Differences between Chemisorbed and Physisorbed Biomolecules on Particle Deposition to Hydrophobic Surfaces †

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · OCTOBER 2005

Impact Factor: 5.33 · DOI: 10.1021/es050204l · Source: PubMed

6 AUTHORS, INCLUDING:

Bruce E Logan
Pennsylvania State University
458 PUBLICATIONS **33,084** CITATIONS

SEE PROFILE

Environ. Sci. Technol. **2005**, *39,* 6371−6377

Differences between Chemisorbed and Physisorbed Biomolecules on Particle Deposition to Hydrophobic Surfaces†

MICHAEL B. SALERNO,‡
SAM ROTHSTEIN,‡
CHISOMAGA NWACHUKWU,‡
HAITHEM SHELBI,‡
DARRELL VELEGOL,‡ AND
BRUCE E. LOGAN*,§

Department of Chemical Engineering and Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, Pennsylvania 16802

This study examines differences between chemisorbed and physisorbed biomolecules on bacterial adhesion to both hydrophobic and hydrophilic surfaces that are biologically nonspecific. Bacteria-sized latex microspheres were used as a simplified model in order to study these factors that affect microbial adhesion. Two biomolecules (protein A, poly-D-lysine) were covalently bound to microspheres in order to study the effect of proteins on particle filtration rates in columns packed with glass beads. When poly-D-lysine or protein A was covalently bonded to the microspheres, sticking coefficients (α) for the microspheres increased by up to an order of magnitude as compared with uncoated latex microspheres. The glass packing beads were then made hydrophobic by covalently attaching silane groups with different carbon-chain lengths (0.2, 1.2, and 2.8 nm). Sticking coefficients for the uncoated microspheres on these silanized packing beads ($\alpha = 0.15$ at 1 mM ionic strength; 0.76 at 100 mM) were larger than those on uncoated glass packing beads (0.02 at 1 mM; 0.15 at 100 mM). In addition, adhesion increased with ionic strength on both hydrophobic and hydrophilic surfaces. Physical adsorption gave different results. When either dextran or protein A was physically adsorbed to both the microspheres and the column, no appreciable change in adhesion was observed. Covalently attaching protein A to the microspheres increased their hydrophobicity, but sticking coefficients were large regardless of the substrate hydrophobicity as a result of biomolecule−surface interactions. This study demonstrates that, at high ionic strength, covalently attached hydrophobic species give much higher sticking coefficients for particles than do physically adsorbed species.

Introduction

Controlling and understanding bacterial adhesion is important in many fields, including prevention of biofilms on medical implants, reduction of drag on ship hulls (*1*, *2*), increased efficiency of water filtration systems (*3*−*5*), and improved efficacy of bioaugmentation methods for treating polluted landfills (*6*−*9*). A number of surface forces (*10*) (e.g., electrostatic and van der Waals interactions, receptor−ligand interactions) (*11*) determine the adhesion of the bacteria. These forces have been shown to depend on solution ionic strength (*12*−*14*), bacterial growth conditions (*15*), substrate physical properties (*16*), and bacterial surface molecular structure (*17*). While discerning the importance of the various forces is difficult when a bacterium is nearly touching the substrate—especially in light of nonuniformly distributed biomolecules on the bacterial surface (*18*)—we hypothesize in this work that bacterial surface proteins have a large impact on increasing adhesion. The attractive forces caused by proteins arise from their having hydrophobic regions and their electrostatic charge.

Traditionally, bacterium−substrate interactions have been described by classical Derjaguin−Landau−Verwey−Overbeek (DLVO) theory. This model accounts for van der Waals (VDW) and electrostatic forces, and it neglects complexities such as surface roughness (*19*−*21*) and charge nonuniformity (*22*−*24*). At separations greater than 50 nm, the attractive VDW forces are dominant but still relatively weak (*25*). As the bacterium approaches closer to the substrate, the VDW attraction becomes larger. However, when both the bacterium and the substrate are negatively charged (the most common case), the VDW attraction is counteracted by repulsive electrostatic interactions (*26*, *27*). Electrostatic interactions become less important at higher ionic strength, and so bacterial adhesion has usually been shown to increase with ionic strength (*28*−*31*).

Nevertheless, there are cases where the DLVO prediction concerning ionic strength fails (*12*), and so extensions to DLVO theory have been developed to account for hydrophobicity (*16*, *32*) and surface free energy (*33*−*35*). Hydrophobic groups on bacterial surfaces have been shown to increase the adhesion of many strains because the groups remove water adsorbed to surfaces (*36*, *37*). Thus, highly hydrated surfaces have actually been found to resist bacterial adhesion (*37*). Some bacteria have been found to be hydrophobic (*35*), while most have been found to be hydrophilic (*14*). One study even showed that adhesion of bacteria was similar to both hydrophobic and hydrophilic surfaces (*38*).

While in some studies the extensions explain adhesion data (*34*, *39*, *40*), this is not always the case (*41*−*43*). One explanation for the mixed success is that nanoscale (*18*, *44*−*46*) or molecular arguments do not appear, even at very small bacterium−substrate separations (*47*). For example, X-ray photoelectron spectroscopy (XPS) experiments suggest that protein molecules are involved in hydrophobic forces (*48*, *49*). Studies have shown that proteins produced by bacteria (e.g., fimbriae, flagella, or enzymes) are important in the initial adhesion stages (*50*). Although the evidence suggests that proteins are important to adhesion, it is not clear whether this results primarily from nonspecific hydrophobic interactions or more specific receptor−ligand interactions (*44*).

Our study aims to clarify the role of biomolecules on bacterial adhesion to both hydrophobic and hydrophilic surfaces that are biologically nonspecific. Our approach is to use colloidal microspheres that have a size similar to many bacteria (i.e., micron size). The microspheres are coated with biomolecules, and thus the colloids have a well-defined surface chemistry and a well-defined geometry.

