Interactions between Motile *Escherichia coli* and Glass in Media with Various Ionic Strengths, as Observed with a Three-Dimensional-Tracking Microscope

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*A. coli* bacteria have been observed to swim along a glass surface for several minutes at a time. Settling velocities of nonmotile cells and a computer simulation of motile cells confirmed that an attractive force kept the bacteria near the surface. The goal of this study was to evaluate whether this attractive force could be explained by reversible adhesion of *E. coli* to the surface in the secondary energy minimum, according to the theory of Derjaguin, Landau, Verwey, and Overbeek (DLVO theory). This theory describes interactions between colloidal particles by combining attractive van der Waals forces with repulsive electrostatic forces. A three-dimensional-tracking microscope was used to follow both wild-type and smooth-swimming *E. coli* bacteria as they interacted with a glass coverslip in media of increasing ionic strengths, which corresponded to increasing depths of the secondary energy minimum. We found no quantifiable changes with ionic strength for either the tendencies of individual bacteria to approach the surface or the overall times bacteria spent near the surface. One change in bacterial behavior which was observed with the change in ionic strength was that the diameters of the circles which the smooth-swimming bacteria traced out on the glass increased in low-ionic-strength solution.

A quantitative understanding of which factors control the extent of bacterial adhesion would be useful for many applications. For example, development of a constitutive equation describing bacterial adhesion to surfaces based on a small set of measurable properties would allow for its incorporation in mathematical models used in the design of bioremediation systems. Our work aims at greater understanding of how changes in the chemical composition of groundwater, such as addition of a surfactant or modification of its ionic strength, affect bacterial adhesion. Such an understanding is important, because these changes can be implemented above ground.

Our objective was to determine if the observed tendency of *Escherichia coli* cells to remain close to glass surfaces for long periods can be explained by electrostatic and van der Waals interactions between the cells and the glass surface. In an unbounded fluid medium, flagellated bacteria such as *E. coli* move in a manner resembling a random walk. That is, they swim in a nearly straight line for about a second (running) and then tumble in place and begin a run in another direction (5). When a bacterium is very close to a large surface (a grain of sand or a microscope slide, for example), its behavior must be altered because the bacterium cannot swim through this surface. In this case, the bacterium is in the DLVO regime: the attractive van der Waals interaction between helical flagellar bundles and the flat half space defined by the glass (27). However, hydrodynamic interactions do not explain why a bacterium will remain near the surface for so long (over 2 min in some cases) when it would be expected to tend to drift away due to Brownian motion and sedimentation (10).

Fymier et al. postulated that the cell remained near the surface because it reversibly adhered to the surface in the secondary free-energy minimum, as described by the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory (10). Researchers have been successful in using the DLVO theory to qualitatively describe the adhesion of bacteria to a variety of surfaces (12, 16, 17, 28, 30, 33). According to the DLVO theory, the interaction between two like-charged surfaces will become more favorable as the ionic strength of the medium is increased.

In general, for two materials of negative surface charge surrounded by a medium of moderate ionic strength, the DLVO theory predicts no interaction at a large distance of separation, as seen in Fig. 2. Attraction between the two materials occurs at a separation of several nanometers, followed by a large repulsion and then a much greater attraction as the surfaces get even closer (29) (this attraction appears too close to the ordinate to be seen clearly in Fig. 2). The first attractive region on the approach to the surface is referred to as the secondary free-energy minimum, and cells which reversibly adhere at this distance from the surface may still exhibit translational motion along it (26). Cells which attach in the primary energy minimum adhere irreversibly (26) and show no translational movement.

To demonstrate the supposition that the observed near-surface swimming behavior of *E. coli* can be explained by the

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DLVO theory, two things must be demonstrated, first, that the tendency of the bacteria to stay near the surface is due to a force, and not simply accumulation due to the presence of an impassable barrier, and second, that altering variables which change the predictions of the DLVO theory correspond to observed changes in the behavior of the bacteria. In this study, the existence of forces holding the bacteria to the surface was verified by measuring the falling velocity of nonmotile bacteria far from the glass surface and comparing it to the falling velocity of motile bacteria near the surface. The existence of a force was also corroborated by predictions of a computer simulation, which calculated the amount of time a bacterium experiencing no forces would spend near the surface. To see if the depths of the energy minima affect a bacterium’s tendency to remain near a surface, cells were observed in the vicinity of the surface and the amount of time they spent swimming near

![Figure 1](image1.png)  
**FIG. 1.** Traces of an HCB437 bacterium swimming near the surface in 50-μm-diameter circles with I of 0.006 M (A), a different HCB437 bacterium under the same conditions (B), an HCB437 bacterium swimming in circles 20 μm in diameter with I of 0.20 M (C), and an HCB437 bacterium swimming in circles 100 μm in diameter with I of 0.006 M (D).

