The Influence of Ionic Strength, pH and a Protein Layer on the Interaction between Streptococcus mutans and Glass Surfaces

By A. ABBOTT,† P. R. RUTTER‡ AND R. C. W. BERKELEY*

Department of Microbiology, University of Bristol, Medical School, University Walk, Bristol BS8 1TD, U.K.

(Received 15 July 1982)

The initial interaction between Streptococcus mutans and hard surfaces has been investigated using a rotating disc technique. The deposition to clean and BSA-coated glass of two strains of S. mutans, FA-1 (serotype b) and KPSK2 (serotype c), which exhibit different surface properties, was studied. Organisms were harvested from cultures grown in a chemostat at a dilution rate of 0.06 h⁻¹ and suspended in NaCl solutions of defined ionic strengths and pH values. The deposition of both strains showed a strong dependence on electrolyte concentration, particularly at low ionic strengths, which was inversely related to the zeta potentials of the organisms. Similarly, the ionic strength at which maximum deposition was first noted (critical coagulation concentration) for the two strains correlated with their relative potentials. Deposition was insensitive to changes in pH at an electrolyte concentration of 0.05 M. The maximum observed deposition did not approach values predicted by theory, suggesting that a further barrier to deposition, other than electrostatic repulsion, might exist. Under all experimental conditions, some of the deposited bacteria were observed to be oscillating, suggesting that they were held at a distance from the collector surface. The cells did not, however, appear to be deposited in a secondary minimum predicted by DLVO theory hence it may be that long-range polymer interactions are also involved in the deposition of these organisms.

INTRODUCTION

Streptococcus mutans is an oral organism found almost exclusively on the teeth and its presence appears to be correlated with dental caries (Fitzgerald et al., 1960; Krasse, 1966; Bowen, 1969; Loesche et al., 1975). In the development of caries the deposition and adherence of S. mutans on to the hard, enamel tooth surface are important processes.

A previous study carried out in this laboratory concerning the initial interaction, referred to as 'deposition', between S. mutans and hard surfaces showed a great variability in this property among a number of strains of the organisms (Abbott et al., 1980). The serotype b strains studied (FA-1, BHT and OMZ51) showed particularly low deposition tendencies which correlated with the possession of high negative zeta potential suggesting that, for these strains, electrostatic repulsion may be important in the initial interaction with the collector.

The present study was designed to establish whether variations in the environmental conditions under which deposition occurred affected the zeta potentials of S. mutans cells, which would influence the electrostatic repulsion between the cells and the collector surface, and whether these variations altered the tendency of the organisms to deposit.

Two strains of S. mutans, FA-1 (a serotype b strain) and KPSK2 (serotype c), were grown in a continuous culture apparatus under conditions of glucose-limitation, and deposition from
suspensions of different pH and ionic strengths to glass and BSA-coated glass, of cells from these cultures, was investigated.

METHODS

Organisms. The strains used in this study were S. mutans FA-1 (serotype b) and S. mutans KPSK2 (serotype c; kindly supplied by J. R. Hunter, C.A.M.R., Porton, U.K.). Stock cultures were maintained by fortnightly subculture on blood agar plates. Culture characteristics were checked before use in experiments using API 20E and 50E identification galleries (Buissiere, 1972) and confirmed using the tests outlined by Hardie & Bowden (1976). The purity of chemostat cultures was checked daily by microscopic examination and culturing on blood agar plates.

Growth conditions. The bacteria were grown in a chemostat (Biotec LP 100) with a 1 litre working volume. The temperature of the culture was maintained at 36 ± 1°C and the pH automatically controlled at 6.5 ± 0.1. Foaming was avoided by gassing directly into the headspace of the chemostat, rather than into the bulk fluid, at approximately 40 ml min⁻¹ with a gas mixture of 95% N₂ : 5% CO₂ (v/v). Medium was pumped into the culture vessel at the dilution rate of 0.06 h⁻¹ by a Miniature Flow Inducer (Watson-Marlow, Bucks., U.K.) and the culture allowed to reach equilibrium for at least 10 mean generation times before samples were removed for experimental purposes.

