₂SO₄ at 40% saturation and kept overnight at 5 C. The sediment obtained by centrifugation was dissolved in 100 ml of distilled water and dialyzed in 4 liters of 0.05 M phosphate buffer (pH 6.8) overnight. The CEP was centrifuged again and stored frozen. Total protein in the preparation was 4.0 mg/ml as measured by the Folin procedure (22). The same preparation was used in this entire study.

Antiserum. Antiserum against CEP was obtained by injecting an equal volume mixture of CEP and Freund incomplete adjuvant (Difco) intravenously into rabbits. During the first week three injections of 0.5 ml each were given, and during the next 2 weeks 1 ml was given in each of six injections.

Anti-HS6 whole serum and anti-group A serum were obtained in rabbits as previously described (23). In the latter case, the anti-HS6 serum was adsorbed with B13 (group d) cells. Antibody globulin against the a-d antigen which had adsorbed to these B13 cells was released at pH 2.0 during 1 h at 5 C. The released globulin was tested for specificity against the a-d antigen by agar gel diffusion and immunoelectrophoresis. Only one band in each case against the a-d polysaccharide antigen was found (15).

Cell-free synthesis of insoluble polysaccharide. In vitro synthesis of insoluble polysaccharide by CEP was measured over a period of 3 h by reading the turbidity increase at 550 nm of the reaction mixture which consisted of 2 ml of 5% sucrose, 10 μlitters of 10% Merthiolate, and 0.05 to 0.5 ml of CEP in 0.05 M phosphate buffer (pH 6.8) and buffer to 6 ml. One to 100 μlitters of 10% Merthiolate was found to have no effect on the rate of polysaccharide synthesis.

The synthesis was also measured by counting the incorporation of sucrose labeled with either 1C-glucose or 1H-fructose (New England Nuclear Corp., Boston, Mass.) into insoluble polysaccharide. The reaction mixture was the same as above except that the labeled sucrose contained either fructose-1-1H (11.43 μCi/mmoll of sucrose) or glucose-1-1C (1.44 μCi/mmoll of sucrose). After the incubation, the insoluble polymer was washed two times with water, dissolved in Mix L scintillation fluid (24), and counted by a Beckman LS-100 instrument.

Adherence of S. mutans cells on glass surface. The adherence of heat-killed or viable cells on glass surfaces was measured. In a sterile Pyrex glass tube (13 by 105 mm), 10 μlitters of 10% Merthiolate, a varying quantity of 0.05 M phosphate (pH 6.8), 0.01 to 0.5 ml of CEP, 0.04 to 3.0 ml of cell suspension, and 0.5 to 2.0 ml of 5% sucrose in buffer were added in the order named. The final volume was 6.0 ml. The solution was mixed gently and incubated at 37 C at a 30° angle for 16 h. The tubes were then rotated three times by hand one by one while at a 30° angle. The suspended cells were poured into a second tube. Six milliliters of buffer was added slowly down the sides of the first tube. The tube was rotated three times again, and the released cells were poured into the second tube. The cells which remained in the first tube were suspended in 6 ml of buffer by vigorous mechanical stirring and by scraping the glass with a rubber-coated rod. The OD of each tube was measured at 55 nm, NaOH (0.5 M) as a suspending medium for the cells (19) was not used because it caused a 25 to 40% drop in OD. This decrease was found with cells from many other serological groups of streptococci in addition to S. mutans. Merthiolate was used in all adherence tests to prevent the growth of contaminants during the 16-h incubation period.

Inhibition of cell-free synthesis of insoluble polysaccharide by antiserum or antibody globulin. CEP (10 μlitters) and 10 to 100 μlitters of complete antiserum or isolated antibody globulin were incubated with 10 μlitters of 10% merthiolate at 37 C for 30 min and then at 5 C for 16 h. The antibody-antigen complex formed was removed by centrifugation. The supernatant was added to 3 ml of buffer and 2 ml of 5% sucrose. The insoluble polysaccharide formation was measured by the increase of turbidity at 550 nm at 37 C for 3 h.

Inhibition of the cell adherence by antiserum or antibody globulin. The reaction mixture was the same as that for the cell adherence test described above except for the addition of 2 to 25 μlitters of antiserum or antibody globulin between the additions of CEP and cell suspension.

