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Energetics of bacterial adhesion

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Summary. For the description of bacterial adhesion phenomena two different physico-chemical approaches are available. The first one, based on a surface Gibbs energy balance, assumes intimate contact between the interacting surfaces. The second approach, based on colloid chemical theories (DLVO theory), allows for two types of adhesion: 1) secondary minimum adhesion, which is often weak and reversible, and 2) irreversible primary minimum adhesion. In the secondary minimum adhesion a thin water film remains present between the interacting surface. The merits of both approaches are discussed in this paper. In addition, the methods available to measure the physico-chemical surface characteristics of bacteria and the influence of adsorbing (in)organic compounds, extracellular polymers and cell surface appendages on adhesion are summarized.
Key words. Bacterial adhesion; long-range forces; short-range forces; electrostatic interaction; DLVO-theory; hydrophobicity; surface Gibbs energy.

Introduction

Surfaces are abound in nature and bacteria eventually colonize them. To prevent removal from a surface, bacteria have to attach to it. Attachment can roughly be divided into two steps. First, the organisms adhere. This initial adhesion is governed by pure physico-chemical surface properties of the bacteria and the solid, and the type of solute [34]. Second, organisms may eventually anchor themselves to a surface using specific appendages or cell surface structures. This process strongly depends on the type of bacterium/surface combination.

Initial adhesion can further be divided into two separate stages, namely reversible and irreversible adhesion. Reversible adhesion may be defined as deposition of bacteria on a surface in such a manner that the bacteria continue to exhibit a two-dimensional Brownian motion, and can be removed from the surface by e.g. the bacterium's own mobility. Irreversibly adhering bacteria no longer exhibit Brownian motion and cannot be removed by a moderate shear force.

In the following we will discuss the physico-chemical models which can describe reversible and irreversible adhesion. In addition, methods to determine the individual

model parameters will be summarized. Finally, the relevance of the models for the prediction of the behavior of microbes in natural systems will be evaluated.

Theory of cell adhesion

Treating bacterial adhesion as a physico-chemical process is complicated by the nature of bacterial cells. Bacteria are far from 'ideal' particles. They have no sharp surface boundary, simple geometry, or uniform molecular surface composition. Internal chemical reactions can lead to changes in molecular composition both in the interior and at the surface, and molecules and ions may cross the bacterium/water interface. These chemical processes continue also after adhesion. Therefore, the adhered cells are rarely in complete physico-chemical equilibrium with their environment. These complicating factors have to be kept in mind when bacterial adhesion is interpreted in physico-chemical terms.

Long-range interactions

Bacteria may be considered as living colloidal particles, and as such they obey the laws of physical chemistry. If a colloidal particle approaches a surface it interacts with that surface. Derjaguin, Landau, Verwey and Overbeek (DLVO) have postulated that the total long-range interaction over a distance of more than 1 nm is a summation of Van der Waals and Coulomb interactions[45] (fig. 1). In this approach the interaction between a particle and a surface is described as a function of the separation distance (for separation distances > 1 nm).

Van der Waals interaction (G_A). Due to correlation in the electron motion, two atoms attract each other if they are a short distance apart. In this interaction, an instantaneous dipole moment in one atom induces an instantaneous dipole moment in the other atom. Generally, the attraction is strong between atoms having high ionization potentials. The energy of such a (dispersive) interaction between two particles at a given separation distance (H) is expressed by the Hamaker constant (A)[19]. The Hamaker constants for the interaction between bacteria (2), and surfaces (1), across a medium (3), A_{132}, are related to the Hamaker constants of the individual components of the system[48]:

$$A_{132} = A_{12} + A_{33} - A_{13} - A_{23}$$
$$\approx (A_{11}^{1/2} - A_{33}^{1/2})^2 (A_{22}^{1/2} - A_{33}^{1/2})^2 \quad (1)$$

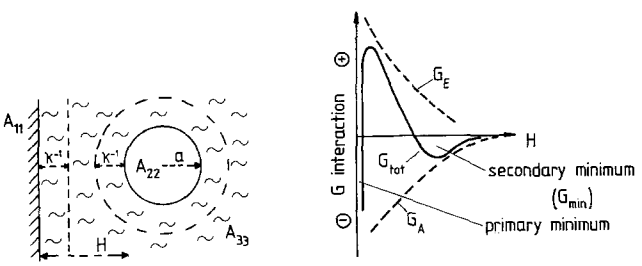
Nir[39] showed, theoretically and experimentally, that in addition to dispersive, (random) dipole-dipole and (random) dipole-induced dipole interactions should also be incorporated into the Hamaker constant. This is especially important for biological surfaces. The numerous (induced) dipoles in these surfaces may increase the Hamaker constant by a factor of about 1.4.

