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CELL SURFACE ENERGY, CONTACT ANGLES AND PHASE PARTITION III. ADHESION OF BACTERIAL CELLS TO HYDROPHOBIC SURFACES

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Summary

The densities of adhesion of *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Serratia marcescens* to five types of plastics were studied in relation to interfacial free energies at the aqueous interfaces of both the bacteria and the plastics. The free energy of adhesion of bacteria to plastic in an aqueous medium is a linear function of partition of the bacteria between the solid surface and the liquid phase. These results show that the thermodynamics of the partitioning of a suspended particle between two immiscible liquid phases also apply to partitioning between a liquid and a solid phase.

The adhesion of cells to solid substrata has been the subject of extensive study because of its relevance to a multitude of phenomena. Cell adhesion is important in the growth of mammalian cells in culture [1], and may be crucial to both normal and abnormal developmental processes, cancer cell growth and metastasis. Attachment of bacterial cells to tissue surfaces or to prosthetic implants is a precursor to pathogenesis in many cases [2, 3, 4, 5]. Bacterial adhesion to plastics is of economic importance in the fouling of marine equipment [6, 7] and in the immobilization of microbial cells for use in enzymatic conversions [8].

Bacterial cells adhere to surfaces by the formation of mechanical attachment organelles and by non-specific mechanisms involving electrostatic and hydrophobic interactions [8, 9]. This paper describes the application of surface physics to the adhesion of bacterial cells to hydrophobic, or low-energy surfaces. This subject has been studied most extensively in relation to the microbial fouling of plastics used in the marine environment. Dexter et al. [7, 10,

11] have studied the attachment of mixed microbial populations to plastics of known surface energy (solid/air interface) and found that, except for extremely hydrophobic surfaces, there is an increase in attachment with increasing solid surface energy. Fletcher [12] and Fletcher and Loeb [13, 14] investigated the adhesion of a marine *Pseudomonas* sp. (NCMB 2021) to a number of solid surfaces and found a generally increasing attachment with decreasing solid surface energy. The attachment of bacteria to solid surfaces immersed in an aqueous medium is, however, more directly related to solid/liquid interfacial free energies than to solid/air surface energies.

This study has investigated the relationship between (a) the changes in interfacial free energies which occur as a bacterial cell attaches to a hydrophobic uncharged solid in an aqueous medium, and (b) the number of cells which attach per unit surface area of the solid substratum. The basic relationship used is that developed previously [15, 16] between partition coefficients and surface free energy changes (Eqn. 1):

$$-\log K_{eq} = \alpha\Delta\gamma + \beta \tag{1}$$

where K_{eq} is the equilibrium partition coefficient ($[\text{cells}]_{\text{surface}}/[\text{cells}]_{\text{medium}}$), $\Delta\gamma$ the change in interfacial free energy accompanying partition and α and β are empirical parameters.

From this point of view, the equilibrium under consideration is that between cells free in suspension and cells attached to the solid substratum. The change in interfacial free energy which corresponds to the process of attachment (ΔG_a) is given by Eqn. 2. And thus, the expected relationship between the free energy of attachment and the equilibrium constant for attachment is given by Eqn. 3. Here, we describe a direct experimental test of Eqn. 3.

$$\Delta G_a = \gamma_{SB} - \gamma_{SM} - \gamma_{BM} \tag{2}$$

$$\log K_{eq} = -\alpha\Delta G_a + \beta \tag{3}$$

where ΔG_a is the free energy of attachment per unit area of attachment, γ_{SB} the interfacial free energy between the solid and the bacterium, γ_{SM} the interfacial free energy between the solid and the aqueous medium, γ_{BM} the interfacial free energy between the bacterium and the aqueous medium and α and β are empirical parameters.

Serratia marcescens and *Staphylococcus epidermidis* were cultured as described previously [15]. *Staphylococcus aureus* was grown in the same medium as *Staphylococcus epidermidis*.

All solid substrata were commercially available plastics. No attempt was made to ensure the absence of fillers or plasticizers, since we were attempting to relate measured values of surface energies to observed densities of adherence. The plastics used were as follows: poly(vinyl chloride), polystyrene, Plexiglas (Clear, Rohm and Haas), polycarbonate (Lexan, General Electric), and high molecular weight polyethylene. All were cleaned with detergents followed by extensive rinsing in distilled water and washing with methanol.