Adsorption of enzymes on cells and their adherence. In order to allow heat-killed cells to absorb CEP enzymes, 1 ml of 2.5% cells was mixed with 10 μlitters of 10% Merthiolate and 5 to 100 μlitters of CEP, and then incubated at 37 C for 30 min. The suspension was centrifuged. The cells were washed with buffer and incubated with 1 ml of 5% sucrose, 10 μlitters of 10% merthiolate, and 5 ml of buffer, and an adherence test of the cells was done as described above. The supernatant which remained was incubated with 1 ml of heat-killed cells, 1 ml of 5% sucrose, and 4 ml of buffer, and the unabsorbed enzyme activity was measured by the adherence test.

Agar gel diffusion analysis. The analysis was performed as described previously (20).

RESULTS

Synthesis of dextran-levan by cell-free enzyme. Information on the activity and substrate specificity of the enzyme preparation (CEP) used for the adherence test is shown in Fig. 1. The synthesis from sucrose of the insoluble polymer by a 10-fold range of enzyme proceeded after a short lag on a linear course over a 3-h
period. By the use of sucrose in which either the glucose or fructose was labeled, it can be seen in Fig. 2 that the insoluble material synthesized by CEP was found to be composed of 2.7% parts of glucose and 1 part of fructose. According to the data shown (Fig. 1 and 2), 1 ml of CEP converted 0.416 μmol of glucose and 0.153 μmol of fructose per min into the insoluble polymer(s). The activity of 0.416 μmol of glucose per min per ml was equal to 8.49 dextranucrase units (DSU) (11), and the specific activity per min per mg of protein was 1.93 DSU.

Adherence by viable and heat-killed cells.

Table 1 shows that cells which had been killed at either 65 or 100°C adhered to glass surface only in the presence of both sucrose and CEP. In the absence of CEP or sucrose they sedimented loosely at the bottom of the tube. The addition of soluble dextran or insoluble polysaccharide synthesized by CEP from sucrose did not increase the adherence in the presence of CEP (Table 1). The viable cells also showed the same results as the heat-killed cells, except that adherence was obtained by the viable cells in the absence of added CEP. In each of the three cases, the cohesive nature of each film of cells was the same. These data indicate that the killed cells adhered to the glass surface by means of polysaccharide synthesized from sucrose by CEP. Adherence by viable cells in the absence of CEP was most likely due to polymer synthesized by cell-bound enzymes. Heat-killed cells no longer possessed these enzymes in an active form. The heat-killed cells, however, still possessed their ability to adhere when polysaccharide was synthesized by CEP from sucrose (Table 1). Therefore, all subsequent experiments were done by using heat-killed cells in order to remove the possibility that glycosyl or proteolytic enzymes not directly concerned with the adherence process might affect the results.

Figure 3 shows the effect of varying quantities of heat-killed cells on the adherence. Approximately 40% of the cells adhered when 10 to 40 mg of cells were used; however, the addition of more than 40 mg of cells resulted in a decrease, possibly because of a lack of sufficient enzyme (and dextran) and a limitation of glass surface.

In order to determine whether adherence of the dextran polysaccharide occurred in the absence of S. mutans cells, varying quantities of CEP were incubated with sucrose for 16 h. It can be seen (Fig. 4) that a constant limited quantity of dextran adhered to the glass, while the remainder sedimented to the bottom of the tube. This value however (OD 0.04), is to be compared with an adherence value of about 0.40 when cells are used under the same conditions (Table 1).

Adherence of enzyme by heat-killed cells.

Heat-killed cells, which did not possess the enzymes required for adherence (Table 1), were preincubated with CEP, centrifuged, and washed two times with buffer. These cells in the presence of sucrose adhered firmly to the glass

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**Fig. 1.** Synthesis of insoluble polysaccharide by CEP. The reaction mixture (6 ml) consisted of 2 ml of 5% sucrose, 10 μl of 10% Merthiolate, and 0.05 to 0.5 ml of CEP. The turbidity increase at 550 nm was measured at 37°C. Amount of CEP used: ◇, 0.5 ml; ●, 0.25 ml; △, 0.1 ml; ▲, 0.05 ml.

