APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2008, p. 2559–2564 Vol. 74, No. 9
0099-2240/08/$08.00+0 doi:10.1128/AEM.02839-07

MINIREVIEW

Specific Molecular Recognition and Nonspecific Contributions to Bacterial Interaction Forces[▽]

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Bacteria adhere to surfaces by virtue of their interaction forces with a substratum surface. A few decades ago, a paper on bacterial adhesion to surfaces typically would either commence with the statement (15) "bacterial adhesion to surfaces is mediated by highly specific, stereo-chemical interactions between complementary components on the interacting surfaces" or (16, 24) "bacterial adhesion is mediated by a complicated interplay between attractive Lifshitz-Van der Waals forces and repulsive or attractive electrostatic and acid-base forces, originating from the interacting surfaces." Generally, the "specific" approach was favored by microbiologists and biochemists, while physico-chemists usually took a "nonspecific" approach.

The two approaches were reconciled with each other (7, 8) by the realization that both interactions originate from the same, fundamental physico-chemical forces (Lifshitz-Van der Waals, electrostatic, and acid-base interaction) (37). Nonspecific, Lifshitz-Van der Waals interactions operate over longer distances (several tens of nanometers) and originate from all atoms in the interacting entities. The summation of the relatively weak pairwise interactions between all atoms in an adhering bacterium and a substratum yields the final interaction force, similar to the origin of the gravitational force of the earth. Specific interactions, making up for molecular recognition between ligand and receptor molecules, operate over spatially well-confined stereochemical regions, established for instance by interactions between acid, electron-accepting and basic, electron-donating groups or oppositely charged domains, at close approach (up to several nanometers).

Characterization of the bacterial cell and substratum surfaces in terms of their zeta potentials and surface free energies (from measured contact angles with liquids) offers the possibility to calculate the electrostatic and Lifshitz-Van der Waals contributions to the interaction force between two entities in an approach called the DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory (5, 16). In the so-called "extended DLVO" theory (38), acid-base interaction forces are accounted for in addition to Lifshitz-Van der Waals and electrostatic forces. Application of physico-chemical theories toward explaining bacterial adhesion to surfaces has not always been successful, not even adhesion to inert (nonbiological) surfaces. After evaluating over 250 references, Bos et al. (5) concluded that the only general conclusion to be drawn was that negatively charged bacteria adhere more rapidly to a positively charged than to a negatively charged substratum surface. The flaws of a physico-chemical approach based on overall surface characteristics become especially clear when considering bacterial adhesion to protein-coated surfaces, as illustrated by the example in Table 1.

First of all, it should be noted from Table 1 that the presence or absence of antigen I/II on the streptococcal cell surface has only minor effect, if any, on the cell surface hydrophobicity by water contact angles or on the bacterial zeta potential. In a nonspecific approach, one would expect similar adhesion of the two strains to a given surface, which is indeed the case for bare glass. Based on nonspecific interactions, however, it cannot be explained why the strain with antigen I/II adheres in almost four-fold-higher numbers than the strain without antigen I/II after the glass is coated with a salivary conditioning film. Clearly, neither the water contact angles nor zeta potentials are able to probe the presence of localized, microscopic attractive domains that constitute the molecular recognition groups on the interacting cell surfaces. With the introduction of the atomic force microscope (AFM), it has become possible to probe the physico-chemical properties of the bacterial cell surface at a microscopic level, including interaction forces between surfaces (9, 12). In this respect, it is important to realize that different interpretations can be given to the word "specific." In microbial adhesion, the word "specific" is generally associated with molecular recognition phenomena, but sometimes it is also used to designate short-range stereochemical interactions, such as acid-base bonding (1), that are in our view not considered to be specific in the sense of molecular recognition (notwithstanding that acid-base interactions can contribute to molecular recognition). Along these lines, binding forces between fibronectin-coated AFM tips and tissue-invasive and noninvasive *Staphylococcus aureus* strains have been compared, rather than establishing contributions of specific molecular recognition and nonspecific forces to the interaction (41).

It is the aim of this minireview to provide a comparison of the specific and nonspecific contributions to the forces that mediate bacterial adhesion to inert and protein-coated surfaces, based on AFM data. To this end, we will first briefly describe how the AFM can be used to measure real-life bac-

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▽ Published ahead of print on 14 March 2008.