† This paper is part of the Charles O'Melia tribute issue.

* Corresponding author telephone: (814)863-7908; fax: (814)863-7304; e-mail: blogan@psu.edu.
‡ Department of Chemical Engineering.
§ Department of Civil and Environmental Engineering.

10.1021/es050204l CCC: $30.25
Published on Web 05/28/2005

Materials and Methods

Colloid Preparation. Fluorescent carboxylated latex microspheres (1.0−1.5 μm diameter, Polysciences, Inc., Warrington, PA), some purchased with protein A (isoelectric point of 5.7) covalently bound and some without protein A bound, were used in all column experiments. The microspheres were either cleaned and used "bare" or cleaned and used after being coated by bonding poly-D-lysine (PDL, isoelectric point of 12.9) to their surface. To bind this protein to the surface of the carboxylated microspheres, a carbodiimide-based reaction was utilized (Carbodiimide Kit, Polysciences, Inc.) to attach the amino terminus of the protein directly to the carboxyl groups on the microspheres. The carboxylated microspheres were reacted with the carbodiimide to produce *O*-acylisourea, an intermediate that the protein then reacted with to become bound to the surface. In some experiments colloids were preequilibrated with biomolecules (protein A, PDL, or dextran) by suspending them in a biomolecule suspension, storing them overnight, centrifuging and resuspending them in fresh solution, and then allowing them to equilibrate for 30 min before use. This allowed for physical adsorption of the biomolecules to the surface.

The amount of protein A bonded to the microspheres was given by the manufacturer (Polysciences, Inc.). The amount of PDL bonded to latex microspheres was measured using a protein assay (BCA Protein Assay Kit, Pierce Biotechnology, Rockford, IL). Microspheres were treated with 300 μg of PDL and then centrifuged, with the supernatant being saved for protein quantification of the residual.

Test Solutions. The latex microspheres were suspended in phosphate buffer solutions (PBS) at a pH of 7.2 at ionic strengths of 1, 10, and 100 mM, using KH_2PO_4 (Fisher Scientific) and K_2HPO_4 (Fisher Scientific) in DI water (Milli-Q system, Millipore, Marlborough, MA). The 1 and 10 mM solutions were prepared by dilution of the 100 mM IS solution containing 1.55 g of KH_2PO_4 and 5.72 g of K_2HPO_4 in 1 L of DI water.

Column Adhesion Tests. Sticking coefficients (α) were measured in mini-column tests as described elsewhere (*51*). Columns were packed with bare glass beads or silane-coated glass beads, and the columns were preequilibrated to a specific solution by rinsing with 10 mL (~20 pore vol) of DI water. The beads were then rinsed with 10 mL of the specific solution. The solutions were either PBS or PBS containing 0.1 μmol/L of a biopolymer (protein A, PDL, or dextran). The sticking coefficient (α) was calculated from the Rajagopolan and Tien filtration model (*52*, *53*):

$$\alpha = \frac{-4a_c \ln(1 - R)}{3(1 - \theta)\eta L} \quad (1)$$

where a_c is the radius of the glass beads (20 μm), R is the fraction of bacteria retained in the column (i.e., the measured parameter), θ is the porosity (0.46 for SiO_2 beads), η is the collision efficiency (calculated for each experiment as described in ref *49*, but typically ~0.025), and L is the length of the slice of glass beads extruded from the column (0.3−0.6 cm). The top layer of the packing material is extruded from the column, sliced off, and analyzed for the amount of microspheres that adhered in that length of column. Although α is theoretically limited to a range of 0−1, α can be larger than unity as a result of underprediction of the number of collisions (via the parameter η) (*54*).

Column Packing. Glass beads (40 μm diameter, soda lime, Polysciences Inc., Warrington, PA) used as the column packing were cleaned by agitation in a 10% sulfuric acid bath for 3 h and then copiously rinsed with DI water prior to use or chemical treatment (*55*). In some experiments beads were treated with one of three different lengths of silane molecules (all from Fisher Scientific) to make them hydrophobic: methyltrichlorosilane (1-silane, 0.2 nm size), octyltrichlorosilane (8-silane, 1.2 nm), or octadecyltrichlorosilane (18-silane, 2.8 nm). The silanization procedure is described elsewhere in detail (*47*), but briefly it consists of adding glass beads (dried in an oven at 100°C for 2 h) into a silane solution (10% w/w in methanol), shaking by hand, and conducting several washing and decanting steps. Treated beads were stored in excess methanol at 4 °C prior to use.

TABLE 1. ζ-Potentials and Hydrophobicity Measurements for the Surfaces[a]

surface	ζ-potential (mV)	MATH test (% hydrophobic)	water contact angle (°)
SiO_2	−49 ± 2		13 ± 3
1-silane	−58 ± 3		72 ± 2
8-silane	−34 ± 3		86 ± 1
18-silane	−43 ± 4		76 ± 1
bare latex	−45 ± 2	10	
latex with protein A	−23 ± 1	54	
latex with PDL	−8 ± 2		

[a] Data for the first four surfaces previously published by our group (*47*). ζ-potential results for the last three surfaces previously published by our group (*46*).

ζ-Potential and Hydrophobicity Measurements. Zeta potentials (ζ) were measured on both latex and glass microspheres 5 times, with 20 cycles for each analysis (ZetaPALS analyzer, Brookhaven Instruments Corporation, Holtzville, NY). Since the large glass beads used in the column experiments (40 μm diameter) settle too quickly for ζ-potential analysis, smaller (4 μm diameter) SiO_2 beads from the same manufacturer (Polysciences, Inc.) were analyzed for ζ-potential. The ζ-potentials of the packing surfaces were −49 mV for silica, −58 mV for 1-silane, −34 mV for 8-silane, and −43 mV for 18-silane (*47*).