**Fig. 2.** Incorporation of glucose and fructose into insoluble polysaccharide. The reaction mixtures were the same as in Fig. 1, except sucrose in which fructose and glucose were labeled with 1.57 × 10⁻¹ μCi of 1H and 1.99 × 10⁻² μCi of 14C, respectively. One-half milliliters of CEP was used. The OD was measured after incubation for 30, 60, and 120 min. Values of 235 count/min of 14C and 1,024 counts/min of 1H were equivalent to each micromole of glucose and fructose. Glucose, O; fructose, ●.
TABLE 1. Adherence of nongrowing Streptococcus mutans cells (strain HS6) to a glass surface

<table>
<thead>
<tr>
<th>Cells (0.5%)</th>
<th>CEP (100 µlitters)</th>
<th>Sucrose (10 mg)</th>
<th>Polysaccharide (mg)</th>
<th>Adhered cells (OD 550 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 ml</td>
<td>–</td>
<td>–</td>
<td>5.0*</td>
<td>0.02</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>–</td>
<td>+</td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>+</td>
<td>5.0*</td>
<td>0.01</td>
</tr>
<tr>
<td>Killed (65 C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 ml</td>
<td>–</td>
<td>–</td>
<td>5.0*</td>
<td>0.06</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>–</td>
<td>+</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>–</td>
<td>5.0*</td>
<td>0.01</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>Killed (100 C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 ml</td>
<td>–</td>
<td>–</td>
<td>5.0*</td>
<td>0.06</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>–</td>
<td>+</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>–</td>
<td>5.0*</td>
<td>0.04</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>–</td>
<td>0.2*</td>
<td>0.07</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>+</td>
<td>+</td>
<td>8.0*</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Total volume was 6 ml containing 1 mg of Merthiolate.
* Soluble dextran (mol wt 2 × 10⁴) from Sigma Chemical Co.
* Insoluble polysaccharide synthesized in vitro by CEP and sucrose.

The results demonstrate that the cells adsorbed the enzyme and that the cell-bound form of the enzyme synthesized polymer from sucrose. Insoluble polysaccharide (synthesized by CEP) did not significantly reduce adherence when added to cells plus sucrose plus the optimal level of CEP (Table 2). Thus, the polymer did not appear to interfere with those cell-surface functions which are required for adherence.

Effect of CEP on enzyme binding. To investigate the effect of excess CEP on the binding of

![Graph](http://iai.asm.org/)

**FIG. 4. Adherence of insoluble polysaccharide on glass surface.** To 2 ml of 5% sucrose and 10 µlitters of 10% Merthiolate, 0 to 0.25 ml of CEP was added, and the final volume was adjusted to 6 ml with the buffer. After incubation of tubes at 37 C for 16 h at 30°, the insoluble dextran which adhered to the glass surface (●) and the dextran in suspension (○) were measured at 550 nm.

**TABLE 2. Requirement of enzyme and sucrose for adherence of heat-killed Streptococcus mutans**

<table>
<thead>
<tr>
<th>CEP</th>
<th>Sucrose (5 mg/ml)</th>
<th>Insoluble polysaccharide (2 mg/ml)</th>
<th>Adhered cells (OD 550 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>1</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>0.10</td>
<td>1</td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>0.50</td>
<td>1</td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td>0.05</td>
<td>1</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>0.10</td>
<td>1</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>0.5C</td>
<td>1</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>0.05</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>0.10</td>
<td>1</td>
<td>1</td>
<td>0.44</td>
</tr>
<tr>
<td>0.50</td>
<td>1</td>
<td>1</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* Data are for 1 ml of 0.5% cells. Requirements for CEP, sucrose, and insoluble polysaccharide are expressed as milliliters.
* Cell suspension was incubated with quantities of CEP as shown above for 30 min at 37 C, washed three times with buffer, and tested for adherence with sucrose or insoluble polysaccharide, or both.
* Synthesized in vitro from CEP and sucrose.
the enzyme by heat-killed cells, the quantity of CEP adsorbed was determined. Complete adherence was obtained with 25 μl of CEP and the adsorption of the enzyme by the cells was nearly complete (Fig. 5). Quantities of CEP above 25 μl resulted in significant enzyme activity in the supernatant. The supernatant, however, showed less activity than expected. This might have been due to a nonspecific adsorption of enzyme which results in a loss of activity. Denaturation of the enzyme under the conditions used appears less likely.