TABLE 1. Two oral streptococci that solely differ in the presence of surface antigen I/II have similar overall surface characteristics[a]

S. mutans strain	Water contact angle (°)	Zeta potential (mV)	Adhesion (10^6 cm^{-2}) to:	
			Glass	Salivary conditioning film
LT11 (with antigen I/II)	29	−20	12.1	9.6
IB03987 (without antigen I/II)	33	−25	11.8	2.5

[a] Surface characteristics include water contact angles, zeta potentials, and the ability in line to adhere equally well to an inert glass surface but not to an adsorbed film of salivary proteins. Data were taken from studies by Xu et al. (40) and Petersen et al. (25).

terial interaction forces and single-bond molecular recognition forces. Data sets referring to bacterial interaction forces and single-bond molecular forces have appeared as separate classes of data in the literature and have never been combined. After a review of both data sets, they are combined in the section Synthesis and Conclusion in order to compare specific- and nonspecific contributions to the forces mediating bacterial adhesion.

BACTERIAL ADHESION FORCES PROBED BY AFM

The AFM provides an excellent tool to measure interaction forces between surfaces. Usually, approach or retraction forces are measured between the tip (27), a colloid probe (28), or a bacterium attached to the AFM's cantilever and a second surface, which can be either an inert protein-coated or immobilized bacterial cell surface (18, 35).

When retracting the probe from a surface, the probe will stay in contact with the surface until the elastic restoring cantilever force of the bent cantilever overcomes the interaction forces between the surfaces under study, yielding an estimate of the attractive force at a certain distance (9).

The AFM can be used in two entirely different ways. (i) By attaching bacteria to the cantilever, interaction forces can be determined between a bacterium and a substratum surface or another immobilized bacterium. These measurements are subject to considerable uncertainty regarding the actual contact area between the interacting surfaces, but this disadvantage has to be weighed against the fact that such measurements mimic real-life conditions (see Fig. 1 for an example). Moreover, probing of interaction forces in the absence of a sharp tip may avoid damage to the inner cell surface, as has been described to happen for naturally immobilized *Staphylococcus epidermidis* cells on glass (21, 22). (ii) By functionalizing the AFM tip and the surface to be probed with relevant biomolecules (13, 17), single-bond molecular recognition forces can be measured by careful analysis of individual detachment events during retraction (see Fig. 2 for an example). The measurement of molecular recognition forces between ligands and receptors is highly precise and relevant to understand the specific contributions to real-life interactions between biological surfaces.

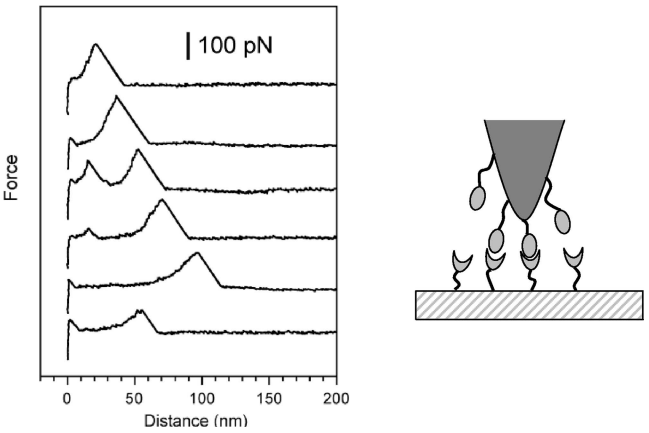

FIG. 2. Example of single-bond interaction forces during retraction. Disruption of each bond yields a small adhesion force during retraction. (Adapted in part from reference 34. Copyright 2003 American Chemical Society).

REAL-LIFE BACTERIAL INTERACTION FORCES

In Table 2 we have compiled some "real-life" bacterial interaction forces in the absence and presence of specific force contributions, as derived from AFM measurements. Nonspecific contributions to the interaction forces are always present, but specific contributions depend on the absence or presence of specific recognition, mediated by target and receptor molecules at the interacting surfaces.