As previously reported (*47*), water contact angles showed that the silanized surfaces used in this study were hydrophobic, whereas the bare silica was highly hydrophilic. In our experiments contact angles of bare or silanized surfaces were measured using the sessile drop method (*56*), using a 3 μL drop of DI water (Table 1). Each sample was photographed and analyzed with the image analysis software, Scion Image (Scion Corp.). Microsphere adhesion to hydrocarbon (MATH) tests were performed to test the relative hydrophobicities of the microspheres. Great care was taken to maintain a consistent protocol for the MATH experiments (*57*, *58*), so that consistent results were obtained (*59*). Hexadecane (0.30 mL; Fisher Scientific) was added to a 1.3 mL sample of the microspheres in 100 mM PBS. The mixture was then vortexed for 2.0 min and then allowed to sit for 10.0 min. A 1.0 mL sample of the aqueous phase was then carefully withdrawn, filtered, and counted using an optical microscope. We then define a "percent hydrophobic" as the percentage of microspheres that end up in the organic (hydrophobic) phase.

Results

Protein A-Coated Microspheres. At an ionic strength of 100 mM, covalently bonding protein A to the surface of the microspheres consistently increased their sticking coefficients to both uncoated glass and silane-treated glass beads (Figure 1). The largest increase in the sticking coefficient (9.5×) was observed for the most hydrophilic surface (bare silica). Sticking coefficients of the protein A-coated microspheres to the three hydrophobic surfaces, relative to uncoated microspheres, increased by a factor of 2−3. Sticking coefficients of protein-A coated latex microspheres to silica and the three hydrophobic surfaces were not significantly different ($p > 0.15$). In the absence of the protein A coating on the

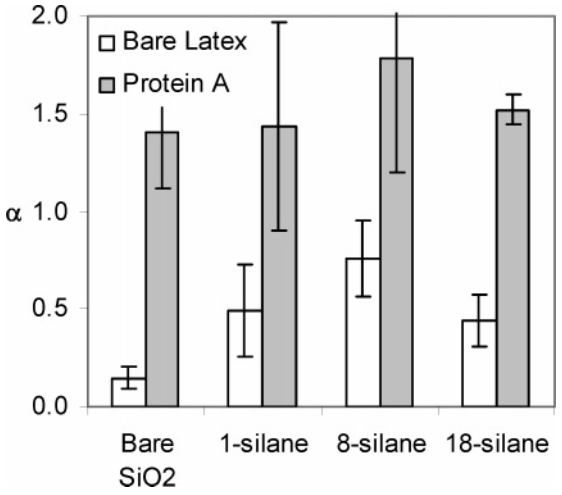

FIGURE 1. Sticking coefficients of bare latex microspheres and protein A-bound microspheres (ionic strength = 100 mM) to silica and silane-treated silica substrates. Protein A greatly increases the adhesion. Error bars are 95% confidence intervals.

microspheres, sticking coefficients for the three hydrophobic surfaces were not significantly different from each other ($p > 0.02$), but all three were significantly larger than the sticking coefficient for the uncoated hydrophilic glass surface ($p < 0.02$).

The presence of covalently bonded protein A on the surface of the colloids made their ζ-potential more positive (−23 mV) as compared with the bare latex microspheres (−45 mV) (Table 1) (*46*). Data from Polysciences showed that on average, there was 6.2×10^{-6} ng of protein A bonded per microsphere. MATH tests showed that relative hydrophobicity increased from less than 10% hydrophobic for the bare latex microspheres to 54% for the microspheres bound with protein A.

A comparison of Figure 2a,b reveals that in fact covalently bound protein A increases adhesion of microspheres to glass beads at ionic strengths from 1 to 100 mM ($p < 0.02$ in 9 of 12 comparisons). In three instances (adhesion to uncoated glass at IS = 1 mM, 8-silane-coated glass at IS = 1 mM, and 18-silane-coated glass at IS = 10 mM), there was no statistically significant change in the sticking coefficient. There is also a general trend of increasing sticking coefficient with ionic strength, both for bare latex microspheres and those bound with protein A. This increase in adhesion can be attributed to a reduction in the repulsive layer thicknesses of the two negatively charged surfaces with an increase in the solution IS.

When protein A was merely present in solution, meaning the protein A was physically bound rather than covalently bound, the adhesion of the microspheres to the column beads was slightly reduced as compared with the covalently bound case (see Figure 3, noting that this is a log scale). For both bare and 8-silane treated silica, in 1 mM ionic strength solution, the difference between physically preadsorbing the protein and covalently bonding the protein was not significant. However, in 100 mM ionic strength, the chemically bound protein A yielded at least 3× greater adhesion values than the physically bound protein A. In addition, in the cases with protein A in solution, the adhesion was actually less than the adhesion when no protein was present (this effect was only significant on bare silica).

Adhesion With Poly-D-Lysine (PDL). PDL, a highly positively charged protein, sharply increased microsphere adhesion to the beads at ionic strengths greater than 1 mM (Figure 4), as compared with Figure 2a. As ionic strength was increased, the sticking coefficient increased as well. On average, there was 1.2×10^{-5} ng/microsphere of PDL bound.