Electrostatic interaction (G_E). Generally, surfaces of particles are charged. Because of electroneutrality, in water the charge on the surface is neutralized by a countercharge that is diffusely distributed around the particle. This system of charge and counter charge can be compared to a condensor and is therefore called an electrical double layer. The thickness of this diffuse double layer (κ^{-1}, fig. 1) is a function of the ion charge and ion concentration. The diffuse layer becomes thinner with increasing ionic strength. As a result, the electrostatic interaction at a given distance of separation between the two surfaces is reduced at higher ionic strength. The Gibbs energy G_E of the electrostatic interaction is determined by the electrokinetic (or zeta) potential of the surfaces[45]. As most natural surfaces and bacteria are negatively charged[28] the electrostatic interaction between bacteria and surfaces at neutral pH is usually repulsive.

DLVO theory. Figure 1 shows a characteristic plot of the total interaction Gibbs energy (G_{tot}, which is a summation of G_A and G_E) as a function of separation (H) between a bacterium and a negative-charged surface[29]. Two minima can be seen in this diagram; a primary minimum close to the surface and a secondary minimum at a greater separation distance (H = 5–20 nm). If a bacterium can reach the primary minimum, short-range forces dominate the adhesive interaction and the DLVO theory cannot be used to predict the interaction energy. The secondary minimum does not usually reach large negative values. Particles captured in this minimum generally show reversible adhesion. For bacterial cells the interaction energy at the secondary minimum is typically between -1 and -20 kT. Energy values below -10 kT result in irreversible adhesion.

Short-range interactions

When bacteria and surface make direct contact (separation distance H = 0) the interaction energy can be cal-



$$G_A = -\frac{A}{6}\left[\frac{2a(H+a)}{H(H+2a)} - \ln\left(\frac{H+2a}{H}\right)\right]$$

$$G_E = \pi\varepsilon\varepsilon_0 a\left(\psi_{13}^2 + \psi_{23}^2\right)\left[\frac{2\psi_{13}\psi_{23}}{\psi_{13}^2 + \psi_{23}^2}\ln\left(\frac{1+e^{-\kappa H}}{1-e^{-\kappa H}}\right) + \ln\left(1-e^{-2\kappa H}\right)\right]$$

$$G_{tot} = G_A + G_E$$

Figure 1. Long-range interactions according to the DLVO-theory. G_A free energy of the van der Waals forces; G_E, free energy of the electrostatic interaction; a, radius of the particle; H, separation distance; κ^{-1}, double layer thickness; A, net Hamaker constant of the system (A_{11} of the surface, A_{22} of the particle, A_{33} of the third phase); ε, dielectric constant of the medium; ε_0, permittivity of free space; ψ, electric potential at the surface (ψ_{13} between surface and medium, ψ_{23} between particle and medium).

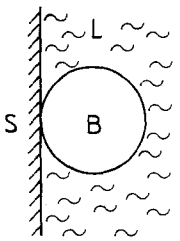


Figure 2. Schematic represention of short-range interactions. The particle (B) makes direct contact with the surface (S) forming a new interface. L = liquid.

culated from the assumption that the interfaces between solid/liquid (SL) and bacterium/liquid (BL) are replaced by a solid/bacterium (SB) interface (fig. 2). The change in the interfacial excess Gibbs energy upon adhesion ($\Delta_{adh}G^{\sigma}$, expressed in $J \cdot m^{-2}$) is described by:

$$\Delta_{adg}G^{\sigma} = G^{\sigma}_{SB} - G^{\sigma}_{SL} - G^{\sigma}_{BL} \quad (2)$$

