Microbial Adhesion Is a Sticky Problem

A greater understanding of the mechanisms by which microorganisms form layers is needed, both for prevention and manipulation

For the great majority of microorganisms the business of adhering to some kind of surface—animate or inanimate—is an essential prerequisite to normal life. And in many ways microbial ecology is concerned with the layers of organisms, which are termed biofilms, that patchwork virtually every available surface in the living and nonliving worlds.

The ubiquitous stickiness of microorganisms has many consequences, the scale of which sometimes strains credibility. For instance, a biofilm just a couple of hundred micrometers thick over the hull of a large ship can retard its speed through the water by as much as 20 percent. The economic impact on an ocean liner and the tactical consequences to a military ship are obvious. A similar film on the inside of a half-inch pipe can cut down flow rate of a liquid by 50 percent. And heat exchange in a steam-driven turbine can be so impaired by this kind of fouling that, by one estimate, a 600,000-kilowatt, fossil-fuel power station may lose $0.5 million worth of energy in a single year.

Wherever there is at least some water in contact with metal surfaces, there are potential problems, as the oil industry knows only too well. There are numerous circumstances in oil recovery and delivery through pipelines that encourage the proliferation of anaerobic biofilms, which can generate ferrous sulfide particles. These particles are so corrosive to mild steel pipes that they can drill tiny holes through even the thickest of walls. This kind of nightmare perforation of oil pipelines is, according to some estimates, causing a loss of $1.0 million of oil each day in Alaska alone: relatively small in economic terms but potentially disastrous environmentally. And the industry is reported to be worried sick about similar developments in undersea pipelines.

These circumstances alone are sufficient to make microbial adhesion a subject of some considerable economic and scientific interest. Add to it, however, the whole panoply of disease processes—for a microorganism must first adhere to its host before it can invade—and the interest becomes compelling. It is, therefore, perhaps surprising that for so ubiquitous and important a process, the molecular mechanisms of microbial adhesion can be described in only the sketchiest of outlines. As a result, methods of preventing the formation of biofilms are at best crude and to a large extent poorly effective.

Not that all attempts at intervention are aimed at prevention, for biofilms have their positive attributes too. For instance, maintenance of water quality in natural systems is a consequence of metabolism in biofilms. And the formation of nitrogen-fixing root nodules in certain plants is initiated by the adhesion of a *Rhizobium* to a root hair, which triggers a series of complex morphological and metabolic modifications. Biotechnologists would very much like to be able to manipulate microbial adhesion at will, because immobilization of bacteria can yield a much more effective and controllable bioreactor.

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One of the barriers to advancing an understanding of microbial adhesion has been an inability to meld together the great diversity of disciplines that have an interest in it. From physical chemists to microbiologists, from engineers to cell biologists, each group has a narrow perspective of the problem that would benefit from exposure to these other views. Such an exposure occurred at a recent Dahlem workshop* on the subject. Forced to pass a week in the intense Dahlem workshop atmosphere, which is so conducive to breaking through interdisciplinary walls, fifty representatives of these various camps began to see their problem in its broader contexts. Said one participant at the concluding session: "We didn't fight, and we even agreed on a few things." That is progress.

To an outsider the subject looks bewildering, varied, and complex. Not only is there a great diversity of surfaces—properly called substrata—on which adhesion might occur, but also the array of microorganisms that might stick is enormous. Moreover, as microorganisms are metabolizing entities, their interaction with potential substrata has an extra dimension of uncertainty. This dimension of uncertainty is redoubled when microbes adhere to living substrata. Even the aficionados did not fail to be impressed. "I had not appreciated the potential variability of it all," conceded David Gingell of the Middlesex Hospital Medical School, London.