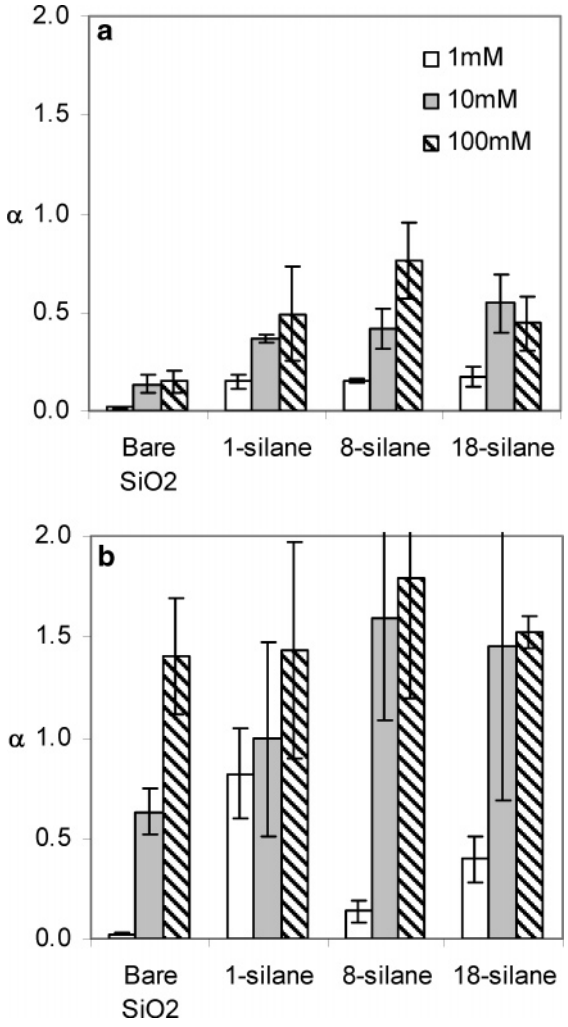

FIGURE 2. Sticking coefficients of bare latex microspheres (a) and protein A-bound microspheres (b) to various substrates. The sticking coefficients increased greatly when the microspheres had bound protein A on them. Error bars are 95% confidence intervals.

In the 1 mM IS solution, physically adsorbing the PDL did not provide as high of adhesion as when the protein was bound to the microspheres. However, in high ionic strength, the two cases yielded equivalent sticking coefficients. The PDL did not promote adhesion when in solution with hydrophobic 8-silane-treated silica. Addition of PDL to the latex microspheres increased their ζ-potential from −45 to −8 mV.

Adhesion With Dextran. The presence of dextran in solution did not significantly affect adhesion (Figure 5; note the log scale). Sticking coefficients of uncoated microspheres to bare glass and 8-silane-coated beads were not significantly altered by the presence of dextran in solution. An increase in ionic strength from 1 to 100 mM increased the sticking coefficients, but this increase was not altered by dextran in solution.

A direct comparison of the effects of the three different biomolecules versus an inorganic system (Figure 6) shows that presence of the different biomolecules in solution (i.e., physically adsorbed rather than covalently bound) did not substantially alter measured sticking coefficients. The only exception to this general trend is in the presence of PDL in solution. The presence of PDL increased the sticking coefficient, but only when the surface was uncoated glass. A summary of the sticking coefficients can be found in Table 2.

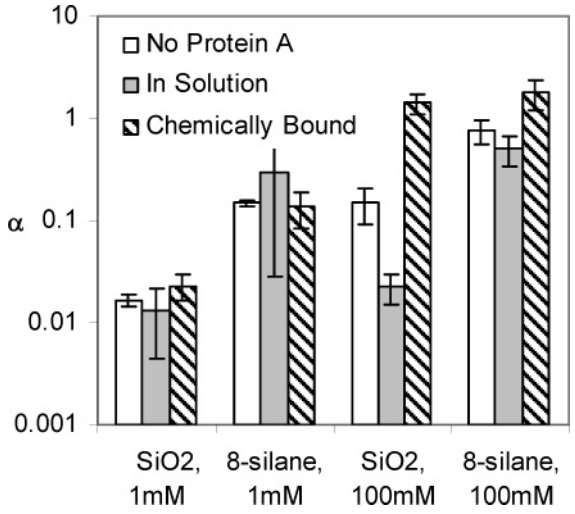

FIGURE 3. Microsphere sticking coefficients, with protein A in solution vs covalently bound to the microspheres. Differences between covalently bound and physically bound protein A appear only at 100 mM ionic strength. Error bars are 95% confidence intervals.

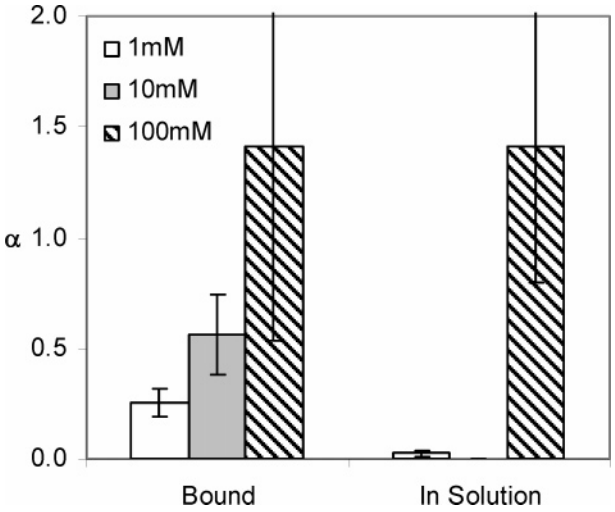

FIGURE 4. Particle deposition to silica, with poly-D-lysine (PDL) in solution vs bound. PDL greatly enhances adhesion at 100 mM, most likely due to a change in the protein's conformation. Error bars are 95% confidence intervals.

