DLVO and steric contributions to bacterial deposition in media of different ionic strengths

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Abstract

The deposition of eight bacterial strains on Teflon and glass in aqueous media with ionic strengths varying between 0.0001 and 1 M was measured and interpreted. Two types of interactions were considered: (1) those described by the DLVO theory, which comprise van der Waals attraction and electrostatic repulsion (bacteria and surfaces are both negatively charged); and (2) steric interactions between the outer cell surface macromolecules and the substrata. As a trend, at low ionic strength (< 0.001 M), deposition is inhibited by DLVO-type electrostatic repulsion, but at high ionic strength (≥ 0.1 M) it is dominated by steric interactions. The ionic strength at which the transition from the DLVO-controlled to the sterically controlled deposition occurs, is determined by the extension of the macromolecules into the surrounding medium, which varied between 5 and 100 nm among the bacterial strains studied. The steric interactions either promote deposition by bridging or inhibit it by steric repulsion. Between Teflon and hydrophobic bacteria, bridging is generally observed. The surface of one bacterial strain contains amphiphilic macromolecules that form bridges with Teflon but induce steric repulsion on glass. The presence of highly polar anionic polysaccharide coatings on the cell impedes attachment on both glass and Teflon. For practice, the general conclusion is that the deposition of most bacteria is: (1) strongly inhibited by DLVO-type electrostatic repulsion in aqueous environments of low ionic strength such as rain water, streams and lakes; (2) controlled by DLVO and/or steric interactions in systems as domestic waste waters and saliva; and (3) determined by steric interactions only in more saline environments as milk, urine, blood and sea water. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Control of bacterial adhesion is an important issue in, e.g. food processing, environmental and medical biotechnology. Despite this practical rele-
vance, the mechanism of the adhesion process has not yet been fully resolved. It is often assumed that adhesion is primarily determined by (1) the interactions described by the DLVO theory of colloid stability; and (2) steric interactions between outer cell surface macromolecules and substratum surface [1–6]. All of these interactions contribute to the total Gibbs energy of interaction $\Delta G_s$ which is a function of the separation distance $h$ between the cell and the substratum [1]. Assessment of the relative importance of the DLVO and the steric interactions to $\Delta G_s (h)$ requires more systematic investigation. In fact, full deconvolution into these constituents is probably not simple because they are not additive. The DLVO contribution comprises the sum of the van der Waals and the electrostatic interactions. For a simple surface, the DLVO contribution to $\Delta G_s (h)$ can be estimated rather accurately, given the electrostatic properties, Hamaker constants and cell geometry. However, bacterial surfaces are not simple, in that the charged layer has a non-zero thickness which may change with $h$. Because bacteria and most synthetic and natural surfaces are negatively charged at ambient pH-values, the electrostatic interaction is, as a rule, repulsive. At low ionic strength, say $\leq 0.001$ M, the long range DLVO-type electrostatic repulsion dominates over the van der Waals attraction, but at high ionic strength the van der Waals attraction dominates [1].

Steric interactions may be repellent or attractive [1,7]. In aqueous media, steric repulsion occurs when the cell-surface macromolecules are hydrophilic and have no affinity for the substratum. On the other hand, if these macromolecules, or parts of them, have an affinity for the substratum, exceeding a certain critical value, bridging may take place [7]. Bridging is generally observed for solids and micro-organisms that are both hydrophobic [6,8–11].

Depending on the ionic strength $I$ of the medium, bound polyelectrolytes may influence adhesion through a combination of electrical and non-electrical interactions [12–14]. Such a combined action is sometimes called ‘electrosteric’. Increasing $I$ reduces all electrostatic forces. An ionic strength of at least 0.5 M is needed to achieve essentially complete suppression of electrostatic interactions [12,14,15]. How this works out for the surface layers on bacteria is a specific phenomenon, depending on the way the polyelectrolyte is bound, its branching and flexibility.

The complexity of steric interactions in bacterial adhesion is illustrated by the observation that they may reduce the rate of deposition but thereafter cause irreversible attachment [1]. The nature of the cell surface coating can, to some extent, be inferred from the isoelectric point (iep) of the bacterial cell [16] and from the water contact angle $\theta_w$ on dried bacterial lawns [6,8,11,17]. The values of these parameters help to distinguish between cell coatings of negatively charged polysaccharides, amphiphilic macromolecules, and non-polysaccharide macromolecules such as lipids and proteins [16].

The adhesion interaction may be quantified by its activation Gibbs energy $\Delta G^\alpha \cdot \Delta G^\alpha$ can be estimated by comparing experimental adhesion results with calculations using a certain model, describing the transport rate of cells from bulk solution to substratum surface. $\Delta G^\alpha$ is related to the adhesion efficiency, i.e. the probability for a (bacterial) particle to adhere upon arrival at a substratum surface [1,18–23]. At high ionic strength ($\sim 0.1$ M) the rate of bacterial deposition is dominated by steric interactions whereas at lower ionic strength electrostatic repulsion retards and controls this rate [1,18,23–27].