**Role of cell surface binding site in adherence.** Evidence to indicate the presence of a binding site on the surface of the *S. mutans* cell was obtained with antibody globulin specific for a surface polysaccharide. This purified anti-a-d globulin reacted with the a-d site of the group a antigen (15). The a-d antigen was identified as a surface polysaccharide, and its immunological specificity was dependent on D-galactosamine (15). The specific a-d antibody globulin produced the same level of inhibition (80%) as did the anti-HS6 serum from which it was purified (Fig. 6). The protein content of the a-d preparation was one-eighth that of the HS6 whole serum. The HS6 serum, after adsorption with B13 cells (which contain the a-d antigen), did not produce a significant inhibition of adherence. Normal rabbit serum gave similar results. These results indicate that the a-d polysaccharide functions as a binding site for the insoluble dextran synthesized by HS6 cells, or it is required in the function of an unknown binding site located in close proximity to the a-d site.

To show that the a-d antibody globulin possessed no inhibitory activity on synthesis of polysaccharide by CEP, the enzyme and antiserum were combined, and after incubation the activity of the supernatant was tested. Neither anti-a-d globulin nor anti-HS6 serum inhibited the synthesis of polysaccharide, whereas anti-CEP serum showed a complete inhibition (Fig. 7). These results show that a second requirement, in addition to polysaccharide synthesis, is necessary for adherence to occur. Also, it is evident that the cell-bound form of CEP is a poor antigen when whole cells are used for immunization.

Further evidence for the separate and distinct activities of the anti-CEP and anti-HS6 whole sera is shown in Fig. 8. Purified *S. mutans* group a polysaccharide (well 3) gave a sharp band of identity with HS6 anti-serum (well 4) but no reaction with anti-CEP serum (well 2). The reaction of CEP (well 1) with its own antiserum (well 2) establishes the presence of several antigens in the CEP preparation; however, none of these antigens gave any significant reaction with HS6 serum (well 4). These results show that the antibodies in the CEP serum were...
polysaccharide synthesis supernatant C. The adherence obtained with heat-killed cells of other immunological groups of streptococci has been determined. The procedure was the same as that used with HS6. S. sanguis (strain 10556) was 80% of that of the S. mutans (HS6) control, whereas S. mitis (strain 903) produced an adherence of only 10%. Two group E strains (5385 and K129) were 9 and 18%, respectively. An adherence of 40% was shown by a group R streptococcal strain (735), whereas those from groups A (T3m and Richards), F (G565), K (Murphy), O (B361), and T (6496) were 10% or less. S. salivarius (group K) produced an adherence equal to 10.7% of the control, and those members of group D were as follows: S. bouvi (8177), 8.5%; S. durans (8307), 26.7%; S. equinus (1090), 17.9%; S. faecium (9790), 13.3%; and S. zymogenes (8176), 40.0%. It is evident from this survey that only an occasional streptococcal strain possesses the ability to adhere to a glass surface to an extent comparable to S. mutans.

DISCUSSION

The present results demonstrate that adherence to smooth glass surfaces by S. mutans cells requires two principal steps: (i) the synthesis of high-molecular-weight water-insoluble polysaccharide, and (ii) the binding of the polymer to an antigenic site located on the surface of the cell.

In regard to polysaccharide synthesis, S. mutans possesses both dextran and levan sucrose activities (2, 3, 5, and 8), and the ratio of these activities may vary from strain to strain (8). CEP synthesized water-insoluble dextran and levan in a 2.7:1 ratio (Fig. 2) over a period of 16 h, except for the first 30 min of incubation. In contrast, no levan synthesis was found during the first 5 h with a group d strain (4). A different concentration of ammonium sulfate was used in each case to precipitate the enzymes, and differences in substrate concentration and quantity of radioactive enzyme were present. Under such conditions differences are to be expected. A 3:1 dextran:levan ratio was obtained by the use of a concentrated dialyze of an S. mutans culture fluid (7). However, it is not clear whether the S. mutans insoluble polysaccharide is a copolymer of glucose and fructose, a mixture of dextran and levan polymers, or covalently bound dextran and levan fractions. Although it has been suggested that levan may enhance the formation and adherence of plaque (14), it is not known whether the incorporation of fructose is necessary to obtain a polymer with adhesive characteristics.