Adhesion-density experiments were performed as follows. The solid sub-

strata were placed in individual flasks with growth medium and a dense inoculum of the organism was added. The flasks were incubated on the gyro-rotary shaker for 24 h at 20°C. By this time, the culture was entering the stationary phase. The substrata were carefully removed and rinsed thoroughly with sterile distilled water to remove loosely attached cells. The samples were fixed for 1 min in Bouin's fixative (71% (v/v) saturated aqueous picric acid, 24% (v/v) formalin and 5% (v/v) acetic acid), stained for 1 min in crystal violet (1%), rinsed in distilled water and allowed to air-dry in sterile petri dishes. Adherence was determined by counting the number of cells per unit area using vertical-illumination optical microscopy.

Solid/liquid interfacial free energies were determined from contact angles of water and growth media on freshly cleaned samples of each plastic and lawns of bacteria collected on Millipore filters [16]. Contact angles were measured by the horizontal-projection technique [15, 16]. Surface tensions of the aqueous media were determined with a Fisher Autotensiomat using the du Nouy method. Interfacial free energies were determined by the equation-of-state approach [17], and the free energy of adhesion was calculated from these using Eqn. 2.

For the purpose of comparison with other studies [8, 10—14, 18], Table I lists the surface energies of the cells and plastics used in this study as determined from contact-angle measurements and the equation-of-state approach [17]. The values obtained for the bacteria are well within the range of values reported for other bacterial species [8]. The values obtained for the surface energy of the sample of high molecular weight polyethylene and for polystyrene are somewhat higher than other values given in the literature, however, this may be due to the presence of plasticizers.

The relationship between the logarithm of the number of cells attached per 100 μm^2 and the free energy of adhesion calculated from Eqn. 2 is given in Fig. 1. There is a linear relationship between these variables for the adhesion of *S. aureus* to all five plastics studied. For both *S. epidermidis* and *Se. marcescens*, there is a linear relationship between the log of the density of adhesion and the free energy of adhesion for four out of the five plastics studied. An anomolous point is obtained for the adhesion of these two organisms to the sample of high molecular weight polyethylene, and it is most probable that this results from the high value of the surface energy found for this sample. Litera-

TABLE I

Material	Surface energy, γ_{SV} (erg/cm^2)
Plastics	
Poly (vinyl chloride)	38.3
Polystyrene	43.8
Plexiglas^R (acrylic)	53.1
Lexan^R (polycarbonate)	54.7
Polyethylene (High M_r)	64.6
Cells	
Serratia marcescens	66.3
Staphylococcus epidermidis	67.9
Staphylococcus aureus	69.7

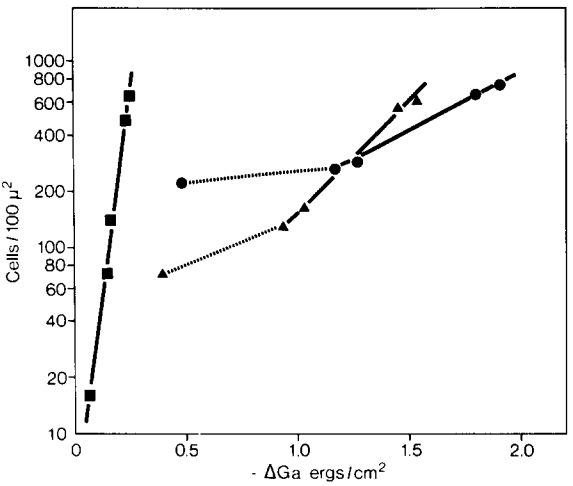

Fig. 1. The density of cells adhering to a plastic surface is a linear function of the free energy of adhesion of the cells to that surface in an aqueous medium.

ture values for the surface energy of pure polyethylene range from 32 to 42 erg/cm^2 [8, 18]. However, the use of these values gives a more negative estimate of ΔG_a than that suggested by the extrapolation of the line obtained with the other solid substrata. An error in the solid/liquid interfacial tension of about 15% or about 0.2 erg/cm^2, could account for this, or there could be some heterogeneities in the material. Nevertheless, these data offer further support for the relationship which was proposed earlier between the free energy of phase partition and surface free energies and demonstrates its applicability to adhesion phenomena.

Eqn. 2 indicates that ΔG_a will be negative when γ_{SB} is less than the sum of γ_{SM} and γ_{BM}. Conversely, ΔG_a could be positive, which would result in little or no adhesion to the surface, and possibly in the detachment of cells from a surface on which they were growing. The reduction in the absolute values of γ_{SM} and γ_{BM} relative to γ_{SB} will cause an increase in ΔG_a, however, the conditions which will result in this are difficult to predict. In general, an agent which will slightly decrease the surface tension of the medium, without coating the cells, may cause the desired changes. These findings and this approach to the direct measurement of cell/medium interfacial free energies may have implications in the development of agents which will prevent the attachment, or cause the detachment, of metastasizing cells or of microbial pathogens from tissues, teeth or prothetic materials.

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