The main objective of the present paper is to assess the relative importance of steric and DLVO interactions for bacterial adhesion in environments of different ionic strengths. To that end, some new results will be reported and the applicability range of simple models will be investigated. It is expected that the outcome depends strongly on the specific nature of the bacterial cell coating; therefore a variety of cells will be considered.

2. Experimental

2.1. Aqueous media, solid surfaces and bacteria

Sources and preparation procedures of the following materials were described previously [8]:
phosphate-buffered saline solutions (PBS) of various ionic strengths, thin transparent sheets of PFA Teflon and glass slides of dimensions 18 × 9 mm², and the bacterial strains listed in Table 1. Cell suspensions are buffered in PBS to maintain the pH 7 during the adhesion experiments. The electrokinetic mobilities \( u \) of the bacteria in 0.01 M PBS are not significantly different from those in 0.01 M KNO₃ solutions of pH 7 (data not shown). This indicates that PBS does not interfere in the electrostatic interaction, e.g. by specific adsorption of phosphate at the bacterial cell surface. None of the bacterial strains, except P4, possess cell surface appendages such as flagellae, fimbriae, etc.

2.2. Cell surface polymer composition

The iep’s of the cells, determined by electrophoresis, their hydrophobicities, expressed by \( \theta_w \), and the polymer composition tentatively inferred from these parameters, were taken from a previous paper [16] and are included in Table 1.

2.3. Transmission electron micrographs

Transmission electron micrographs of cross-sections of cells of strains C5 and C6 were made to examine their cell surface polymer structure. The specimen were negatively stained with ruthenium red and uranyl acetate according to the method of Handley [28].

2.4. Water wettability of the substrata

According to their wettabilities by water a macroscopic surface of glass is hydrophilic (\( \theta_w = 12° ± 2° \)) and that of Teflon is hydrophobic (\( \theta_w = 105° ± 1° \)) [8].

2.5. Electrokinetic characterization of bacteria and surfaces

Electrophoretic mobilities of the bacteria and streaming potentials of the substratum surfaces were measured according to Rijnaarts et al. [8] in PBS with ionic strengths varying between 0.001

<table>
<thead>
<tr>
<th>Strain</th>
<th>Other designation</th>
<th>( \theta_w )</th>
<th>iep</th>
<th>Type of polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Arthrobacter sp. DSM 6687</td>
<td>15 (1)</td>
<td>1.7</td>
<td>AP</td>
</tr>
<tr>
<td>C2</td>
<td>Coryneform DSM 6685</td>
<td>29 (1)</td>
<td>2.6</td>
<td>AP</td>
</tr>
<tr>
<td>C3</td>
<td>Rhodococcus sp. C125</td>
<td>70 (5)</td>
<td>3.0</td>
<td>AMPH</td>
</tr>
<tr>
<td>C4</td>
<td>Rhodococcus erythropolis A177</td>
<td>87 (5)</td>
<td>2.8</td>
<td>AMPH</td>
</tr>
<tr>
<td>C5</td>
<td>Corynebacterium sp. DSM 6688</td>
<td>89 (1)</td>
<td>3.2</td>
<td>NP</td>
</tr>
<tr>
<td>C6</td>
<td>Corynebacterium sp. DSM 44016</td>
<td>103 (6)</td>
<td>3.8</td>
<td>NP</td>
</tr>
<tr>
<td>C7</td>
<td>Gordona sp. 1775/15</td>
<td>115 (5)</td>
<td>3.3</td>
<td>NP</td>
</tr>
<tr>
<td>C8</td>
<td>Gordona sp. DSM 44015</td>
<td>117 (4)</td>
<td>3.4</td>
<td>NP</td>
</tr>
<tr>
<td>P1</td>
<td>Pseudomonas oleovorans ATCC 29347</td>
<td>17 (1)</td>
<td>1.7</td>
<td>AP</td>
</tr>
<tr>
<td>P2</td>
<td>Pseudomonas fluorescens p62</td>
<td>25 (1)</td>
<td>3.6</td>
<td>NP</td>
</tr>
<tr>
<td>P3</td>
<td>Pseudomonas sp. Strain B13</td>
<td>32 (1)</td>
<td>2.2</td>
<td>AP</td>
</tr>
<tr>
<td>P4</td>
<td>Pseudomonas putida mt2</td>
<td>40 (4)</td>
<td>3.2</td>
<td>NP</td>
</tr>
</tbody>
</table>

Superscripts:

1. The sources of organisms other than DSM or ATCC strains are described by Rijnaarts et al. [8].
2. Standard deviation in \( \theta_w \) determined by at least three independent measurements is presented in parenthesis.
3. Standard error in values of iep is 0.2.
4. The types of the cell surface polymers were deduced from iep and \( \theta_w \) values, and literature data on cell wall composition, discussed in a previous study [16]. These are: anionic polysaccharidic (AP), with iep \( \leq 2.8 \) and \( \theta_w \leq 32° \); amphiphilic (AMPH), most likely hydrophobic polysaccharidic, with \( 2.8 \leq \text{iep} \leq 3.0 \) and \( 70° \leq \theta_w \leq 87° \); non-polysaccharidic (NP), which are probably proteinaceous (\( 25° \leq \theta_w \leq 40° \)) or lipid-like (\( 89° \leq \theta_w \leq 117° \)) having an iep \( \geq 3.2 \).
and 0.1 M. Values of \( u \) for the various bacteria in media of \( I = 0.01 \) M ranged from \(-1.03 \times 10^{-8}\) to \(-3.34 \times 10^{-8}\) m\(^2\) V\(^{-1}\) s\(^{-1}\). Electrophoretic mobilities were converted into electrophoretic potentials of the bacteria (\( \zeta_b \)) using the Helmholtz–Smoluchowski equation and the electrophoretic potentials (\( \zeta \)) of the substrata were derived from the streaming potentials using the corresponding equations. In order to obtain values for \( \psi^d \approx \zeta \) in the, experimentally inaccessible, ranges of high (1 M) and low (10\(^{-4}\) M) ionic strength, an extrapolation procedure was developed and is described in Appendix A.

A more accurate assessment of \( \zeta_b \) requires knowledge of the surface conductance at the bacterial cell wall [29]. However, such data are not available for most of the systems. Hence, the calculated values of \( \zeta_b \) may be systematically underestimated to an unknown extent. However, the \( \zeta_b(I) \) dependencies for the various bacteria, relative to each other (Fig. 10), are realistic.

2.6. Activation Gibbs energies of adhesion (\( \Delta G^\ast \))

Activation Gibbs energies of adhesion (\( \Delta G^\ast \)) were obtained from deposition experiments at ionic strengths varying between 0.0001 and 1 M following method 1 described in reference [8]. For each adhesion measurement, sealed vials with a volume of 9 cm\(^3\) containing a piece of substratum surface submerged in a bacterial suspension in PBS were prepared in triplicate and incubated for four hours at room temperature. The initial bacterial concentration \( c_b \) was \(5 \times 10^8\) cells per cm\(^3\). After incubation, washing procedures were performed which reduced the suspended cell concentration by at least a factor of 150 and avoiding shear forces. These procedures kept the adhered cells for all but one (strain P1/Teflon) case irreversibly bound to the surfaces [1,8]. Directly after the washing step, the surfaces were removed from the vial. Adhesion, \( \Gamma \) (cells m\(^{-2}\)), was determined by counting attached cells under a light microscope. In principle, experimental values of \( \Delta G^\ast \) can be obtained from the ratio of the measured rate of deposition to that calculated for cells moving uninhibited from bulk suspension to the surface. In the absence of convection, this transport is controlled by diffusion. For the incubation times and cell concentrations considered here, \( \Delta G^\ast \) values can be obtained from a single value of \( I \) and the previously published value of the effective diffusion constant \( D_e \) of the cells [8], according to Equation A3 of [1], which after rearrangement becomes

\[
\Delta G^\ast/kT = \ln[2c_b(tD_e/\pi)^{1/2}] \quad (1)
\]

where \( k \) (J K\(^{-1}\)) is Boltzmann’s constant, \( T \) (K) the absolute temperature and \( t \) (s) is the incubation time.

3. Results

3.1. Activation Gibbs energy of adhesion as a function of ionic strength

By way of example, the adhesion of strain C3 on glass and Teflon is shown in Fig. 1. At \( I > 0.01 \) M, the deposition on Teflon is diffusion-limited and not inhibited by any repulsion. Lowering \( I \) to values \(< 0.01 \) M results in a strongly reduced adhesion. The adhesion of this organism on glass is also enhanced by increasing the ionic strength; it levels off at \( I > 0.01 \) M but, unlike for Teflon, it does not reach the level corresponding to diffusion-controlled deposition. From these data, using Eq. (1), \( \Delta G^\ast \)-values are obtainable. According to this procedure, the adhesion data for the strains C1, C3, C5, C6, P1, P3 and P4 on Teflon and glass were converted into \( \Delta G^\ast \)-values. Results are given in Fig. 2(A–D). There is a wide strain-substratum specific variation in the \( \Delta G^\ast-I \) dependency. For instance, at \( I = 0.1 \) M \( \Delta G^\ast \) varies between 0 and 5 kT, increasing in the order: NP and AMPH/Teflon < NP/glass < AP/Teflon < AP and AMPH/glass (explanation of abbreviations is given in the caption to Fig. 2). The NP-coated pseudomonad P4 shows a rather deviating \( \Delta G^\ast-I \) behaviour, especially with Teflon. The cells of this strain possess a flagellum. Under a microscope it was observed that, at low ionic strength, some of the attached cells on both substrata make fierce rotating movements. Apparently, a fraction of the
cells attach by their flagellae. At $I = 1$ M, none of the attached cells displayed motility.

3.2. Electron microscopy

The EM micrographs (Fig. 3(A–B)) indicated a difference in the extension of cell surface macromolecules between strains C5 and C6. The thickness of the polymer matrix outside the cytoplasmic membrane is 30 nm for C5 and 50 nm for C6.

3.3. Electrokinetic data

The results of the electrophoresis and streaming potential measurements are presented in Figs. 9 and 10.

4. Discussion

4.1. Contributions to the interaction

Interaction between bacteria and substrates is governed by a number of forces of which only the sum effect is measurable in adhesion experiments. As there seems to be some dispute in the literature on this issue, let us identify the main ones.