![Figure 2](image2.png)  
**FIG. 2.** Interaction energies between a glass surface and *E. coli* cells. (A) Interaction with HCB1 bacteria as a function of distance at three ionic strengths; (B) interaction with HCB437 bacteria as a function of distance at two ionic strengths.
and parallel to it was measured for each of several ionic strengths. The depths of the secondary free-energy minima were calculated from measured electrophoretic mobilities and physical property data of the bacteria, glass, and liquid media.

**MATERIALS AND METHODS**

**Bacteria and growth conditions.** Three different strains of *E. coli* were used in these experiments. The swimming behavior of each strain is described in Table 1. All bacteria were cultured in tryptone broth (10). Five hundred microliters of bacterial stock was added to 50 ml of autoclaved broth in a 250-ml shake flask, and this solution was incubated for ~6 h (until mid-exponential phase) at 30°C and shaken at 100 rpm (0.48 × g) on a Labline Environshaker. For tracking experiments, cells were harvested at mid-exponential phase, filtered, and resuspended according to the method of Berg and Turner (6) and were used as the appropriate buffer at a cell density of about 10^8 cells/ml (4). Phosphate buffers (1) of different ionic strengths (I) were created by diluting a stock buffer (I = 0.202 M) with distilled and deionized water and were used as the media for tracking experiments. The ionic strengths used in these experiments were 0.020, 0.060, 0.020, and 0.006 M, but not every ionic strength was tested with every bacterial strain.

**Bacterial sedimentation velocity test.** Nonmotile HCB137 bacteria were observed in the tracking microscope in media of different ionic strengths to determine if there was a difference between the average amount of time a bacterium spends near the surface. The behavior of randomly motile particles was simulated with the parameter values of run length, average turn angle, and tumbling probability determined for HCB1 (5) and HCB437. A cellular dynamics computer simulation program, which was recently published (10), was used for this simulation. Randomly motile particles were allowed to interact with the pore walls in a no-flow simulation. The boundary condition at a solid surface specifies that a particle which will penetrate a wall at a porous matrix, was used for this simulation. Randomly motile particles were tracked for over 2 min.

**Measurement of electrophoretic mobility.** The electrophoretic mobilities of the cells and glass used in these experiments were measured with a Coulter Delsa 440 electrophorimeter. The cells were prepared as described above but at a lower cell density (~10^7 cells/ml). Glass was prepared by cleaning it with distilled water, ethanol, and heptane, air drying and crushing it to a fine powder with an agate mortar and pestle (10, 13, 21), and suspending it in the appropriate buffer. Measurements were made at 30°C. The measured electrophoretic mobilities and calculated zeta (ζ) potentials for both the *E. coli* and the glass are in the range of those reported by other authors (13, 14, 18, 31), although no direct comparisons can be made, as the bacterial strains, type of glass, and measurement conditions differ. The ζ potential was calculated from the electrophoretic mobility by the Smoluchowski equation (15), \[ \zeta = \frac{\mu_g (\zeta_g / \varepsilon)}{e} \], where \( \mu_g \) is the electrophoretic mobility, \( \zeta_g \) is the viscosity, and \( \varepsilon \) is the dielectric solubility of the solution. The time for an individual bacterial trace to determine how long each bacterium spent swimming parallel to the surface. To do this, a unit vector was constructed in the direction pointing from one data point to the following data point and the dot product of this vector and the unit normal to the plane of the glass was calculated. A tolerance of ±0° from parallel was used, because this value gave the best agreement with the amount of time the bacterium swam parallel as judged from the visualizations of the bacterial traces. Reported values are averages of the fractions of the total times tracked that individual cells remained near the surface. The definition of near was set at 10 µm from the surface, although a 5-µm cutoff was used and led to similar results for HCB1. The 10-µm cutoff was used to allow for two possible errors introduced by the tracking microscope: surface calibration drift and the inexact bacterial positions.

The first problem arises because as the apparatus warms up, the perceived location of the glass surface drifts downward at a rate up to 0.5 µm/min (9). This rate is too low not to affect individual bacterial traces, but over the entire observation period for a population of bacteria, cells appeared to be further from the surface than they actually were. This problem was combated by frequent recalibration. Since the microscope tracks a bacterium as it if were a point particle and may track at a point anywhere along the bacterium’s length, the second problem results in an inherent uncertainty of ±2 µm in the bacterium’s position at any time (3).