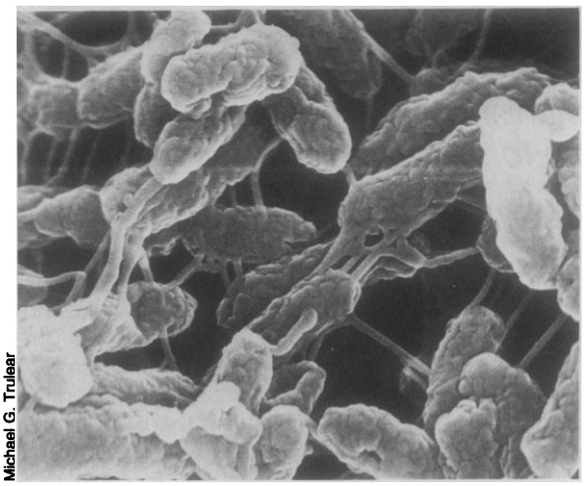
Microbial adhesion can be usefully divided into two phases: the mechanism(s) of initial adhesion and the ecology of the established biofilm. The former was the prime focus of attention at the Dahlem meeting. Issues such as how (or whether) a microorganism senses a surface and what underlies the difference between specific and nonspecific adhesion attracted a lot of interest.

With the ingenuousness of an economist who begins an exposition on a favored economic model with the phrase, "imagine an ideal world," physical chemists have been known to approach the adhesion problem with "imagine a perfect, simple system." Then, equipped with a mathematical model—known for arcane reasons as DLVO theory—that was developed to describe quantitatively the interaction of inanimate colloidal particles with smooth surfaces, have attempted to scrutinize microbial adhesion.

This obviously limited approach is taken, not because physical chemists are hopelessly naïve, but because to do otherwise would quickly mire the explorer in territory where neither quantitative nor qualitative deductions could be made.

DLVO theory has presented a clear picture of the outcome of certain "long range" forces—specifically, electrostatic and van der Waal's—in the intereaction between a particle and a surface. Charged with the same sign, a particle and a surface may approach each other until a strong repulsive force begins to operate as the two near. At intermediate ionic strengths of the suspending medium, however, there is just before this energy barrier rises a low energy trough, known as the primary minimum. A particle can hover at this point, which may be some 70 angstroms from the surface,

*Proceedings of the workshop, which was held 15 to 20 January in West Berlin, will be published later this year. Dahlem Konferenzen, Wallotstrasse 19, D-1000 Berlin 33, F.R. Germany.



Michael G. Trulear

Biofilm portrait

Scanning electron micrograph of an experimental biofilm composed of Pseudomonas aeruginosa. *Magnification ×15,000.*

with some statistical probability of breaking loose.

For a bacterium, residence, however brief, at a primary minimum represents a great opportunity for establishing long-term attachment. Rather than being smooth, as in the physical chemists' imagination, the surface of a bacterium is likely to be festooned with polymer chains or even to have long appendages projecting from it. Because degree of electrostatic repulsion depends on radius of curvature, the appendages, such as flagella or pili, can readily penetrate the energy barrier that separates the bacterium from the surface and can potentially participate in any of a variety of short-range interactions, such as hydrogen bonds and those of dipole-dipole forces, with the surface and other molecules there. It is these short-range forces between molecules on the bacterial surface and those on the substratum that are the key to long-term adhesion.

Physical chemists, being smart people, are of course acutely aware of the limitation of their simple systems and are striving to construct more complex physical and mathematical models that are less distant from reality. It might be possible to adsorb protein or polysaccharide polymers onto latex particles, for instance, or to anchor polymers into phospholipid vesicles. "This would make our models more complicated and more akin to biological systems," said Paul Rutter, a physical chemist with British Petroleum, England.

So, the question of how microorganisms get to surfaces can at least be approached somewhat in terms of the laws of physics. There has been an interest in the past in the idea that bacteria might be tracking gradients of nutrients that tend also to concentrate at surfaces. But it now appears that relative to the distance that bacteria swim in their swim-tumble-swim random walk mode of mobility, the nutrient gradient hugs very close to the surface and cannot therefore influence the direction of an organism's movement.

The same limitation applies to the electrostatic and van der Waal's forces, so a bacterium is unable to sense where a surface is and then swim toward it. "Microorganisms have no choice but to go to surfaces," commented Ian Robb of Univelever Research, England, because hydrodynamic forces will tend to take them to surfaces and once there they tend to stick. Stickiness, incidentally, appears to be enhanced in starved cells, which is obviously an adaptive property.