1. Electrostatic interaction ($G_{el}$) defined as the isothermal reversible electric work in bringing bacterium and substratum from infinity to $h$. It is caused by the surface charge on the glass or Teflon and by the bulk charge on the polyelectrolytic bacterial outer surface. As the precise distribution of the latter charge is not known, no a-priori assessment of $G_{el}$ is possible, but since we are interested in the rate at which barriers are passed, computation of $G_{el}$ on the basis of z-potentials and diffuse double layer theory is a viable option.

2. Van der Waals interaction ($G_{vdW}$). This is classical, and determined by the geometry of the system, and the Hamaker constants of bacterium (mainly its surface), water and substratum $G_{el}$ and $G_{vdW}$ together constitute the DLVO regime.

3. Steric interaction ($G_{ster}$) are all those forces resulting from interactions between uncharged chains and/or chain elements. They have three contributions, an osmotic (mixing) term, an entropic volume restriction term and, if chains can protrude far enough to reach the substratum, a binding contribution. These three contributions are not additive [30]. The most important parameters are the segment density distribution and the Flory–Huggins interaction parameters $\chi_{ij}$ which measure the interaction between pairs of groups $i$ and $j$ across the solvent.

Other fundamental types of interactions are unlikely. Sometimes interaction of Gibbs energies of the nature

$$G^s(h) = A \exp(-h/\chi)$$  \hspace{1cm} (2)

are introduced [31] and claimed to be of an acid–base nature. This is a misnomer. Eq. (2) originates from the stacking of solvent molecules onto repulsive walls, i.e. it is a solvent structure-mediated interaction and has nothing to do with acidity or basicity. At very short distances this interaction assumes an oscillatory nature. That [2] has been ‘successfully’ applied to describe steric interactions finds its origin in the fortuitously exponential decay of steric forces with distance; with two
parameters there is enough room for adjustment, but [2] then acquires a purely empirical nature.

The question remains whether the real solvent structure-mediated force has to be included. Given its range (a few solvent layers), which is short compared to the thickness of the polyelectrolytic coat, the answer is negative.

As a result, the state of the art is that we are left with DLVO and steric forces. In [31], Van Oss states that these contributions are additive.

However, according to more recent theories [32] they are not additive, because charging of macromolecules affects the segment density distribution and the $\chi$’s. Faced with this basically unsolvable problem we have introduced the picture of an infinitely steep $G_{\text{ster}}^{\text{adh}}$ decay. This simplifies the treatment to a one-parameter issue (the ‘steric thickness’), but it is solidly anchored in steric interaction theory and experiments, which show that the $G_{\text{ster}}^{\text{adh}}$ decay is several orders steeper than that of $G_{\text{el}}$ and $G_{\text{vdW}}$.

Fig. 2. Activation Gibbs energy of adhesion $\Delta G^*$, derived from deposition experiments for the various bacteria (Table 1) on Teflon and glass, as a function of the ionic strength of the medium. The abbreviations indicate the following types of cell coatings: AP, anionic polysaccharides; AMPH, amphiphilic compounds, most likely lipopolysaccharides; NP, non-polysaccharide compounds such as proteins or lipids.
Fig. 3. Transmission electron micrographs of thin sections of the strains C5 (left) and C6 (right), negatively stained with uranyl acetate and ruthenium red: bar = 150 nm; white arrow indicates cytoplasmic membrane; black arrow indicates cell exterior. The macromolecular matrix outside the cytoplasmic membrane is about 30 nm for strain C5 and ~50 nm for strain C6.

DLVO contribution to $\Delta G^\sigma(h)$. The DLVO contribution to $\Delta G^\sigma(h)$ was calculated for each bacterium–substratum–ionic strength combination studied, assuming constant potentials, $\psi^d$, for which we took the measured or extrapolated values of the $\zeta$-potentials for the bacterium and substratum surfaces (see Appendix A). The argument for choosing the constant potential rather than the constant charge model is that the time scale at which association–dissociation of surface groups relax is shorter than that for cell-substratum encounter. The boundary values of the interval over which the Hamaker constant $A_{bs(w)}$ varies, and estimates of the effective radius $R_e$ of the cells, used in the calculations, were taken from ref. [8]. Typical results of the DLVO modelling are represented by the curves for strain C3 shown in Fig. 4(A–B). The differences between glass and Teflon originate from the influence of the