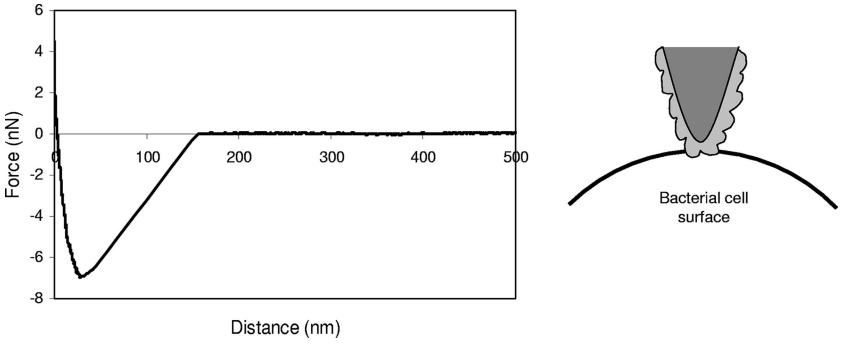

FIG. 1. Example of interaction forces between a protein-coated tip and a bacterial cell surface during retraction. Interaction forces of multiple bonds between adsorbed proteins and bacterial cell surfaces are accumulated into one adhesion force peak.

TABLE 2. Bacterial interaction forces in the absence and presence of specific recognition[a]

Bacterial adhesion parameter	Bacterial interaction (nN):		Reference
	With specific contribution	Without specific contribution	
Streptococci to salivary films			40
pH 5.8	Median, 0.0 Range, −1.2	Median, 0.0 Range, −0.1	
pH 6.8	Median, −0.4 Range, −2.9	Median, −0.1 Range, −0.4	
Streptococci to laminin films			6
pH 5.8	Median, 0.0 Range, −5.0	Median, 0.0 Range, −1.5	
pH 6.8	Median, −0.1 Range, −4.9	Median, −0.1 Range, −2.1	
Coaggregation between actinomyces and streptococci	Mean, −3.0 to −4.0	Mean, −1.0	26
Aggregation between enterococci	Mean, −2.3 to −2.6	Mean, −1.2 to −1.5	39
Summary			
pH dependence	Increases with pH	Increases with pH	
Force value	−3 to −5	0 to −2	

[a] Note that some of the older data were analyzed according to parametric statistics (force contributions represented by mean values), whereas more recent data analyses make use of nonparametric statistics (force contributions represented by median and range values of the distribution).

The data for streptococcal adhesion to salivary and laminin films involve *Streptococcus mutans* LT11 and *S. mutans* IB03987, strains with and without the specific recognition surface protein antigen I/II, respectively. Antigen I/II is involved in the adhesion of streptococci to salivary films and extracellular matrix proteins, like laminin. Streptococci with antigen I/II adhere stronger to salivary pellicles and laminin films than streptococci without antigen I/II, while furthermore the adhesive forces increase with increasing pH. Nonparametric statistics have demonstrated these differences in median force values to be significant (6, 40), although the largest effects are seen on the range values of the distributions.

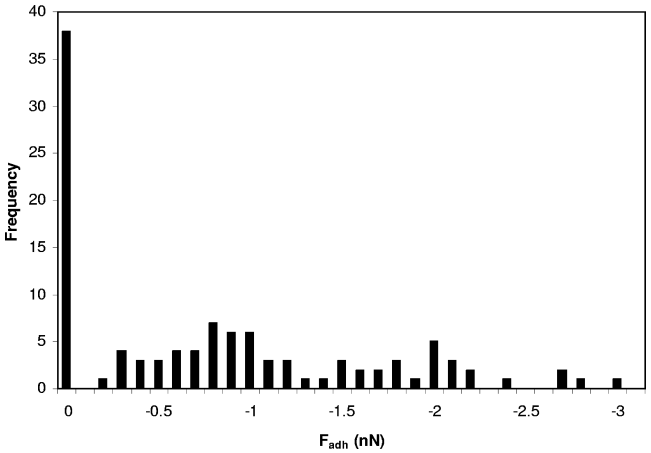

FIG. 3. Nonparametric distribution of adhesion forces (F_{adh}) between a protein-coated AFM tip and a bacterial cell surface, showing a trimodal distribution with a large range value.