A complex growth medium was used consisting of the following constituents (l⁻¹): Casamino acids (Difco), 2.5 g; yeast extract (Difco), 2.5 g; KH₂PO₄, 136 mg; K₂HPO₄, 348 mg; NaCl, 10 mg; MgSO₄.7H₂O, 200 mg; FeSO₄.7H₂O, 10 mg; MnSO₄.4H₂O, 10 mg; adenine, 10 mg; guanine, 10 mg; and uracil 10 mg. This medium was sterilised in 15 l quantities by autoclaving for 2 h at 10 lbf in⁻². The limiting nutrient, glucose, was dissolved in 1 litre deionized water, autoclaved separately and added aseptically to the sterile bulk.

Cell suspensions. Bacterial suspensions for the rotating disc and microelectrophoresis experiments were prepared by diluting fresh samples from the chemostat, sonicated using a 2 cm probe at low amplitude to peak using an MSE 100 W Ultrasonic Disintegrator) to disperse the cells without lysing them, in a salts solutions consisting of the salts in the growth medium adjusted to 0.05 M with NaCl. The pH of the suspensions was adjusted to values chosen to correspond with the range found in saliva (Gron, 1973).

The influence of ionic strength on the deposition of the cells was studied using washed cells suspended in NaCl solutions of various ionic strengths.

Collectors for deposition. Bacteria were deposited on to clean glass coverslips (19 mm, no. 3, Chance-Propper) and glass coverslips coated with a monolayer (see Edwards & Rutter, 1980) of BSA. All the glass coverslips were degreased in acetone before soaking in a 50 : 50 mixture of concentrated HCl/concentrated HNO₃. The coverslips were thoroughly rinsed three times in double distilled water from an all-glass still and steamed for 30 min before use. The BSA-coated collectors were prepared by immersing clean glass coverslips in 0.5% (w/v) aqueous BSA fraction V (Sigma), adjusted to pH 6.5 and stirred for at least 3 h followed by overnight washing in stirred double distilled water (2 l). The presence of an adsorbed layer of BSA was confirmed by staining a sample coverslip with Coomassie blue (Bio-Rad) and the adsorption was therefore assumed to be essentially irreversible, as has been shown for adsorption to silica (Edwards & Rutter, 1980).

Electrophoretic mobility measurements. The electrophoretic mobilities of the cell suspensions were determined using a Rank Brothers mark 11 microelectrophoresis apparatus. Thin walled, narrow bore, cylindrical and flat cells with platinum electrodes were used at 21 ± 2°C and mobility was observed under dark ground illumination. Zeta potentials were derived from the mobility data using the tables of Ottowill & Shaw (1972) based on computations by Wiersema et al. (1966).

Deposition at the surface of a rotating disc. The apparatus and experimental procedure has been described briefly (Abbott et al., 1980); a fuller description and discussion of theoretical aspects is given by Abbott (1981). Essentially the rotating disc technique relies on the ability to be able to predict accurately the collision rate, under a given set of conditions, between particles in suspensions and a collector at the surface of the disc. This is made possible by the defined hydrodynamics of a disc rotating in a fluid. Calculation of the ratio of the number of collisions to the observed numbers of deposited cells, the stability ratio W, gives a measure of the tendency of a particle to deposit on a surface. When W is unity the collector surface is acting as a perfect sink.

RESULTS AND DISCUSSION

Influence of ionic strength on deposition

With electrolytes such as NaCl it can be shown that an increase in electrolyte concentration is paralleled by a fall in the electrophoretic mobility of particles due to compression of the electrical double-layer (Shaw, 1980). If deposition is controlled solely by physico-chemical parameters, such as van der Waals' attractive forces and electrostatic repulsion forces as
Interaction of *S. mutans* with hard surfaces

![Graph 1](image1)  
**Fig. 1**. Influence of ionic strength (*I*) on deposition of *S. mutans* to glass. Experiments were carried out in NaCl suspensions of pH 6.5 at 20 °C. Deposition is represented by log *W*, where *W* is the ratio of the theoretical number of collisions to the observed number of deposited particles. ○, *S. mutans* FA-1 grown in a glucose-limited chemostat culture at a dilution rate of 0.06 h⁻¹. ●, *S. mutans* KPSK2 grown in a glucose-limited chemostat culture at a dilution rate of 0.06 h⁻¹. □, negatively charged polystyrene latex of 0.4 μm radius. Bars represent ± 2 S.D. about the mean. Arrows indicate the critical coagulation concentration.