Because a bacterium which reversibly adheres to the surface should continue swimming along it for some time, we also analyzed each bacterial trace to determine how long each bacterium spent swimming parallel to the surface. To do this, a unit vector was constructed in the direction pointing from one data point to the following data point and the dot product of this vector and the unit normal to the plane of the glass was calculated. A tolerance of ±10° from parallel was used, because this value gave the best agreement with the amount of time the bacterium swam parallel as judged from the visualizations of the bacterial traces. Reported values are averages of the fractions of the total times tracked that individual cells swam parallel to the surface.

The surface excess concentration is a measure of how much more (or less) cells are concentrated near the surface than in the bulk. The surface excess concentration was calculated with the following equation, suggested by LeVan et al. (19):

\[ \text{Surface excess concentration} = \frac{C_{\text{surface}} - C_{\text{bulk}}}{C_{\text{bulk}}} \]

where \( C_{\text{surface}} \) is the concentration of cells at the surface, \( C_{\text{bulk}} \) is the concentration in the bulk, and \( k \) is the surface energy.

**TABLE 1. Swimming behavior of bacterial strains used and their ζ potentials**

<table>
<thead>
<tr>
<th>Strain (reference)</th>
<th>Behavior</th>
<th>ζ potential (mV) with I (M) of:</th>
<th>0.20</th>
<th>0.06</th>
<th>0.02</th>
<th>0.006</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCB1 (AW405) (2)</td>
<td>Wild type</td>
<td>7.47 ± 10.1</td>
<td>7.47</td>
<td>-29.5</td>
<td>NM*</td>
<td></td>
</tr>
<tr>
<td>HCB437 (32)</td>
<td>Smooth swimming</td>
<td>-4.77</td>
<td>NM</td>
<td>NM</td>
<td>-22.82</td>
<td></td>
</tr>
<tr>
<td>HCB137</td>
<td>Nonmotile (no flagella)</td>
<td>-11.02</td>
<td>-25.22</td>
<td>-42.61</td>
<td>-34.90</td>
<td></td>
</tr>
</tbody>
</table>

* NM, not measured.

**TABLE 2. Average fractions of time spent swimming near or parallel to the surface by HCB1 and HCB437 organisms at various ionic strengths**

<table>
<thead>
<tr>
<th>Strain</th>
<th>% of time near surface* with I (M) of:</th>
<th>0.20</th>
<th>0.060</th>
<th>0.020</th>
<th>0.006</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCB1</td>
<td>38 ± 22</td>
<td>39 ± 12</td>
<td>33 ± 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCB437</td>
<td>80 ± 45</td>
<td>92 ± 20</td>
<td>57 ± 23</td>
<td>16 ± 4</td>
<td>12 ± 6</td>
</tr>
</tbody>
</table>

* The numbers of cells in samples with I at 0.202, 0.060, and 0.020 were 37, 35, and 27, respectively.

+ The numbers of cells in samples with I at 0.202 and 0.006 were 29 and 55, respectively.

+ The ± values give the 95% confidence limits.
of the glass surface. The computer simulation makes no allowance for possible attraction between the bacterial cells and the glass surface. As can be seen in Table 3, in all cases, actual bacterial cells spent longer times near the surface than did their simulated counterparts. The simulation may tend to overestimate the time spent near the surface with respect to the times measured with the tracking microscope, because the computer cannot lose track of particles while cells can sometimes escape from the tracking microscope.

**DLVO theory predictions.** Figure 2 shows the predicted interaction energy profiles for cells approaching the glass surface, as calculated from the DLVO equations given by Norde and Lyklema (26) and the measured ζ potentials, which are shown in Table 1. Negative interaction energies indicate an attraction between the bacteria and the surface, while positive interaction energies indicate repulsion. The secondary minimum must be at least $-1.5 \text{ K T}$ in depth in order to overcome a bacterium’s thermal energy (30). Therefore, bacterial adhesion in the secondary minimum is expected for HCB1 at all ionic strengths and for HCB437 only at the highest ionic strength.

Due to the approximations made in the use of these equations and some of the parameter values, these predictions are only semiquantitative. For example, the equations assume that the bacterium is a smooth sphere, on which all charge is evenly distributed. The *E. coli* cells are actually ellipsoids with numerous appendages, such as flagella. There may also be localized points of positive charge (such as the flagella), even though the bacterium as a whole is negatively charged. However, the DLVO theory predictions will indicate the relative strength of the adhesion of the bacteria, such that more, and stronger, adhesion in the secondary energy minimum is expected at the high ionic strength than at the low ionic strength.