When $\Delta_{adh}G^{\sigma}$ is negative, adhesion is thermodynamically favored, and will proceed spontaneously.
If the molecular composition of the interface, the pressure, and the temperature do not change, equation (2) may be written, as a balance of interfacial tensions (γ, expressed in $J \cdot m^{-2}$):

$$\Delta_{adh}G = \gamma_{SB} - \gamma_{SL} - \gamma_{BL} \quad (3)$$

It should be noticed that equations (2) and (3) only apply if both interacting surfaces make direct contact. If it is assumed that only 1 % of the cell surface is in direct contact with the solid surface $\Delta_{adh}G$ will be of the order of 600–6000 kT[29]. This interaction is thus irreversible and much stronger than adhesion in the secondary minimum. However, short-range interactions can only become effective when long-range interactions allow a particle to approach a surface. A high maximum in G_{tot} would prevent such an approach.

Cell surface characteristics

Hydrophobicity

The term 'hydrophobicity' is often used in the interpretation of bacterial adhesion. Hydrophobicity of a certain component indicates its tendency to interact with water. More specifically, hydrophobicity originates from the fact that water-water contacts are thermodynamically more favorable than contacts between two non-polar groups or between a non-polar group and water, i.e. it is a feature of non-polar groups tending to be rejected from an aqueous medium rather than being positively attracted to one another. Generally, the excess Gibbs energy of a surface decreases with increasing hydrophobicity. With increasing hydrophobicity of a surface, $\Delta_{adh}G$ (eq. 3) will become more negative, resulting in higher adhesion strength.

Methods used to determine bacterial hydrophobicity

Method	References
Contact angle of a drop of liquid on a layer of cells	2, 6, 17 27, 36, 37
Partitioning of cells in an aqueous/hydrocarbon two-phase system	10, 27, 36, 37
Partitioning of cells in an aqueous two-phase system	17, 27, 48
Salt aggregation	10, 26
Partitioning of hydrocarbons	25, 32, 40
Hydrophobic interaction chromatography	10, 22, 26 36, 37
Bacterial adhesion as a function of the interfacial energies of the solid and liquid	2, 15
Direction of spreading of a drop of liquid	46

The relation between hydrophobicity and Van der Waals interactions can be deduced from eq. (1). From this equation it is obvious that the Hamaker constant for the interaction between surface and bacterium is smaller if A_{11} and A_{33} or A_{22} and A_{33} are more alike. The more hydrophobic a bacterium or surface is, the more its individual Hamaker constant deviates from that of water, and the larger the Hamaker constant for the total interaction will become.
The hydrophobicity of surfaces can only be characterized semi-quantitatively by assessing the preference for water compared to another phase (e.g. air or hexadecane)[27]. The methods for evaluating hydrophobicity (table) have not yet been evaluated systematically. The general trend is that very hydrophobic and very hydrophilic cells behave similarly in all tests. However, cells with intermediate surface hydrophobicities behave differently in different tests[9, 10, 27, 36, 37]. There seems to be a consensus on using contact angle measurements as the relatively best method for characterizing bacterial hydrophobicity. The reliability of this test may be improved by combining it with hydrophobic interaction chromatography[36] or a hydrocarbon/water partitioning test[36].
The surface hydrophobicities of different bacterial strains show large variations. Water contact angles range from 10° to 120°[4, 7, 24, 35]. Usually, cells in the early stationary phase are used for adhesion and hydrophobicity measurements. But it has been shown regularly that bacteria may become more hydrophobic[12, 18, 32] and show increased adhesion[12, 14, 33, 47] during the exponential growth phase and at high dilution rates in a chemostat. Although the reason for this phenomenon is not yet known, this fact should not be neglected when adhesion experiments under various conditions have to be interpreted.

Electrophoretic mobility

The extent of the electrostatic interaction can be deduced from the electrophoretic mobility of the cells[28]. A high electrophoretic mobility corresponds with a high electrokinetic potential (ψ).

The electrokinetic potential originates from the charged groups in the bacterial cell wall. Carboxy groups contribute predominantly to the charge of the bacterial surface. At neutral pH the cells are usually negatively charged. The isoelectric point is normally around pH 3 [37].