![Graph 2](image2)  
**Fig. 2**. Variation of zeta potentials of *S. mutans* with ionic strength (*I*). Zeta potentials were derived from electrophoretic mobility data using the tables of Ottowill & Shaw (1972) based on computations of Wiersema et al. (1966). Electrophoretic mobility measurements were made in NaCl suspensions at pH 6.5, 20 °C using a Rank Brothers Microelectrophoresis apparatus. The radii of the bacteria, used to determine zeta potentials, were 0.4 μm for FA-1 (○) and 0.3 μm for KPSK2 (●). These were measured using a Vickers-A.E.I. image splitting eye-piece on a conventional Wild microscope. Bars represent ± 2 S.D. about the mean.

Described by DLVO theory for the stability of lyophobic sols (Deryagin & Landau, 1941; Verwey & Overbeek, 1948), an increase in electrolyte concentration should bring about an increase in deposition rate to a maximum after which the rate should remain constant. Increased deposition rate is accompanied by a reduction in stability ratio (*W*) which was clearly illustrated in the present study by observations on the deposition of model negatively charged polystyrene latex particles (Fig. 1). Similar observations have been recorded elsewhere (Hull & Kitchener, 1969; Clint et al., 1973; Rutter & Abbott, 1978).

The deposition of the cells (Fig. 1), showed a dependence on electrolyte concentration, at low ionic strengths, which was inversely related to the zeta potentials of the organisms (Fig. 2) in accordance with theory. Similarly the ionic strength at which maximum deposition was first noted (the critical coagulation concentration) for the two strains correlated with their relative potentials.

The results reinforce the previous conclusions (Abbott et al., 1980) that, at low ionic strengths, electrostatic repulsion plays an important role in the deposition tendencies of these strains of *S. mutans*.

At higher ionic strengths a deposition maximum was reached but it was not as high as expected on theoretical grounds (*W* ≠ 1). This was in contrast to the much better approximation between the theoretically predicted results for negatively charged latex particles and the experimental values obtained. The reasons for the small discrepancy between the predicted and experimentally determined values for latex are discussed elsewhere (Abbott, 1981). That bacterial cells do not behave as predicted at high ionic strengths suggests that a further barrier to deposition occurs over and above that posed by electrostatic repulsion. The outer surface of the
streptococcal cell is not a fixed structure but may have an associated diffuse layer of polymeric material (Wicken & Knox, 1977). This layer may itself be responsible for a further repulsion between the cell and the collector surface. An interesting observation from the deposition experiments may be relevant to this point. It was seen, under all the experimental conditions, that up to half of the deposited bacteria were oscillating. They appeared to be positioned a small distance away from the surface, but were held strongly enough to resist removal by washing. Due to the fact that electrostatic repulsion forces generally decay with distance faster than van der Waals’ attraction forces, DLVO theory predicts that it is possible for overall attraction between a particle and a surface to occur both at a very small (\(< 1 \) nm) separation (primary minimum) and at a separation distance of several nm (secondary minimum) as illustrated in Fig. 3. It was conceivable that the oscillating bacteria might have been held at the secondary minimum position. This suggestion was tested by replacing the suspending medium by one of a lower ionic strength. Cells deposited at a secondary minimum would be expected to be lost on lowering the ionic strength due to an expansion of the electrical double-layer, thereby shifting the balance between attraction and repulsion towards a repulsive interaction. On diluting the suspending medium, however, cells were not lost from the surface indicating that they were probably not held in a secondary minimum.