The nonparametric, wide distributions generally observed in interaction force measurements by AFM (see Fig. 3 for an example) suggest that the surface characteristics are not homogeneously distributed over all bacterial cells probed during AFM. Indeed, culture heterogeneities are quite common and with the introduction of instrumentation that can measure properties of individual bacteria, like AFM, this is becoming more and more obvious. Furthermore, so-called "zeta sizing" has demonstrated that subpopulations with different cell surface charges exist within axenic cultures (3, 36). Different subpopulations within one culture can also differ in flagellation (29), natural competence (11), or autofluorescence (19). It is likely that the largest force values, as indicated by the range values in the nonparametric AFM interaction force distributions, represent a subpopulation that must be considered most relevant for adhesion: if only 1% of a culture would be represented by the range value, in a suspension of 10^6 bacteria per ml, this would represent 10^4 bacteria per ml with a strong affinity for a substratum! Alternatively, a nonparametric, wide interaction force distribution may reflect a heterogeneity over the surface of one individual bacterium (6, 13, 26, 40), such as for instance described for *Pseudomonas putida* cells, where AFM has demonstrated a range of adhesion affinities and polymer lengths on a single bacterium (10). However, also if the wide nonparametric distributions would be indicative of heterogeneity over a single cell surface, this would still point to the importance of the range value since the highest force values measured on a single cell surface are most relevant in

TABLE 3. Overview of single-bond molecular recognition forces

Molecular pair	Interaction force (nN/single bond)	Reference
Avidin-biotin	0.160	21
Avidin-iminobiotin	0.085	14
Streptavidin-biotin	0.257	
Avidin-desthiobiotin	0.094	
Streptavidin-iminobiotin	0.135	
Protein D-galactose	0.038	32
Protein D-mannose	0.054	
Vascular smooth muscle cell receptor-fibronectin	0.039	30
Arg-Gly-Asp ligands–integrins on osteoblast	0.032–0.097	20
Saccharomyces carlsbergensis-carbohydrate	0.121	33
S. carlsbergensis–mannose-specific lectin	0.117	
Mycobacterium bovis-heparin	0.053	13
Bacillus subtilis spore-antibody CotA	0.055	31
Fv fragment of antilysozyme-lysozyme	0.050	4
Interaction force on avg	0.095	

terms of mediating adhesion. AFM has also been applied to measure the forces between (co-)aggregating bacteria (26) and shows that in case streptococci and actinomyces have specific recognition molecules for each other, they coaggregate more strongly (−3 to −4 nN) than when the specific recognition molecules are lacking(−1 nN). Analogously, enterococci may or may not have so-called aggregation proteins at their surface. Pairs with aggregation proteins aggregate more strongly (−2.3 to −2.6 nN) than strains without the aggregation protein (−1.2 to −1.5 nN (39). Moreover, when the specific recognition molecules on strains with aggregation proteins were blocked using antibodies, the interaction force decreased to values observed for strains lacking the aggregation protein.

Overall, inspection of Table 2 shows that bacterial interaction forces in the absence of specific recognition amount on average 0 to −2 nN. When specific contributions exist in addition to the always present nonspecific contributions, the interaction forces reach average values of −3 to −5 nN. Referring to Table 2, it is concluded that a factor of a 2 to 3 difference in interaction force can have a tremendous impact on bacterial adhesion to surfaces.

SINGLE-BOND MOLECULAR RECOGNITION FORCES

In Table 3, we have compiled single-bond molecular recognition forces, as derived from AFM using functionalized tips. The interaction between avidin and streptavidin with biotin analogs is one of the most well-known specific recognition phenomena, and although there are differences between single-bond interaction forces among different biotin analogs (Table 3), the order of magnitude is in the subnanometer region for all analogs studied (14, 23), but well above the generally accepted lower limit for reliable single-bond force measurements of 0.005 nN.