Discussion

When no biomolecule was present, the adhesion of the latex microspheres to the three hydrophobic surfaces was 3−10× greater than to hydrophilic silica (Figure 1). However, with protein A bound to the microspheres, the sticking coefficients for all four surfaces were large and were not statistically different from each other. This implies that the issue of substrate hydrophobicity is less important when protein A is present. However, this disagrees with many previous studies, which have shown that, for certain strains, bacterial adhesion does in fact depend on contact-surface hydrophobicity (*16*, *35*). A possible reason for this discrepancy is that our system has isolated the effect of a single protein and, therefore, reduces the confusion of having the complexity of a true bacterial surface. Interestingly, the hydrophobicity of the particle increases when protein A is covalently bound, and the adhesion of the microspheres to both hydrophilic and hydrophobic substrates also increases when protein A is covalently bound. Our data also show that the protein-coated microspheres do not require a biospecific surface to interact with in order to increase adhesion. Previous

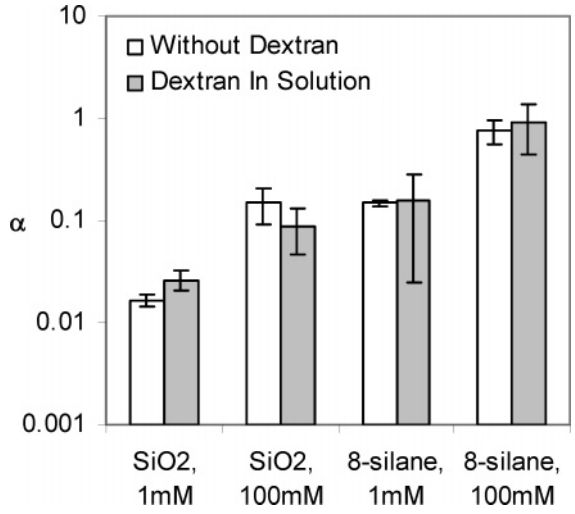

FIGURE 5. Particle deposition with and without dextran in solution. While ionic strength tends to increase sticking coefficients, the dextran has a negligible effect. Error bars are 95% confidence intervals.

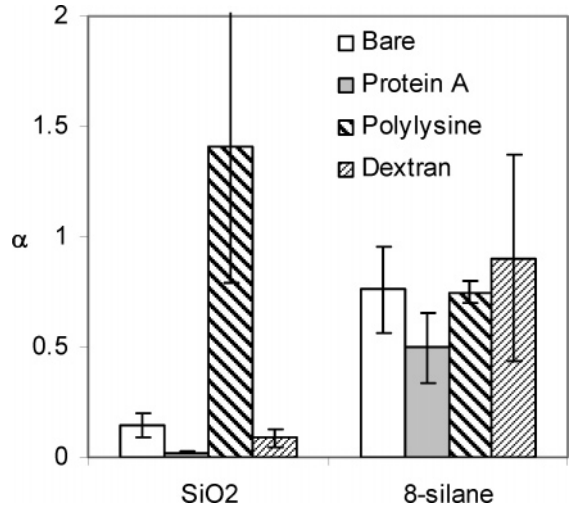

FIGURE 6. Effects of biomolecules in solution at 100 mM. Dextran does not increase adhesion for the physically bound case. Protein A increases adhesion only for the covalently bound case and not for the physically bound case as shown. PDL increases adhesion for both physically bound and covalently bound, but only on the bare silica. Error bars are 95% confidence intervals.

work by other investigators has shown, using real time microscopy, that many types of bacteria attach to hydrophobic, methyl-terminated self-assembled monolayers (SAM), while the one exception was a *Pseudomonas* strain, which showed attachment to a hydrophilic, hydroxyl-terminated SAM (*60*). A possible explanation posed by the authors was that the bacteria utilized different components of attachment with different surfaces (*61*). For example, bacteria may bind primarily via polysaccharides to hydrophilic surfaces but bind to hydrophobic surfaces by proteins or lipids (*11*).

Adhesion increased with ionic strength for all experimental conditions. This is consistent with traditional DLVO theory, as increasing the ionic strength decreases the Debye length and hence suppresses electrostatic repulsions between negatively charged latex microspheres and silica beads. The effect of ionic strength was much stronger when protein A was present (Figure 2). This is attributed to a rearrangement of the protein on the surface of the microspheres, creating a more condensed configuration, which would allow the microspheres to better approach the surfaces. Our result that

TABLE 2. Summary of Sticking Coefficients of Microspheres on both Silica and 8-Silane at 1 and 100 mM Ionic Strengths

			chemically bound		physically adsorbed		
ionic strength	surface	bare latex	protein A	PDL	protein A	PDL	dextran
1 mM	silica	0.016 ± 0.002	0.022 ± 0.006	0.255 ± 0.061	0.012 ± 0.008	0.026 ± 0.013	0.026 ± 0.006
	8-silane	0.148 ± 0.011	0.138 ± 0.054		0.290 ± 0.262	0.152 ± 0.073	0.153 ± 0.128
100 mM	silica	0.148 ± 0.056	1.403 ± 0.288	1.415 ± 0.878	0.442 ± 0.133	1.413 ± 0.618	0.089 ± 0.042
	8-silane	0.760 ± 0.194	1.789 ± 0.593		0.498 ± 0.158	0.749 ± 0.054	0.903 ± 0.468

ionic strength is an important factor in bacterial adhesion is supported extensively in the literature (*12*−*14*).

At low ionic strength, the presence of protein A, whether covalently bound to the microspheres or only in solution, did not have an appreciable affect on particle deposition in the columns (Figure 3). This is most likely because at this low ionic strength, the protein was shorter than the Debye length; therefore, electrostatic repulsion was still important. At high ionic strength, however, the bound protein had a large impact on adhesion, whereas the protein in solution increased adhesion only slightly. The fact that the physically bound protein A had a weaker effect is likely due to desorption of the protein from the surfaces. As is common in ion exchange chromatography columns, which will elute proteins when high ionic strength solutions are injected (*62*), our system may be showing similar behavior by desorbing the protein and hence not increasing colloid deposition significantly when protein is merely present in solution.