The next question, then, is what happens to a microorganism once it is attached to a surface? The strength of adhesion, it has been observed, usually increases the longer a microorganism remains there. As Robb pointed out, this is often taken to imply that the organism is producing further adhesion polymers that are increasing the linkages with the surface, which may indeed be true. He reminded participants, however, that even plastic or rubber particles will increase their adhesion to surfaces the longer they stay there, presumably because they are slowly being flattened against the surface under the influence of van der Waal's forces. The two adhering surfaces begin to lose their separate identities, and this must happen in microbial adhesion too.

Nevertheless, microorganisms do produce exocellular polymers that eventually play a major role in the microecology of the biofilm. Indeed, in some biofilms as much as 90 percent of the carbon is contained in exocellular polymers. Although this figure is high for most systems, it became clear to many that a full understanding of biofilms depends crucially on a fuller understanding of polymer gels. Kevin Marshall, of the University of New South Wales, who was the prime instigator of the Dahlem conference, said, "I have become much more aware of a great need to do much more polymer chemistry. I'm sure this has been an important shift in perceptions through this meeting."

Even relatively simple polymers on a bacterial surface can effect extremely tight adhesion, through the culmination of very many weak attachments. Robb showed that even with as few as 30 percent of its total potential contacts touching a substratum, a typical polymer might still be making 10,000 contacts. The total adsorption energy would be significant, even if the figure for individual contact points was extremely small.

Garth Jones of the University of Michigan, like many biologists, was much impressed by this calculation, and it caused him to change his perception of the relative importance of specific as against nonspecific adhesion in nature. "I used to think that specific adhesion mechanisms, of the lock and key variety, were more important than I do now," he said. "But it is clear you can have very tight adhesion even with nonspecific adhesion." Jones's prejudice perhaps reflected his preoccupation with adhesion to living surfaces, where specific adhesion is apparently more common than it is in attachment to inanimate surfaces.

Many aspects of microbial metabolism can become altered when attachment to substrata occurs, but the data on this matter are, to say the least, messy and contradictory. However, Michael Silverman, of the Agouron Institute, La Jolla, described one dramatic and incontrovertible change that occurs in a microorganism once it attaches to a surface. *Vibrio parahaemolyticus*, a marine microorganism involved in marine biofouling, has two types of flagella, a polar flagellum and a lateral flagellum. The organism uses the former to swim through water while the latter grows only once contact with a surface has occurred, when it is used for adhesion and swarming over the surface.

Silverman and his colleagues have been doing genetic analysis on the lateral flagellar genes, and they find that the genes are switched on some 30 minutes after contact with a surface has occurred. In this case, as in others, it seems clear that the organism has sensed the presence of the surface: the question is, how? Deformation of the cell membrane would be one obvious signal of contact, except that bacterial cells are enclosed in tough outer membranes that are relatively resistant to deformation. Some kind of chemical message is another possibility. For instance, Marshall and

his colleagues were recently able to induce metabolic changes in an organism of the type that occur when it contacts a surface, simply by adding detergent to the suspending fluid. In natural conditions the organisms might take up detergent molecules that tend to adhere to surfaces, he speculates.

Although the question of bacterial sensing of surfaces pricked a great deal of interest at the meeting, there were very few data with which to address it.

Another topic of some speculation was the possibility that organisms might be able to change the nature of their adhesion polymers—generally known as adhesins—according to prevailing circumstances. One known, outstanding example of this is *Neisseria gonorrhoeae*, which can apparently modulate the chemical composition of both its pili and some of its surface polymers to a considerable extent. The effect is that the organism can attach to and penetrate several different types of host cell in its pathogenic cycle and to a large extent can escape immunological attack.

No one knows how general this phenomenon might be. "It's a wonderful speculation that microorganisms might have the capacity to adapt to circumstances by shuffling genes for adhesin molecules," said Silverman. "My bet is that these adhesins aren't as static as it might appear." David White of Florida State University thinks it is a beguiling idea: "We are only just beginning to realize how important it might be." Marshall, however, guesses that it won't turn out to be a major phenomenon in bacterial adhesion.