Protein D is found in the alveolar fluid, where it takes part in the immune defense of the lungs against invading pathogens. The protein has various carbohydrate recognition domains which can take part in the specific interactions with carbohydrates (32). The interaction between fibronectin and $\alpha_5\beta_1$-integrin is important for the focal adhesion of vascular smooth muscle cells. The unbinding force of 0.039 nN was measured for a single bond between fibronectin and $\alpha_5\beta_1$-integrin (30). The Arg-Gly-Asp (RGD) sequence within a protein has considerable influence upon the final binding force with integrins; forces between 0.032 and 0.097 nN have been observed (20). Aggregation of yeast like *Saccharomyces carlsbergensis* is important in their flocculation, which controls fermentation in brewing and wine making. The lectin-carbohydrate single-bond interaction forces originating from *S. carlbergensis* amounts of 0.117 to 0.121 nN (33). A 0.055-nN unbinding force was measured for protein A from the outer surface of a bacillus spore (31). Antigen binding of individual Fv fragments of antilysozyme antibodies (Fv) to lysozyme was accompanied by a single-bond interaction force of 0.050 nN (4).

Overall inspection of Table 3 shows that for specifically interacting molecules, single-bond interaction forces reach, at the most, several tenths of an nN.

SYNTHESIS AND CONCLUSIONS

The aim of this review is to provide a comparison of the specific and nonspecific contributions to the forces that mediate bacterial adhesion to inert and protein-coated surfaces. Although, of course, exact values depend on the strain-substratum combination, it can be concluded that in the absence of specific contributions, bacterial interaction forces operate in the regimen up to −2 nN. In the presence of specific contributions, forces about 2 to 3 times stronger are observed, which implies that the specific contribution to an interaction force amounts between −1 and −5 nN. At the single-bond level, the molecular recognition forces that make up for a specific contribution to the interaction forces in bacterial adhesion also differ depending on the ligand-receptor system evaluated, but here too a general conclusion can be drawn, namely that these forces operate in the subnanometer regime and on average amount to 0.095 nN, albeit with variation dependent on the type of ligand-receptor pair involved.

By comparison of the specific contribution to bacterial interaction forces (Table 2) with the single-bond recognition forces (Table 3), it can be calculated the specific contribution must involve 10 to 50 specific ligand-receptor bonds (that is the number of ligand-receptor pairs interacting with 0.095 nN per pair, that make up for a total specific contribution of −1 to −5 nN). The pH dependence of the specific contribution to the interaction forces measured points to the electrostatic nature of these interactions, which most likely involves ion pairing. Exact spatial stereochemistry between recognition molecules then allows for the final specificity in bacterial selection for a given substratum surface.

The final question to be addressed is whether 10 to 50 ligand-receptor bonds involved in adhesion of one bacterium to a substratum surface is a realistic estimate. We previously addressed this question for streptococcal adhesion to laminin-coated substrata (6), but this review allows more general evaluation. Based on a contact area between a micrometer-sized

bacterium and the AFM tip of 2×10^{-3} μm^2, the distribution of 10 to 50 ligand-receptor pairs over this contact area would yield the conclusion that 15×10^3 to 75×10^3 sites would be present over an entire bacterial cell surface. Assuming a projected area of 100 nm^2 per binding site, as valid for a molecule like immunoglobulin G (IgG) (2), this implies that a bacterial cell surface is covered fully by specific binding sites, and in fact requires that the specific binding sites are arranged along structural surface features in order to allow a fit. Very often this is indeed the case (6).

The above conclusion of full coverage depends strongly on the size of the specific binding site assumed, and a projected area of the binding site 2 to 3 times smaller would yield the conclusion of partial surface coverage of the bacterial cell surface by the specific binding sites. This may, in certain cases, be more realistic, especially because full coverage of a bacterial cell surface by a class of specific recognition molecules should in principle be reflected strongly in the overall properties of the bacterial cell surface. As in general (see also Table 1), the absence or presence of specific recognition molecules is hardly expressed in overall physico-chemical cell surface properties as hydrophobicity and charge, we consider it more likely that the estimated 15×10^3 to 75×10^3 sites per bacterial cell surface only yield partial surface coverage: i.e., the projected surface area of a ligand-receptor pair should be considerably smaller than 100 nm^2.

To conclude, this review provides a further elaboration of our understanding of bacterial adhesion mechanisms and points to the need to evaluate adhesion mechanisms on a microscopic or even nanoscopic level, in addition to evaluations based on macroscopic characteristics such as surface hydrophobicity and charge.

ACKNOWLEDGMENT

This review is dedicated to Paul G. Rouxhet, on the occasion of his retirement, 28 September 2007.

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