A number of other possible explanations for the phenomenon of oscillation exist. Firstly it has been suggested that lipoteichoic acids at the surfaces of S. mutans cells might mediate attachment to negatively charged surfaces via Ca\(^{2+}\) bridges (Rolla, 1976), however, we have found that deposition of glucose-limited cells is not enhanced in the presence of 0.05 M CaCl\(_2\) (Abbott, 1981). Another explanation could be one of transient bridging via extracellular polymers which are known to be present on the surfaces of S. mutans when grown in both glucose and sucrose media (Grenier et al., 1977; Iacono et al., 1976; Gibbons & Banghart, 1976; Nalbadian et al., 1974). Polymer-mediated interactions can occur at much greater range than van der Waals’ and electrostatic interactions and very small amounts of adsorbed high molecular weight polymers can induce attachment in deep minima at long range (Rutter, 1980). Polymer bridging can occur under conditions of relatively high surface charge and low electrolyte concentration when DLVO theory cannot support the existence of a secondary minimum of sufficient magnitude to retain a particle in the close proximity of a surface. Attachment of bacteria to surfaces via cell surface polymers is well documented (Harris &
Interaction of S. mutans with hard surfaces

Table 1. Influence of pH on the zeta potentials and deposition to glass of chemostat-grown S. mutans in 0.05 M-NaCl

Zeta potentials were derived from electrophoretic mobility data using the tables of Ottowill & Shaw (1972). Electrophoretic mobility measurements were made using a Rank Brothers Microelectrophoresis apparatus. Mean values ± 2 s.d.s (20 replicates) are given. Deposition is represented by W, the ratio of theoretical collisions to observed deposition. Mean values (and the s.e. of the means, shown in parentheses) were calculated from determinations of W (8 replicates) on three samples from each of two cultures prepared at different times.

<table>
<thead>
<tr>
<th>Organism</th>
<th>pH</th>
<th>Negative zeta potential (mV)</th>
<th>Deposition to glass (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans FA-1</td>
<td>4-5</td>
<td>25.8 ± 2.4</td>
<td>18.1 (0.77)</td>
</tr>
<tr>
<td></td>
<td>5-0</td>
<td>26.7 ± 2.5</td>
<td>21.0 (0.82)</td>
</tr>
<tr>
<td></td>
<td>5-5</td>
<td>24.1 ± 3.0</td>
<td>20.6 (1.02)</td>
</tr>
<tr>
<td></td>
<td>6-0</td>
<td>26.1 ± 4.0</td>
<td>28.3 (2.93)</td>
</tr>
<tr>
<td></td>
<td>6-5</td>
<td>25.8 ± 2.3</td>
<td>15.8 (0.74)</td>
</tr>
<tr>
<td></td>
<td>7-0</td>
<td>25.0 ± 2.2</td>
<td>25.8 (0.34)</td>
</tr>
<tr>
<td></td>
<td>7-5</td>
<td>25.7 ± 4.0</td>
<td>33.7 (1.14)</td>
</tr>
<tr>
<td>S. mutans KPSK2</td>
<td>4-5</td>
<td>10.9 ± 2.1</td>
<td>6.9 (0.07)</td>
</tr>
<tr>
<td></td>
<td>5-5</td>
<td>11.9 ± 1.5</td>
<td>7.8 (0.09)</td>
</tr>
<tr>
<td></td>
<td>6-5</td>
<td>13.5 ± 2.2</td>
<td>5.5 (0.26)</td>
</tr>
<tr>
<td></td>
<td>7-5</td>
<td>13.8 ± 4.3</td>
<td>5.2 (0.03)</td>
</tr>
</tbody>
</table>

Mitchell, 1973; Fletcher & Floodgate, 1973; Costerton et al., 1978; Rutter & Abbott, 1978). It has not yet, however, been possible to undertake a systematic electron microscope study of the cell surfaces of these organisms grown in the chemostat in order to establish whether this explanation is likely or not. It may be worth noting, however, that the conformation and diffusion characteristics of surface macromolecules of bacteria can be influenced by changes in ionic strength and pH of the environment (Tenney & Stumm, 1965; Doyle et al., 1974) and that therefore the dependence of deposition on electrolyte concentration may reflect an effect on surface polymers as well as on surface potentials.