Binding PDL to the microspheres resulted in large increases in sticking coefficients on bare silica, regardless of ionic strength. PDL was used as an example of a large (134 kDa MW), highly positively charged (charge of +900 at neutral pH) protein (*46*). This was in contrast to protein A, which is a moderately sized protein (around 55 kDa) and is slightly negatively charged (charge is −6.8 at neutral pH). Therefore, based on electrostatics alone, the observed increase in adhesion was expected since the positive molecules would add to the attraction between the microspheres and the surfaces. Physically adsorbing the PDL to the silica and microspheres caused a similar increase in adhesion at high ionic strength but did not affect adhesion at low ionic strength. It is likely that the microspheres and silica were both coated with enough PDL that it actually caused electrostatic repulsion. On 8-silane-treated surfaces, adsorbing the PDL had no appreciable effect at either 1 or 100 mM. Most likely, the PDL did not adsorb to the 8-silane due to the hydrophobic surface not attracting the highly charged, solvated, hydrophilic molecules. We did not test the 1-silane and 18-silane for this experiment since the behavior of the colloids would be similar to the 8-silane cases.

Depletion forces due to the biomolecules in solution were also considered as possibly contributing to the behavior of the colloids. However, an estimate at the experimental conditions revealed that depletion forces were less than 0.01 pN. Therefore, they most likely did not affect the adhesion of the colloids. Bridging effects are also possible in this system, especially for PDL. However, if bridging were important, we would expect to see evidence of it in all cases with PDL. Instead, the only significant increase in adhesion due to physisorbed PDL occurred at 100 mM on bare silica, which can be attributed to electrostatics.

For all four cases tested, having dextran (MW 140 kDa) in solution with the microspheres and columns did not cause a statistically significant change in adhesion. Dextran was used as a model polysaccharide since the surfaces of Gram-negative bacteria are composed not only of proteins and phospholipids but also polysaccharides. Our result is opposite to previous work that showed dextran decreases adhesion of carboxylated-modified latex microspheres in soil systems (*63*). This discrepancy is most likely due to the confounding effects of metal oxides, humic acids, and other impurities prevalent in a soil system but not present in our controlled experiments. Our work also shows that having any of the biomolecules in solution (i.e., only physical adsorption) with the surfaces does not create significant changes in adhesion, except in the case of a polycationic protein in low ionic strength. Along with this one exception to the rule, the large increases in sticking coefficient were seen only for cases in which the proteins were chemically bound to the microspheres. This indicates that biopolymers in solution (such as those that may be excreted by bacteria to form a conditioning film, which our system does not have) may not be required for bacterial adhesion.

In conclusion, we have shown that a protein covalently bound on a microsphere's surface can significantly increase its adhesion in a packed column. Attaching protein A to the microspheres increases their hydrophobicity, yet the overall adhesion is independent of substrate hydrophobicity; the microspheres adhered just as well to a hydrophilic surface as to a hydrophobic one, despite the lack of biospecific interactions with the substrates. It can be concluded, therefore, that proteins play a significant role in the attachment of bacteria to non-biospecific substrates.

Acknowledgments

This research was funded by the National Science Foundation CRAEMS program (Grant CHE-0089156). This research was also funded in part by the Penn State Biogeochemical Research Initiative for Education (BRIE) (NSF IGERT Grant DGE-9972759). We thank Mary Parent for help with the MATH experiments. It is an honor to publish this paper in a special issue of the Journal honoring Professor Charles R. O'Melia. His work on particles and filtration has been an inspiration to us over the years, and we sincerely appreciate not only his work in this area but especially his leadership and contributions to the environmental engineering profession.

Literature Cited

(1) Marshall, K. C.; Stout, R.; Mitchell, R. Mechanism of the initial events in the sorption of marine bacteria to surfaces. *Can. J. Microbiol.* **1971**, *17*, 1413.

(2) Dempsey, M. J. Marine bacterial fouling—a scanning electron-microscope study. *Mar. Biol.* **1981**, *61*, 305.

(3) Logan, B. E.; Hilbert, T. A.; Arnold, R. G. Removal of bacteria in laboratory filters: models and experiments. *Water Res.* **1993**, *27*, 955.

(4) Cho, J.; Amy, G.; Pellegrino, J. Membrane filtration of natural organic matter: initial comparison of rejection and flux decline characteristics with ultrafiltration and nanofiltration membranes. *Water Res.* **1999**, *33*, 2517.

(5) Korbutowicz, M. K.; Nowak, K. M.; Winnicki, T. Analysis of membrane fouling in the treatment of water solutions containing humic acids and mineral salts. *Desalination* **1999**, *126*, 179.

(6) Marlow, H. J.; Duston, K. L.; Wiesner, M. R.; Thompson, M. T.; Wilson, J. T.; Ward, C. H. Microbial transport through porous media: the effects of hydraulic conductivity and injection velocity. *J. Hazard. Mater.* **1991**, *28*, 65.

(7) Steffan, R. J.; Sperry, K. L.; Walsh, M. T.; Vainberg, S.; Condee, C. W. Field-scale evaluation of in situ bioaugmentation for remediation of chlorinated solvents in groundwater. *Environ. Sci. Technol.* **1999**, *33*, 2771.