Fig. 4. DLVO contributions to $\Delta G^\sigma(h)$ calculated for the combination of *Rhodococcus* strain C125 (strain C3, Table 1) with glass (A) and Teflon (B) at different ionic strengths, using the extremes of the range for the Hamaker constant: $3.4 \times 10^{-21} J \leq A_{bs(w)} \leq 8.8 \times 10^{-21} J$ for glass and $7.2 \times 10^{-23} J \leq A_{bs(w)} \leq 2.0 \times 10^{-22} J$ for Teflon. The lower, most negative parts of the curves correspond to the highest value of $A_{bs(w)}$ in both figures. The maxima of the curves at $I \leq 0.1$ (not shown) amount to hundreds to thousands of $kT$-units.
Hamaker constant. The Hamaker constant of glass is about two to three times higher than that of Teflon. However, since the values for the Hamaker constants of Teflon and water are very close, the values for \( A_{\text{w/w}} \) differ largely, by a factor of 40–50, between glass and Teflon [1]. According to the DLVO-contribution (Fig. 5(A)), the ionic strengths above which adhesion is to be expected, either by suppression of the maximum in \( \Delta_{\text{DLVO}}G^\omega(h) \) or by the formation of a secondary minimum in \( \Delta_{\text{DLVO}}G^\omega(h) \), are different between glass and Teflon. Primary minimum adhesion may occur if the maximum of \( \Delta_{\text{DLVO}}G^\omega(h) \) is suppressed below, e.g. 5 \( kT \) [1]; this condition is not reached for most bacteria and Teflon, even up to \( I = 1 \) M, and for glass only at \( I > 0.1 \) M (Fig. 5(B)). A significant secondary minimum, i.e. deeper than 3 \( kT \) [1], exists for most bacteria and glass at \( I > 0.002 \) M and for most bacteria and Teflon at \( I \geq 0.8 \) M (Fig. 5(C)). Hence, if the deposition would be controlled by DLVO interactions, secondary minimum adhesion on glass is to
be expected at 0.002 M < I < 0.1 M and primary minimum adhesion at I > 0.1 M. With Teflon adhesion in the secondary minimum would occur at I > 0.8 M and for primary minimum adhesion I has to be increased beyond 1 M. In passing, the large difference between I needed to produce secondary minimum adhesion on glass and Teflon is proof of the large difference between the in-water Hamaker constants for these systems.

DLVO and steric interactions. At low ionic strength, e.g. < 0.001 M, the overall DLVO interactions are strongly repulsive (Fig. 4(A–B)). The barriers may amount to thousands of kT (Fig. 5(B)). However, the inhibition of deposition is far less than predicted by these high DLVO-barriers. Rather, ΔG^s values of ~ 5 kT are derived (Fig. 2(A–D)). This difference must be caused by attractive steric interactions, probably by bridging. The distance over which steric interaction effectively inhibits deposition lies between h_s, the position of the secondary minimum, and h_{sat}, the position where Δ_{DLVO}G^s(h) - Δ_{DLVO}G^s(h = h_s) equals 5 kT, i.e. the maximum value found experimentally for ΔG^s (Fig. 5(D–E)). This is confirmed by the high steric barriers obtained for h_2 for I = 0.001 M, this distance is larger than 100 nm for all cases. Hence, to cause (irreversible) attachment, very long polymers are needed to bridge the cell–substratum separation. Only strain P4 on Teflon is able to cover such long separations efficiently (Fig. 2(A)), probably by its flagellum. Except for this system the conclusion is that at low ionic strength DLVO-type electrostatic repulsion is the dominant interaction inhibiting adhesion and that cell surface macromolecules only have a minor influence. However, at high ionic strength ΔG^s strongly depends on the type of cell coating (Fig. 2(A–D)). For some systems (panel A), electrostatic repulsion is already fully suppressed at 10^{-2} or 10^{-1} M ionic strength, but for others electrolytes continue to exert their screening action even up to 1 M. This is typical for a polyelectrolytic layer [14] and results from the electrolyte-dependent spatial distribution of charges. This applies to all depositions on glass (panels B and D) and to AP on Teflon (panel C). From the distinction with NP and AMPH on Teflon (panel A) it is inferred that there must be apolar groups on NP and AMPH that have enough affinity for the Teflon surface to ensure bridging.

The results for the Rhodococcus strain C125 (C3), presented in Fig. 6(C, D), exemplify different cases of steric interactions as schematically depicted in the Fig. 6(A–B). At high I, the adhesion at Teflon is not retarded. Hence, ΔG^s = 0 which indicates bridging (Fig. 6(B), curve b). Glass adhesion is inhibited, even at high I, implying steric repulsion: ΔG^s > 0 (Fig. 6(B), curve a). This repulsion is greater than the relatively strong van der Waals attraction to glass (Fig. 4(A)).

In view of the discussion given above, a value for the ionic strength, I_s, may be indicated for the transition of DLVO-controlled adhesion into sterically-controlled adhesion. The value of I_s is derived from the condition where the extension λ of the steric interaction just exceeds the range of the DLVO contribution as indicated by the position of the secondary minimum h_2 (Fig. 5(D–E)). Values of λ were determined using the h_2(I) dependency and the adhesion results (Fig. 6(C–D)). For strain C3, the value of λ for bridging with Teflon is calculated to be 38 nm and a comparable value of 46 nm is derived for the steric repulsion at the glass surface. The values of I_s for strain C3 at Teflon and glass are 0.013 and 0.004 M, respectively.