Experimental evidence

In order to evaluate the relevance of short-range and long-range interactions for bacterial adhesion, the general trends observed in adhesion experiments reported in literature will be briefly summarized here:

1) Adhesion increases with increasing hydrophobicity of the bacterium and/or solid surface [6, 9, 12, 13, 15, 27, 36].

2) Adhesion decreases with increasing electrostatic repulsion [13, 28, 29, 33].

3) If the influence of hydrophobicity and electrophoretic mobility on adhesion are studied simultaneously, hydrophobicity is often found to be the dominating characteristic [13, 28]. A distinct influence of the electrokinetic potential is only observed in case the solid and/or bacterial surfaces are hydrophilic [30].

4) Normally, adhesion is found to be reversible [6, 13, 29, 33]. This indicates that, in thermodynamic terms, the interaction between bacterium and surface is relatively weak ($\Delta_{adh}G > -10$ kT/cell $= -4.10^{-20}$ J/cell).

5) Irreversible adhesion is only observed when the electrostatic interaction is attractive or weakly repulsive [3, 18, 41]; or when both bacterium and solid surface are strongly hydrophobic [5, 28]. Sometimes irreversible adhesion occurs after an initial reversible adhesion stage [7].

6) Several observations indicate or show (e.g. internal reflection microscopy) the presence of a water layer between an adhering cell and the surface. This water layer increases with decreasing ionic strength [1, 16, 33, 41].

7) From the measurement of adhesion isotherms, a $\Delta_{adh}G$ value of -2 to -6 kT/cell has been derived [24, 29].

All these observations can be reasonably well described in terms of long-range interactions (i.e. the DLVO theory). Observations that 1) the adhesion Gibbs energy is relatively small (reversible adhesion), and 2) there is some distance between an adhering bacterium and a solid surface, suggest that adhesion takes place in the secondary minimum. According to the DLVO theory, theoretical calculations also show that for conditions relevant for bacterial adhesion, secondary minimum adhesion is expected, with an adhesion Gibbs energy of -1 to -20 kT per cell and a separation distance of 5–20 nm [8, 29, 33]. There is also agreement between experimental observations and theory that primary minimum adhesion is to be expected in the case of very strong Van der Waals attraction (i.e. both surfaces are hydrophobic). The adhesion energy in the primary minimum can be calculated on the basis of short-range interactions. Bacteria, initially adhering in the secondary minimum, may in the course of time reach the primary minimum, either by simply passing the energy with barrier (if it is not too high) or by protruding fibrils, fimbriae, etc. through the energy barrier [7, 8, 33]. Fimbriae have considerably smaller radii than the whole cell. Since the electrostatic repulsion energy depends more strongly on the particle radius than the Van der Waals attraction (i.e. G_{tot} is smaller for smaller particle radii), individual fimbriae can adhere readily in the primary minimum. As a result they will bridge the gap between surface and bacterium [23, 29, 35]. The occurrence of secondary minimum adhesion is not necessarily due to electrostatic repulsion. There are a few indications that surface polymers may sterically hinder a close approach of the two surfaces and force the particles to adhere in the secondary minimum [1, 31].

Modification of surfaces

In natural environments, besides bacteria and solids, dissolved (in)organic components are present as well. This material may adsorb onto the bacterial and/or solid surface. As a result, either a highly hydrated polymeric layer or a compact polymeric layer or layer(s) of small adsorbed molecules will be formed. Loosely structured layers will lead to steric interactions. At low surface coverage, with parts of the polymer chains protruding into solution and part of the surface available for adsorption, polymer bridging may lead to irreversible adhesion. At high surface coverage steric repulsion between the polymer chains may prevent strong adhesion. Small molecules and polymers that adsorb in compact layers, which is generally the case with globular proteins, are expected to affect adhesion mainly through changes in the hydrophobic and electrostatic surface characteristics. Indeed, experimental data [30] have confirmed that adsorbed proteins influence the adhesion of bacteria by modifying the hydrophobic and electric properties in a way predicted by the DLVO theory.