(8) Harvey, R. W.; Garabedian, S. P. Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer. *Environ. Sci. Technol.* **1991**, *25*, 178.
(9) Champ, D. R.; Schroeter, J. Bacterial transport in fractured rock: a field-scale tracer test at the Chalk River Nuclear Laboratories. *Water Sci. Technol.* **1988**, *20*, 81.
(10) Israelachvili, J. N. *Intermolecular and Surface Forces*; Academic Press: London, 1992.
(11) Fletcher, M. Bacterial attachment in aquatic environments: a diversity of surfaces and adhesion strategies. *Bacterial Adhesion*; Wiley-Liss: New York, 1996; pp 1−24.
(12) Camesano, T. A.; Logan, B. E. Influence of fluid velocity and cell concentration on the transport of motile and nonmotile bacteria in porous media. *Environ. Sci. Technol.* **1998**, *32*, 1699.
(13) Bolster, C. H.; Mills, A. L.; Hornberger, G. M.; Herman, J. S. Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces. *J. Contam. Hydrol.* **2001**, *50*, 287.
(14) Li, B.; Logan, B. E. Bacterial adhesion to glass and metal-oxide surfaces. *Colloids Surf., B* **2004**, *36*, 81.
(15) Gallardo-Moreno, A. M.; Gonzalez-Martin, M. L.; Brugue, J. M.; Perez-Giraldo, C.; Sanchez-Silos, R.; Gomez-Garcia, A. C. Influence of the growth medium, suspending liquid and measurement temperature on the physico-chemical surface properties of two *Enterococci* strains. *J. Adhes. Sci. Technol.* **2003**, *17*, 1877.
(16) Van Loosdrecht, M. C. M.; Lyklema, J.; Norde, W.; Schraa, G.; Zehnder, A. J. B. Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. *Appl. Environ. Microbiol.* **1987**, *53*, 1898.
(17) Schaer-Zammaretti, P.; Ubbink, J. Imaging of lactic acid bacteria with AFM—elasticity and adhesion maps and the relationship to biological and structural data. *Ultramicroscopy* **2003**, *97*, 199.
(18) Jones, J. F.; Feick, J. D.; Imoudu, D.; Chukwumah, N.; Vigeant, M.; Velegol, D. Oriented adhesion of *Escherichia coli* to polystyrene particles. *Appl. Envrion. Microbiol.* **2003**, *69*, 6515.
(19) Shellenberger, K.; Logan, B. E. Effect of molecular scale roughness of glass beads on colloidal and bacterial deposition. *Environ. Sci. Technol.* **2002**, *36*, 184.
(20) Gotzinger, M.; Peukert, W. Particle adhesion force distributions on rough surfaces. *Langmuir* **2004**, *20*, 5298.
(21) Sun, N.; Walz, J. Y. A model for calculating electrostatic interactions between colloidal particles of arbitrary surface topology. *J. Colloid Interface Sci.* **2001**, *234*, 90.
(22) Feick, J. D.; Velegol, D. The electrophoresis of spheroidal particles having a random distribution of zeta potential. *Langmuir* **2000**, *16*, 10315.
(23) Feick, J. D.; Velegol, D. Measurements of charge nonuniformity on polystyrene latex particles. *Langmuir* **2002**, *18*, 3454.
(24) Feick, J. D.; Chukwumah, N.; Noel, A. E.; Velegol, D. Altering surface charge nonuniformity on individual colloidal particles. *Langmuir* **2004**, *20*, 3090.
(25) Busscher, H. J.; Sjollema, J.; van der Mei, H. C. Relative importance of surface free energy as a measure of hydrophobicity in bacterial adhesion to solid surfaces. In *Microbial Cell Surface Hydrophobicity*; Doyle, R. J., Rosenberg, M., Eds.; American Society for Microbiology: Washington, DC, 1990; p 335.
(26) Verwey, E. J. W.; Overbeek, J. Th. G. *Theory of the Stability of Lyophobic Colloids*; Elsevier: Amsterdam, 1948.
(27) Madigan, M. T.; Martinko, J. M.; Parker, J. *Brock Biology of Microorganisms*, 9th ed.; Prentice Hall: Upper Saddle River, NJ, 1997.
(28) Weerkamp, A. H.; Uyen, H. M.; Busscher, H. J. Effect of zeta potential and surface energy on bacterial adhesion to uncoated and saliva-coated human enamel and dentin. *J. Dent. Res.* **1988**, *67*, 1483.
(29) Rijnaarts, H. H. M.; Norde, W.; Lyklema, J.; Zehnder, A. J. B. DLVO and steric contributions to bacterial deposition in media of different ISs. *Colloids Surf., B* **1999**, *14*, 179.
(30) Zita, A.; Hermansson, M. Effects of IS on bacterial adhesion and stability of flocs in a wastewater activated sludge system. *Appl. Environ. Microbiol.* **1994**, *60*, 3041.
(31) Deshpande, P. A.; Shonnard, D. R. Modeling the effects of systematic variation in IS on the attachment kinetics of *Pseudomonas fluorescens* UPER-1 in saturated sand columns. *Water Resour. Res.* **1999**, *35*, 1619.
(32) van Oss, C. J. Hydrophobicity of biosurfaces—origin, quantitative determination and interaction energies. *Colloids Surf., B* **1995**, *5*, 91.
(33) Jucker, B. A.; Zehnder, A. J. B.; Harms, H. Quantification of polymer interactions in bacterial adhesion. *Envrion. Sci. Technol.* **1998**, *32*, 2909.
(34) Poortinga, A. T.; Bos, R.; Busscher, H. J. Reversibility of bacterial adhesion at an electrode surface. *Langmuir* **2001**, *17*, 2851.
(35) Ong, Y. L.; Razatos, A.; Georgiou, G.; Sharma, M. M. Adhesion forces between *E. coli* bacteria and biomaterial surfaces. *Langmuir* **1999**, *15*, 2719.
(36) Pringle, J. H.; Fletcher, M. Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. *Appl. Environ. Microbiol.* **1983**, *45*, 811.