**Time spent swimming near and parallel to the glass surface.** The determination of near and parallel was designed to show if the tendency of individual cells to approach the surface was affected by the ionic strength of the solution. Table 2 shows the percentage of time each strain of bacteria spent swimming near the glass surface at each ionic strength. Note that for each strain, there are no significant differences in the amounts of time bacteria spent near the surface as the ionic strength changed. The smooth-swimming bacteria always remained near the surface longer than their tumbling counterparts; however, this result was also predicted by the computer simulation (Table 3), which did not account for surface interactions.

**Surface excess concentration.** Table 4 gives the overall surface excess concentrations for both strains in media of different ionic strengths. This calculation was designed to measure the overall tendency of a population of bacteria to approach the glass surface. As can be seen in Table 4, for a given strain, there is no significant difference in bacterial behavior at different ionic strengths. Again, the smooth swimmers (HCB437) have a larger surface excess, as expected.

**Qualitative changes in behavior with ionic strength.** There were two major changes in bacterial behavior with ionic strength.

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**Table 3.** Average and maximum times spent near the surface for tracking experiments with HCB1 and HCB437.

<table>
<thead>
<tr>
<th>Bacteria*</th>
<th>I (M)</th>
<th>Avg time within 10 μm of surface(s)</th>
<th>Maximum time within 10 μm of surface(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP with properties of HCB1 (wild type)</td>
<td>0.202</td>
<td>5.75 ± 3.32</td>
<td>55.7</td>
</tr>
<tr>
<td>HCB1 (exptl)</td>
<td>0.060</td>
<td>8.13 ± 2.50</td>
<td>36.0</td>
</tr>
<tr>
<td>HCB437 (exptl)</td>
<td>0.020</td>
<td>6.79 ± 3.86</td>
<td>50.0</td>
</tr>
<tr>
<td>RMP with properties of HCB437 (smooth swimming)</td>
<td>3.55 ± 0.48</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>HCB437 (exptl)</td>
<td>0.202</td>
<td>15.63 ± 8.79</td>
<td>135.6</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>13.88 ± 3.06</td>
<td>65</td>
</tr>
</tbody>
</table>

* RMP, randomly motile particles in computer simulation.

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**Table 4.** Surface excess concentrations for HCB1 and HCB437 in media of different ionic strengths.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Surface excess concn (μm⁻²·yr⁻¹) with I (M) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>HCB1</td>
<td>0.0825 ± 0.19</td>
</tr>
<tr>
<td>HCB437</td>
<td>1.78 ± 0.34</td>
</tr>
</tbody>
</table>

* The ± values give the 95% confidence limits. NM, not measured.
which are not reflected in the data presented thus far. The first change in behavior was that larger numbers of irreversibly adhered cells were found at the higher ionic strengths than at the lower ionic strengths. Irreversible adhesion is operationally defined as cells which are in focus with the plane of the glass but exhibit no translational or Brownian motion. However, these cells were not counted in tracking experiments. The second change in behavior is illustrated in Fig. 1, where bacterial traces from experiments with HCB437 are shown. In addition to the 50-μm-diameter circles which were observed by Frymier et al. (10) (in the highest ionic strength solution), 20-μm-diameter circles were also observed. At the lowest ionic strength, 100-μm-diameter circles were also observed. Although the 20- and 50-μm-diameter circles were seen at both ionic strengths, the 100-μm diameter circles were seen only in the experiments using low ionic strengths.

**DISCUSSION**

The results of the sedimentation velocity test indicate that some force which inhibits sedimentation must be acting on bacteria in the immediate vicinity of the glass. Qualitative observations confirm this, such as cells which left the surface did so by swimming away from it and not by slowly drifting downwards, as would be expected if they were settling. The results of the computer simulation further suggest that some force holding the actual bacteria near the surface exists, since simulated uncharged particles spend much less time near the surface than do their experimental counterparts. One might suggest that bacteria concentrate near the surface due to the impassability of the surface, i.e., cells whose trajectories would have carried them through the surface simply turn and swim along it. The computer simulation, which mimicked this wall effect but did not include any mechanism for attraction, demonstrated that impassability alone was not sufficient to account for the time cells spent near the surface.

At first glance, there appears to be a contradiction between the computer simulation results and surface excess calculations for the HCB1 strain. Although the computer simulation indicates that there may be an attraction to the surface, the 95% confidence interval brackets a surface excess of 0 at all ionic strengths, which is the case when there is no attraction to the glass surface. We believe that there is an attraction to the surface exhibited by these bacteria but that it is masked by the large deviations which result from the tumbling behavior of the wild type. The tumbling probability of HCB1 follows a Poisson distribution, meaning that the average run length and the standard deviation of the run lengths are equal (5). Since the amount of time spent near the surface is at least partially related to run time (bacteria seem more likely to exit the surface with a tumble), it seems reasonable that the time near the surface and surface excess data have large variations. Note that, in all cases, the smooth-swimming bacteria have much smaller confidence intervals than the wild type.