The enzyme preparation in the present study contained three or more antigens (Fig. 8). These may represent enzymes required for polymer synthesis. It is unlikely that a single enzyme is responsible for dextran synthesis because...
branch points in the polymer have been identified (1). In such cases, an enzyme responsible for formation of a branch point and another for polymer formation would be required (17). It is logical that a similar number of enzymes are required for the synthesis of levans. Consequently, the terms dextranosucrase and levansucrase refer to a mixture of enzymes.

The present results demonstrate by immunological procedures that an antigenic polysaccharide component located on the surface of the S. mutans cell is essential for adherence to occur. This conclusion is based upon the fact that (i) the synthesis of insoluble polysaccharide does not require the presence of streptococcal cells, and (ii) antibody globulin against the a-d polysaccharide antigen strongly inhibited the adherence in a mixture of heat-killed cells, cell-free enzyme(s), and sucrose. The specificity of the a-d antigen is also shown by the lack of inhibition by the a antibody. Both of these globulins occur in the same serum, and their separation (15) has provided a demonstration of the participation of the a-d site in adherence. These procedures have thus separated the adherence process into its two principal parts: polysaccharide synthesis and the receptor site function of the S. mutans cells.

It is clear from Fig. 3 and 4 that the S. mutans cells adsorbed the synthetase enzymes and that the a-d antibody globulin inhibited adherence without any inhibitory effect on the synthesis of the polymer (Fig. 6). The addition of insoluble dextran to killed cells plus enzyme did not produce adherence (Table 2), thus it is evident that the polymer must be synthesized on the cell surface in order for the polymer to participate in adherence. An "active" form of the polymer may be produced which binds simultaneously to the enzyme(s) and the a-d polysaccharide site. It thus appears that the enzymes and the a-d site function together in order to complete the adherence process. It is not apparent whether the enzymes bind directly to the cell surface a-d site, or whether there is a separate enzyme site in close proximity to the a-d site which might be blocked by a-d globulin. The specificity of the receptor site, however, is indicated by the failure of the blocked a site to interfere with adherence (Fig. 6).

The possibility cannot be entirely eliminated that a binding site other than a-d and which might be common to all S. mutans cells is responsible for adherence. However, the absence of other antibodies in the a-d globulin preparation, based on agar gel diffusion and immunoelectrophoresis, would appear to make such a possibility very unlikely. Further evidence against the existence of a common site has been tested by adsorption of anti-HS6 serum with FA1 (group b) cells. The adsorbed serum contained antibody which was inhibitory to adherence, whereas the globulin released from the FA1 cells had no effect (see Methods for experimental procedures) (15).

These results indicate that a binding site which functions in adherence is found in strains which possess the a-d site and that an antigenic site which is common to all strains probably does not exist. Thus it is probable that other immunologically specific S. mutans strains, and other dextran-producing streptococcal strains occasionally found in dental plaques, possess similar binding sites which may differ from each other in serological specificity. Whether these include the S. mutans group b (16) or c antigens is not known.

Nongrowing viable S. mutans cells have been reported to agglutinate in the presence of sucrose or upon the addition of high-molecular-weight dextran (6). The necessity for several hours of incubation for agglutination in sucrose has been attributed to dextranosucrase activity present in a cell-bound form in S. mutans (7). In the present study, the ability of viable cells to adhere (without added CEP) could also be attributed to the presence of polymer synthetase enzymes on the cells. The specific adsorption of enzymes from CEP by heat-killed cells resulted in the restoration of the adherence ability of such cells (Table 2). However, it is seen in Table 1 that the adherence of viable cells was only about one-half that of heat-killed cells which had adsorbed CEP. It appears that the heat treatment may have denaturated the CEP protein and at the same time exposed new sites for the adsorption of cell-free enzyme. Consequently, the additional adherence obtained was probably due to the action of additional bound enzyme.

The relationship of agglutination to adherence is of interest in regard to the present results. Heat-killed S. mutans cells will not agglutinate when dextran is added (7), indicating that a binding site, if required, has been destroyed by heat treatment. However, the ability of heat-killed cells to adhere has been demonstrated (Table 1), and also the necessity for polymer synthesis in the presence of the cells (Table 2) indicates that the mechanisms of agglutination and adherence possess different requirements.

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LITERATURE CITED