Relation between steric effects and type of cell surface polymer. All adhesion data were converted to ΔG^s-values and plotted as a function of h_2 (Fig. 7(A–D)). The values derived for λ are summarized in Table 2. The height of the steric barrier ΔG^s for the various types of cell surface polymer increases in the order (Fig. 7(A–D), Table 2): non-polysaccharide (lipids or proteins) or amphiphilic compounds with Teflon < non-polysaccharide compounds with glass < anionic polysaccharides with Teflon < anionic polysaccharides or amphiphilic compounds with glass. Bacteria coated with non-polysaccharides or amphiphilic polymers bridge at Teflon surfaces, as judged from ΔG^s = 0.

Anionic polysaccharide cell coatings. Anionic polysaccharides are highly soluble in water and therefore they exert a steric repulsion on most surfaces in an aqueous environment [7,12,14]. This is confirmed by the high steric barriers observed for the combinations of the strains P1, P3 and C1 with glass and Teflon (Fig. 7(C–D), Table
2). These surface polyelectrolytes protrude over a considerable distance into the surroundings of the cell, as indicated by $\lambda$-values up to several tens of nanometers.

Amphiphilic cell coatings. In previous studies [8,16] evidence was obtained for the amphiphilic character of the surfaces of strains C3 and C4. The hydrophilic part seems to dominate the steric interaction of strain C4 causing a repulsion at both glass and Teflon [16]. The hydrophilic moieties on the surface of C3 impede adhesion on glass while the lipophilic parts cause bridging with Teflon. Adhesion of bacteria by different types of polymer moieties on surfaces differing in hydrophobicity has been observed by others as well [33].

![Diagram](image-url)

Fig. 6. (A–B) Illustration of the magnitude and the effective extension $\lambda$ of steric interactions. Two types of steric contributions $\Delta G^s(h)$ to the Gibbs energy of interaction $\Delta G(h)$ can occur: the macromolecules either induce steric repulsion (a) $\Delta G^s > 0$ or cause bridging (b) $\Delta G^s = 0$; (C–D) Deposition and the extensions of DLVO ($h_2$) and steric ($\lambda$) interactions. The deposition of strain C3 (Rhodococcus C125) onto Teflon (C) and glass (D) and the calculated extension of the DLVO interaction as a function of the ionic strength $I$ of the medium. The short-dashed lines indicate the deposition level expected for purely diffusive transport of cells to the substratum. The long-dashed lines indicate how $\lambda$ and $I_s$ can be derived from the $h_2$–$I$ curve obtained from adhesion experiments.
Non-polysaccharide cell coatings. The surface of C6 cells are known to contain hydrophobic long chain mycolic acids [17]. In an aqueous medium these hydrophobic macromolecules would condense into a thin rather compact layer at the cell surface so that they are not able to span separations of 25–33 nm as indicated by their relatively low \( \lambda \)-values (Table 2). Hence, other component(s) may be responsible for the adhesion. The reduced adhesion on glass when increasing \( I \) from 0.1 to 1 M (Fig. 7(B)) suggests that this additional component is a polyelectrolyte. The relative high water contact angle of the C6 cell surface, the absence of strong steric repulsion at glass and the relatively high iep of 3.8 point to macromolecules other than highly polar polysac-
charides [16]. Possible candidates are carboxylated lipids or peptidolipids known to exist on surfaces of mycobacteria [34,35], which are closely related to coryneform bacteria. The adhesion pattern of strain C5 is similar to that of C6, except for the extension of the steric interactions, which are much shorter for C5 (Fig. 7(A–B), Table 2). Possibly, the cells of strain C5 have a bare peptidoglycan-mycolic acid cell wall matrix [16,17].

The difference in steric extension between the strains C5 and C6 is in line with the EM micrographs (Fig. 3(A–B)), although a complete match of the steric range and the EM results is not to be expected for the following reasons: (1) the sample preparation for EM involves dehydration of the cell wall which is known to cause (partial) collapse of outer polymer structures [36]; (2) the location in the cell envelope at which the rather compact peptidoglycan fabric changes into a more loosely structured polymer layer is not well defined; (3) the effective steric extension is smaller than the actual length of some of the cell surface macromolecules (Fig. 6(A), [37]). Assuming a peptidoglycan layer of 20 nm thick, which is a reasonable estimate for Gram-positive organisms [36], the loosely organized outer polymer surface appears to be ~10 nm for strain C5 and 30 nm for strain C6. These values follow the same trend as those derived for \( \lambda \) which are 7 ± 1 nm for C5 and 29 ± 4 nm for strain C6 (Table 2).

The adhesion behaviour of strain P4 is obviously controlled by its flagellum. Bridging separations are relatively large, 165 nm for Teflon and 50 nm for glass (Fig. 7(A–B)). However, even attachment by the flagellum does not make adhesion 100% effective as indicated by deposition barriers of 0.6 \( kT \) on Teflon and 1.2 \( kT \) on glass. In addition to the flagellum, strain P4 has a non-polysaccharide coating on its outer membrane LPS structure [16]. De Flaun et al. [37] showed that a non-adhering Pseudomonas mutant lost its ability to form flagellae. Hence, the role of flagellae, relative to other cell surface structures, in the adhesion of these types of micro-organisms deserves further study.