Although an attraction between the bacteria and the glass surface appeared to exist, it did not follow the expected trends predicted by the DLVO theory. We found neither a change in the amount of time bacteria spent swimming near the surface nor a change in the bacterial tendency to approach the surface with changing ionic strength. Even though our DLVO theory predictions are only semiquantitative, we expected to see a significant difference between behaviors at the different ionic strengths. Rutter and Vincent claim that it is reasonable to assume that the time a bacterium remains in the secondary minimum is proportional to $\exp[G_{\text{max}}/kT]$, where $G_{\text{min}}$ is the depth of the secondary minimum (29). If this conclusion is so, and if *E. coli* adheres in the secondary minimum during circling as proposed, we would expect a difference of 5 orders of magnitude between the times HCB1 bacteria spent near the surface in the high- and low-ionic-strength solutions. We also would expect the time spent near the surface to be infinite (i.e., irreversible adhesion) for HCB437 in the high-ionic-strength solution. Although some cells were observed to irreversibly adhere, most cells did not.

Other authors have reported increased adhesion and even increased reversible adhesion as the ionic strength of the medium increased. For example, Jewett studied the penetration of *Pseudomonas fluorescens* PH through filters and found that the fractional penetration increased over 100% within the same range of ionic strengths considered here (16). van Loosdrecht et al. observed increased adhesion of many species of bacteria, including *E. coli* to polystyrene disks with increasing ionic strength (30). Rijnaarts et al. (28), Johnson et al. (17), Martin et al. (24), and Gannon et al. (12) have observed greater bacterial breakthrough in columns packed with various materials as the ionic strength of the medium decreased. Meinders et al., who did not use ionic strength to vary the predictions of the DLVO theory, also found that the deposition efficiencies of several types of bacteria in a parallel-plate flow chamber were correlated with the depth of the secondary energy minimum (25). However, of those studies none directly observed the behavior of individual bacteria as they interacted with a surface. Although our qualitative observations of increasing numbers of bacteria resting immobile on the glass as ionic strength increases are more consistent with previously reported results, when bacteria exhibiting translational motion are exclusively considered, these changes with ionic strength disappear.

One change in bacterial swimming behavior with changing ionic strength that we did observe was the unexpected appearance of circles of larger diameters at the lowest ionic strength. This observation pertains solely to the smooth-swimming bacteria, which trace out nearly perfect circles on a glass surface. Although the wild type may remain near the surface for long times, also sometimes circling, its ability to tumble prevents it from swimming in complete circles. With their model of bacterial swimming behavior near a planar surface, Ramia et al. were able to predict that a bacterium swimming at a small, constant distance from the surface would swim in a circular pattern (27). The circular pattern results from the fact that the helical bacterial flagellum generates thrust in all three dimensions as it spins and that an asymmetry in the thrust in the $x$ direction (normal to the swimming direction and the plane of the surface) is introduced by the solid surface (27). Ramia et al. predict that the radius of the circle should be similar to the length of the entire organism (bacterium plus flagella), which for their model was about 10 μm (27).

The total length of a bacterium in our experiments was about 10 μm; therefore, the trace in Fig. 1C, which shows a bacterial trace 20 μm in diameter, agrees well with Ramia’s predictions. However, this does not explain why circles 50 μm in diameter are just as common at the high ionic strength (Fig. 1A and 1B) or why circles 100 μm in diameter appear at the lowest ionic strength (Fig. 1D).

In the model of Ramia et al., the bacteria swim at a minimum dimensionless distance, roughly equivalent to the helical radius of the model flagella (27), which is between 200 and 300 nm in *E. coli* (23). It is reasonable to assume that as the bacterium swims at a greater distance from the glass surface, the hydrodynamic effect of the glass surface on that bacterium will decrease. Therefore, bacteria swimming very close to the surface will trace out circles of small diameter while bacteria swimming further from the surface will trace out circles of
larger and larger diameters, until they are far enough away that there is no hydrodynamic effect and they swim in a straight line. Perhaps the bacteria which swim in 50-μm diameter circles are further from the glass and those that swim in 100-μm diameter circles are still far. This may indicate that there is some decrease in attraction between the bacteria and the surface at low ionic strength, although this decrease is much smaller than expected. The fact that circles of all three diameters occur at the low ionic strength may be explained by the fact that the bacteria are of a range of sizes and therefore may have slightly more or less attraction for the surface than the mean size considered in the calculations.

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