The prospect of trying to isolate and characterize the huge array of adhesin polymers deployed by microorganisms is daunting enough. If microorganisms are able to shuffle through a file drawer of adhesin genes and slip appropriate ones into the reading apparatus at appropriate times, the problem passes into a yet more complex dimension. There was a lot of talk at the Dahlem meeting of the need to understand the molecular and steric grain of the bacterial surface in order to understand the nature of adhesion. How much more of a challenge it will be if that grain were to be in a swirling state of flux.—**ROGER LEWIN**

A New Way to Assign ^{31}P Chemical Shifts

Phosphorus-31 nuclear magnetic resonance (NMR) spectrometry promises to be a valuable tool for studying the conformation of macromolecules such as DNA, RNA, and phospholipids. The reason, says Philip Bolton of Wesleyan University, is that the chemical shift of the ^{31}P signal may be dependent on torsional forces at the phosphorus-oxygen bond. The technique has not been used to any significant extent, however, because it has been very difficult to associate each signal with a specific phosphorus in the macromolecule. "There are about 35 macromolecules whose ^{31}P spectra have been published," Bolton says, "and virtually every possible assignment for each signal has been made."

In NMR spectroscopy with protons or carbon-13, this problem can usually be overcome because of the large catalog of known chemical shifts available. If this does not work, it is usually possible to replace one or more atoms with an isotope that does not have a magnetic dipole moment and therefore does not give an NMR signal. Replacement of a proton with deuterium, for example, removes the signal of the target atom from the spectrum, and thereby provides unequivocal identification. There is no comparable catalog of chemical shifts for ^{31}P compounds and the only available isotope, ^{32}P, has both a high radioactivity and a short half-life. Some assignments can be made if resolved and assigned ^{1}H NMR spectra are available, but this is a difficult and tedious process.

Bolton, John Gerlt of Yale University, and Ming-Daw Tsai of The Ohio State University have recently independently found a technique to assign chemical shifts unequivocally; Bolton and Gerlt published back-to-back (and cooperative) papers in a recent *Journal of the American Chemical Society* [**106**, 437 and 439 (1984)], while Tsai published somewhat earlier [*ibid.* **105**, 5455 (1983)]. Each investigator used oxygen-17 to label specific phosphorus atoms.

The technique had previously been used in ^{31}P spectrometry of small molecules. When ^{31}P is directly bonded to ^{17}O in such molecules, a phenomenon known as "scalar relaxation of the second kind" occurs because of quadrupolar relaxation of the ^{17}O. As a direct result of this relaxation, the NMR signal for ^{31}P is broadened and becomes less intense. This makes it possible to pick out the signal for the atom to which ^{17}O is attached. But, says Gerlt, "several people have predicted that this kind of relaxation will not occur if the phosphorus-oxygen bond is in a macromolecule."

In fact, the scalar quadrupole relaxation probably does nor occur in most macromolecules. A direct dipolar relaxation unexpectedly does occur, however, with precisely the same result. Bolton and Andrew Joseph used this technique with a complex of polyinosine and polycytidine, for example, to obtain chemical shifts for the ^{31}P nuclei in each polynucleotide. A similar study with a complex of polyadenosine and polyuridine showed no selectivity, however, between spectra in which poly(A) was labeled and in which it was not. This finding, says Bolton, "implies that the residues of both homopolymers may have alternating conformations."

Gerlt, Matthew Petersheim, and Shujaath Mehdi labeled the DNA oligomer CpGpCpG (C, cytidine; G, guanosine) at each of the two outside phosphorus nuclei separately to identify each of the chemical shifts for the molecule. They were then able to use these labeled oligomers in the presence of actinomycin D to show that the drug intercalates between the center guanosine and cytidine units. Tsai and his colleagues were able to use the technique to identify the signal from P_α in the spectrum of adenosine diphosphate bound to arginine kinase. They were also able to demonstrate broadening of ^{31}P peaks in phospholipid bilayers.

This technique, says George Gray of Varian Corporation, "opens up a greater use of ^{31}P spectrometry" because it makes it possible to assign chemical shifts with a relatively small amount of effort. Perhaps the first step, though, is developing a catalog of chemical shifts for phosporus nuclei in various compounds. All three groups are currently directing a good deal of effort to that problem.—**THOMAS H. MAUGH II**