Suppression of electrosteric effects at high ionic strength. At \( I \geq 0.1 \) M, attachment is predominantly controlled by steric interactions. The decrease in \( \Delta G^* \), as observed for the strains C1, C3, C6, P1 and P3, when changing \( I \) from 0.1 to 1 M (Fig. 7(B–D)), is as expected since these cells are coated with polyelectrolytes. At such high values of \( I \) electrostatic repulsion is suppressed [7,12,14,15]. Because the number of polyelectrolyte molecules per unit bacterial surface area is fixed, this also leads to a shrinking polyelectrolyte

<table>
<thead>
<tr>
<th>Cell-coating(^b)</th>
<th>Strain</th>
<th>Steric barrier ( \Delta G^* ) (kT)</th>
<th>Effective steric length ( \lambda ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glass</td>
<td>Teflon</td>
<td>Glass</td>
</tr>
<tr>
<td>Anionic polysaccharides:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>4.8</td>
<td>3.2</td>
<td>&gt;30</td>
</tr>
<tr>
<td>P3</td>
<td>3.2</td>
<td>2.8</td>
<td>&gt;50</td>
</tr>
<tr>
<td>C1</td>
<td>2.8</td>
<td>3.2</td>
<td>25</td>
</tr>
<tr>
<td>Amphiphilic macromolecules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>4.1</td>
<td>2.4</td>
<td>n.d.(^c)</td>
</tr>
<tr>
<td>(hydrophobic polysaccharides)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>3.3</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Non-polysaccharide macromolecules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>1.0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>(lipids and proteins)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>0.8</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>P2</td>
<td>1.1</td>
<td>0</td>
<td>n.d.(^c)</td>
</tr>
<tr>
<td>P4</td>
<td>1.2</td>
<td>0.6</td>
<td>50(^d)</td>
</tr>
</tbody>
</table>

\(^a\) The properties listed are the height of the steric barrier \( \Delta G^* \) and the range \( \lambda \) of the steric interactions.

\(^b\) The most probable type of polymer present on the cell surface was deduced from \( \Theta_u \) and iep values, and literature data on cell wall composition [8].

\(^c\) n.d., not determined.

\(^d\) It is very likely that these bridging distances are partially determined by the flagellum of this organism.
coat and allows the cells to approach the sub-stratum surface more closely which results in a stronger contribution of the van der Waals attraction (Fig. 4(A–B)). The van der Waals attraction being stronger for glass than for Teflon [1] could very well be the reason that the reductions in $\Delta G^\sigma$ upon increasing $I$ to 1 M are more pronounced for glass (Fig. 7(B, D)) than for Teflon (Fig. 7(C)).

Adhesion behaviour of micro-organisms in natural aqueous environments. Among the strains studied, the variation in the range over which DLVO-interactions are effective by varying the ionic strength between 0.001 and 0.1 M (Fig. 5(D–E)) is comparable to the variation in the range covered by steric interaction (Fig. 7(A–D), Table 2), i.e. between a few nanometers and, e.g. 100 nm. As a consequence, values for $I_c$ vary over this same $I$-range. Hence, understanding the adhesion behaviour of a given bacterial species in a given environment requires assessment of both steric and DLVO contributions for each case. Nevertheless, some general trends can be deduced from the results presented in this paper.

Teflon is very hydrophobic so that all bacterial surface polymers containing hydrophobic groups tend to adsorb on it, thereby forming bridges. That bridging prevails (over DLVO-type attractions) is also caused by the relative low Hamaker constant. In contrast, glass is very hydrophilic, which, in an aqueous environment, is unfavourable for bridging. Consequently, with glass steric interaction will often be repulsive, which may be partly compensated by the stronger van der Waals attraction caused by the larger Hamaker constant. Thus, these two model surfaces, glass and Teflon, represent rather two extremes in the series of negatively charged substrata that are encountered in most natural and technical environments. Hence, the ionic strengths at which the DLVO/steric interaction transition occurs for Teflon and for glass demarcate the ionic strength interval where this would occur for almost any other negatively charged clean surface. The $I$-intervals, thus defined, are shown in Fig. 8(A) for the bacteria studied, and these $I$-intervals may be compared with the ionic strengths of various aqueous media (Fig. 8(B)).

Thus, in an environment of low ionic strength, like in rain water, rivers and lakes, adhesion of most bacteria is impeded by DLVO-type electrostatic repulsion. Only flagellated micro-organisms (e.g. strain P4) are able to bridge cell-substratum separations in these systems. In, for example, (domestic) waste waters and saliva deposition may be dominated by steric and/or DLVO interactions and both types of interactions have to be considered for an assessment of bacterial adhesion. In more saline systems, such as milk, blood, urine, and, even more so, sea water, adhesion is in most cases controlled by steric interactions. In sea water electrosteric effects are also suppressed and micro-organisms have only one option to prevent the immobilisation, namely by forming a hydrophilic coating that causes a non-electrostatic steric repulsion.