Influence of pH on deposition

The influence of pH on the zeta potentials of the two strains (Table 1) was in agreement with results obtained by Olsson & Glantz (1977). Interestingly, the deposition of both strains was relatively insensitive to changes in pH at the ionic strength of 0.05 M. The data from the ionic strength experiments suggest that at this ionic strength the deposition of strain FA-1 is controlled by its surface charge characteristics but that of strain KPSK2 may be independent of these factors. The insensitivity of both deposition and zeta potential to changes in pH for strain FA-1 is thus consistent with the findings from the ionic strength experiments. The lack of correlation between zeta potential and deposition at different pHs for strain KPSK2 may also be consistent with the ionic strength experiments on this organism in that they reflect an independence from surface charge characteristics for deposition at an electrolyte concentration of 0.05 M. Measurements of the zeta potentials of the glass and BSA-coated glass surfaces also showed a relative insensitivity to change in pH over the range studied, ruling out an effect of the potential of the collector surfaces on deposition.

Influence of a protein layer on deposition

There was little apparent difference between the deposition of strain FA-1 on to clean or BSA-coated glass, whose zeta potentials under the experimental conditions employed were similar, so only the data for deposition on to glass is illustrated. In the case of strain KPSK2, however, the deposition to BSA-coated glass was lower (P < 0.001) than that to glass under all experimental conditions (Table 2). Similar observations were made on the deposition of batch cultured cells of these strains (Abbott et al., 1980). It is likely, in the case of S. mutans FA-1, where deposition is very low due to the strong influence of zeta potential, that any modifying effect of a protein layer on attachment is so small as to be undetectable. For S. mutans KPSK2 the BSA layer seems to
impair attachment and this may be via a steric effect on the diffuse surface macromolecules of the cell. This inhibition is less obvious at low ionic strengths where electrostatic repulsion is more important in the deposition of this organism. It has been reported previously that BSA inhibits the attachment of a marine pseudomonad to polystyrene (Fletcher, 1976).

### References


Table 2. Influence of a protein layer on deposition of S. mutans KPSK2 to glass

Deposition is represented by W, the ratio of theoretical collisions to observed deposition. Mean values (and the s.e. of the means) were calculated from determinations of W (8 replicates) on three samples from each of two cultures prepared at different times.

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Deposition to glass (W)</th>
<th>Deposition to BSA-coated glass (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-001 m-NaCl, pH 6-5</td>
<td>27.5 (1.54)</td>
<td>31.6 (1.55)</td>
</tr>
<tr>
<td>0-0025 m-NaCl, pH 6-5</td>
<td>16.8 (0.72)</td>
<td>20.5 (1.69)</td>
</tr>
<tr>
<td>0-005 m-NaCl, pH 6-5</td>
<td>8.1 (0.21)</td>
<td>14.1 (0.48)</td>
</tr>
<tr>
<td>0-01 m-NaCl, pH 6-5</td>
<td>5.5 (0.32)</td>
<td>8.7 (0.19)</td>
</tr>
<tr>
<td>0-025 m-NaCl, pH 6-5</td>
<td>5.0 (0.12)</td>
<td>7.6 (0.35)</td>
</tr>
<tr>
<td>0-05 m-NaCl, pH 4-5</td>
<td>6.9 (0.07)</td>
<td>11.2 (0.56)</td>
</tr>
<tr>
<td>0-05 m-NaCl, pH 5-5</td>
<td>7.8 (0.09)</td>
<td>10.7 (0.35)</td>
</tr>
<tr>
<td>0-05 m-NaCl, pH 6-5</td>
<td>5.5 (0.26)</td>
<td>9.8 (0.18)</td>
</tr>
<tr>
<td>0-05 m-NaCl, pH 7-5</td>
<td>5.2 (0.03)</td>
<td>11.0 (0.59)</td>
</tr>
<tr>
<td>0-5 m-NaCl, pH 6-5</td>
<td>5.8 (0.22)</td>
<td>8.4 (0.16)</td>
</tr>
</tbody>
</table>
INTERACTION OF S. MUTANS WITH HARD SURFACES