Specific attachment and biofilm formation

Many bacteria have special surface appendages in the form of long filaments extending into the solution (e.g. fimbriae, pili, or fibrils). Such structures are often responsible for more-or-less specific bacterial adhesion phenomena, and are therefore referred to as 'adhesins'. Mutant strains without such adhesins usually show a decreased adhesion [11, 21, 23, 36].

As stated before, fimbriae can overcome electrostatic repulsion barriers. For example, fimbriated *Neisseria gonorrhoeae* are more adhesive than non-fimbriated ones. When, however, the surface charge signs of cells and surface are opposite, both cell types adhere equally well [21]. For the attachment of bacteria to specific surfaces, macromolecular groups on the cell surface (receptors) have to bind to (or recognize) molecular structures of the other specific solid surface. As fimbriae (or pili) can reach the solid surface more easily than whole cells,

the tip of the fimbria is a good site for receptor structures[11, 23].

It must be noted that the presence of adhesins on a bacterial cell surface changes the overall surface characteristics and thereby also the overall adhesion properties of the cell. Usually, fimbriated cells are found to be more hydrophobic than non-fimbriated cells[23, 25, 36], though sometimes the opposite is reported[22].

The interpretation of the influence of cell-surface appendages depends largely on the number of fibrils per cell, and their topographic distribution. Adhesion of cells with a dense layer of fimbriae will probably depend mostly on the overall cell surface characteristics. The adhesion of cells with only a few fimbriae protruding into solution will mainly be determined by the characteristics of the individual fimbriae.

Cells adhering to a surface can grow and form a biofilm. Processes governing biofilm formation and stability may be very different from those that are of relevance for the initial steps of adhesion. For adhesion, cell-surface interactions are important, whereas for biofilm formation cell-cell interactions also play a significant role. Electron microscopic observations suggest that in most cases bacterial colonization of surfaces is associated with the presence of polymeric cell capsules, usually polysaccharides. However, in the majority of studies the presence of capsules or slime layers has been shown to decrease surface hydrophobicity or adhesion[4, 12, 32, 42, 43, 48]. Brown et al.[4] observed widespread adhesion from mixed populations in a carbon-limited chemostat culture, without any evidence of extracellular polymer production. A nitrogen-limited culture adhered only poorly, despite a large production of extracellular polymers. These and other results[48, 50] suggest that capsules may reduce initial adhesion.

Colonization is a much slower process than adhesion. During colonization experiments bacteria adhering in the secondary minimum therefore have time to produce polymers which are able to bridge the gap between cell and surface, thereby causing irreversible adhesion. In cases where enough polymers are formed, the cell is surrounded by a capsule or slime layer. Instead of stimulating adhesion, these gel-like, viscous structures bridge gaps and cement layers of cells to surfaces. Though adhesion of encapsulated cells can be described by the DLVO-theory, there is no unifying concept as yet which can predict qualitatively and quantitatively permanent attachment in biofilms. Permanent attachment, and also detachment of biofilms (sloughing) depend on a variety of environmental and cell-specific physiological conditions. The precise action of these conditions on biofilm formation is still unknown.

Transport of microorganisms through soil

When cells are transported through soils or aquifers they may attach to the soil particles. This means that bacterial surface characteristics may influence to a certain extent the spreading of bacteria in a soil environment. Adhesion of bacteria to river sediments has been found to follow physico-chemical predictions very well[30]. It should be noted that the transport of cells in a sediment or soil cannot be described by adhesion solely. The transport of cells will also be strongly influenced by filtration effects[20].

Conclusion

Adhesion is initially a reversible process governed by long-range interactions. According to the DLVO-theory, initial adhesion takes place predominantly in the secondary minimum. The depth of this minimum (i.e. the adhesion energy) can in principle be determined on the basis of macroscopic surface characteristics. The DLVO-theory can also be used to predict whether or not the bacterium can approach a surface close enough for short-range interaction forces to become effective. In the latter case, irreversible adhesion may occur in the primary minimum.

Irreversible adhesion and/or surface colonization are related to specific bacterial characteristics, e.g. production of exudates, or the presence of 'adhesins' that bridge the gap between bacterium and surface. Therefore, deeper knowledge on structure-function relationships is needed to explain subsequent stages of the adhesion process.

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