(37) Pringle, J. H.; Fletcher, M. The influence of substratum hydration and adsorbed macromolecules on bacterial attachment to surfaces. *Appl. Environ. Microbiol.* **1986**, *51*, 1321.
(38) Pedersen, K.; Holmstrom, C.; Olsson, A.; Pedersen, A. Statistic evaluation of the influence of species variation, culture conditions, surface wettability and fluid shear on attachment and biofilm development of marine bacteria. *Arch. Microbiol.* **1986**, *145*, 1.
(39) Jucker, B. A., Harms, H., Zehnder, A. J. B. Polymer interactions between five gram-negative bacteria and glass investigated using LPS micelles and vesicles as model systems. *Colloids Surf., B* **1998**, *11*, 33.
(40) Abu-Lail, N. I. and Camesano, T. A. Elasticity of *Pseudomonas putida* KT2442 surface polymers probed with single-molecule force microscopy. *Langmuir* **2002**, *18*, 4071.
(41) Truesdail, S. E.; Lukasik, J.; Farrah, S. R.; Shah, D. O.; Dickinson, R. B. Analysis of bacterial deposition on metal (hydr)oxide-coated sand filter media. *J. Colloid Interface Sci.* **1998**, *203*, 369.
(42) Meinders, J. M.; van der Mei, H. C.; Busscher, H. J. Deposition efficiency and reversibility of bacterial adhesion under flow. *J. Colloid Interface Sci.* **1995**, *176*, 329.
(43) Redman, J. A.; Walker, S. L.; Elimelech, M. Bacterial adhesion and transport in porous media: role of the secondary energy minimum. *Environ. Sci. Technol.* **2004**, *38*, 1777.
(44) Lower, S. K.; Hochella, M. F.; Beveridge, T. J. Bacterial recognition of mineral surfaces: nanoscale interactions between *Shewanella* and α-FeOOH. *Science* **2001**, *292*, 1360.
(45) Xu, L. C.; Vadillo-Rodriguez, V.; Logan, B. E. Residence time, loading force, pH and ionic strength affect adhesion forces between colloids and biopolymer-coated surfaces. *Langmuir* **2005**, in press.
(46) Xu, L. C.; Logan, B. E. Atomic force microscopy colloid probe analysis of interactions between proteins and surfaces. *Environ. Sci. Technol.* **2005**, *39* (10), 3592.
(47) Salerno, M. B.; Logan, B. E.; Velegol, D. Importance of molecular details in predicting bacterial adhesion to hydrophobic surfaces. *Langmuir* **2004**, *20*, 10625.
(48) Van der Mei, H. C.; Busscher, H. J. The use of X-ray photoelectron spectroscopy for the study of oral streptococcal cell surfaces. *J. Adv. Dent. Res.* **1997**, *11*, 388.
(49) van Oss, C. J.; Docoslis, A.; Giese, R. F. Free energies of protein adsorption onto mineral particles—from the initial encounter to the onset of hysteresis. *Colloids Surf., B* **2001**, *22*, 285.
(50) Danielsson, A.; Norkrans, B.; Bjornsson, A. On bacterial adhesion—the effect of certain enzymes on adhered cells of a marine *Pseudomonas* sp. *Botanika* **1977**, *No. 20*, 13.
(51) Gross, M. J.; Albinger, O.; Jewett, D. G.; Logan, B. E.; Bales, R. C.; Arnold, R. G. Measurement of bacterial collision efficiencies in porous media. *Water Res.* **1995**, *29*, 1151.
(52) Rajagopalan, R.; Tien, C. Trajectory analysis of deep-bed filtration with the sphere-in-cell porous media model. *AIChE J.* **1976**, *22*, 523.
(53) Logan, B. E.; Jewett, D. G.; Arnold, R. G.; Bouwer, E.; O'Melia, C. R. Clarification of clean-bed filtration models. *J. Environ. Eng.* **1995**, *121*, 869.
(54) Martin, M. J.; Logan, B. E.; Johnson, W. P.; Jewett, D. J.; Arnold, R. G. Scaling bacterial filtration rates in different sized porous media. *J. Environ. Eng.* **1996**, *122*, 407.
(55) Burks, G. A.; Velegol, S. B.; Paramonova, E.; Lindenmuth, B. E.; Feick, J. D.; Logan, B. E. Macroscopic and nanoscale measurements of the adhesion of bacteria with varying outer layer surface composition. *Langmuir* **2003**, *19*, 2366.
(56) Adamson, W.; Gast, A. P. *Physical Chemistry of Surfaces*, 6th ed.; Wiley-Interscience: New York, 1997.
(57) Rosenberg, M. Bacterial adherence to hydrocarbons: a useful technique for studying cell surface hydrophobicity. *FEMS Microbiol. Lett.* **1984**, *22*, 289.
(58) Lichtenberg, D.; Rosenberg, M.; Sharfman, N.; Ofek, I. A kinetic approach to bacterial adherence to hydrocarbon. *J. Microbiol. Methods* **1985**, *4*, 141.

(59) Parent, M. E.; Velegol, D. *E. coli* adhesion to silica in the presence of humic acid. *Colloids Surf., B* **2004**, *39*, 45.
(60) Wieneck, K. M.; Fletcher, M. Bacterial adhesion to hydroxyl- and methyl-terminated alkanethiol self-assembled monolayers. *J. Bacteriol.* **1995**, *177*, 1959.
(61) Fletcher, M.; Marshall, K. C. Bubble contact angle method for evaluating substratum interfacial characteristics and its relevance to bacterial attachment. *Appl. Envrion. Microbiol.* **1982**, *44*, 184.
(62) Kopaciewicz, W.; Rounds, M. A.; Fausnaugh, J.; Regnier, F. E. Retention model for high-performance ion-exchange chromatography. *J. Chromatogr.* **1983**, *266*, 3.
(63) Camesano, T. A.; Unice, K. M.; Logan, B. E. Modeling dynamic blocking of colloids in porous media using intracolumn deposition patterns and breakthrough curves. *Colloids Surf., A* **1999**, *160*, 291.

Received for review January 31, 2005. Revised manuscript received April 7, 2005. Accepted May 1, 2005.

ES050204L