Appendix A. Analyses of electrokinetic data of cells and solid surfaces

The main purpose of this analysis was to obtain reliable extrapolation of $\zeta$ to $I=1$ or 0.0001 M where electrokinetic measurements are not feasible. For this we used the Stern-layer capacitor concept of the electrical double layer:

$$\psi^o - \psi^d = \sigma^o / C$$

(A1)

where $\psi^o$ and $\psi^d$ are the potentials (V) at the surface and at the fluid-side of the Stern layer, respectively, $\sigma^o$ the surface charge density (C m$^{-2}$), and $C$ is the Stern-layer capacitance (C V$^{-1}$ m$^{-2}$). For smooth and non-conducting interfaces, one can estimate the thickness of the Stern-layer $\delta^s$ from $C$ [38] if an estimate for the dielectric permittivity in that layer is available. However, for bacterial cell surfaces the situation is more complex. Macromolecules may extend into the solution and may shift the plane of shear outwards or lead to surface-associated charge within this plane. Surface conductance and specific adsorption of the counter-ions may also complicate interpretation. Despite these limitations,
the Stern-layer model provides a possibility to estimate \( \zeta \) for \( I \)-ranges where \( \zeta \) cannot be determined electrophoretically.

Applying the Gouy–Chapman theory with \( \zeta \approx \psi^d \), it follows that

\[
\sigma^d = (2\kappa eRT/F) \sinh \left( \frac{zF\zeta}{2RT} \right) \tag{A2}
\]

where \( \kappa \) is the reciprocal Debye-length (m\(^{-1}\)) and is defined as

\[
\kappa^2 = -2F^2 I/\varepsilon RT \tag{A3}
\]

\[
I = \frac{z^2}{8\varepsilon RT} \left( -C(\psi^o - \zeta) \right)^2 \sinh(zF\zeta/2RT) \tag{A4}
\]

where \( R \) is the gas constant (J K\(^{-1}\)), \( \varepsilon \) the dielectric permittivity for water (C\(^2\) J\(^{-1}\) m\(^{-3}\)), \( F \) the Faraday constant (C mol\(^{-1}\)), and \( z \) the valency of the symmetrical electrolyte. \( \zeta \) cannot be explicit as a function of \( I \). Therefore, for computational purposes we used:

The values of \( \psi^o \) and \( C \) were obtained by fitting Eq. (A4) to experimental \( \zeta \) and \( I \) values.
Solids. The model fits the experimental data well for glass and Teflon (Fig. 9). The $C$-values are $-17 \pm 2 \text{ mF cm}^{-2}$ for glass and $-23 \pm 2 \text{ mF cm}^{-2}$ for Teflon. Assuming that $C = \varepsilon^s/\delta^s$ and $\varepsilon^s \approx 0.1 \varepsilon$ (where $s$ stands for Stern-layer) [38], these values correspond to a thickness of the Stern-layer of 0.40 and 0.28 nm, for glass and Teflon, respectively. These values are close to the radius of 0.36 nm of the hydrated Na$^+$-ion [39], which was the main counterion in the systems studied.

Bacteria. The results for the bacteria are shown in Fig. 10. The data points for C1, C2, C5 and C8 at $I = 0.1 \text{ M}$ and C3, C4, C5 and C6 at $I = 0.001 \text{ M}$ were excluded from the fit procedure. Thus, the coefficients of variation in the parameter estimates was always <15%, except for strain C2. This gives an impression of the applicability of the charge-free Stern-layer-model to bacteria. Non-ideal electrokinetic behavior was quantified by the extent to which $C$ depends on $I$. $C$ is approximately constant for $10^{-2.5} \leq I \leq 10^{-1.5} \text{ M}$, in all but one (C2) case. Outside this interval, the presence of macromolecule-bound charges interferes. It is surprising that the charge-free Stern layer model works so well for such complicated systems. Most likely this is caused by the virtual compensation of all charges in this layer [40]. At $I = 0.0001$ and 1, $\zeta$ was estimated with Eq. (A4) and the fitted $\psi^o$ value, accounting for this non-ideality by using values of $C$ obtained from extrapolating $C = f(I)$. For most bacterial strains (C1, C5, C8, P1 and P3), $C^s$ ranges between 20 and 50 mF cm$^{-2}$, which is in the usual range for non-porous surfaces ([38], Section 3.10). Strain C2 has an exceptionally high $C^s$ (>200 mF cm$^{-2}$); such high capacitances are observed for surface charge distributions with a certain depth; relatively low capacitances (6–7 mF cm$^{-2}$) are observed for the pseudomonads P2 and P4, for which surfaces the ratio $\varepsilon^s/\delta^s$ must be relatively low. Future research is required to elucidate the possible effects of the chemical nature and the structure of the outer cell surface on the electrokinetic behavior of bacteria.

5. Conclusion

Comparing experimental deposition data at various ionic strengths with DLVO model calculations allows us to estimate the extensions and magnitudes of DLVO- and steric contributions to the initial stage of bacterial adhesion. Our interpretation was semi-quantitative. At this level of approach a number of trends could be established. The development of a more quantitative model for microbial deposition that unifies DLVO- and macromolecular adsorption theories is a major challenge for future research.

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Fig. 10. The zeta-potential $\zeta$ for the bacteria listed in Table 1 as a function of the ionic strength $I$ of the medium. The curves resulted from fitting the capacitor model (Eq. (A4)) to the experimental data (points): solid curves, coryneform bacteria; dashed curves, pseudomonads.

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