

Comparison between the Adhesion to Solid Substrata of *Streptococcus mitis* and That of Polystyrene Particles

H. M. UYEN, H. C. VAN DER MEI, A. H. WEERKAMP, AND H. J. BUSSCHER*

Dental School, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

Received 8 September 1987/Accepted 9 December 1987

The adhesion of *Streptococcus mitis* to solid substrata from phosphate suspensions with various ionic strengths was studied and compared with the adhesion of polystyrene particles. At all ionic strengths, the interfacial free energy of adhesion governed the relative number of bacteria or polystyrene particles adhering at equilibrium, except that in a low-ionic-strength buffer, adhesion occurred less frequently because of increased electrostatic repulsion. Large differences between bacterial and polystyrene particle adhesion were observed, as indicated by the ratio of bacteria to polystyrene particles adhering, which decreased from 30 to 4 with a change from low to high ionic strength.

It is tempting to apply the same physicochemical principles to the adhesion of microorganisms to solid substrata as apply to the adhesion of inert particles. Several investigators have described bacterial adhesion in terms of physicochemical parameters such as zeta potentials (3, 5-7, 9, 12) or surface free energies (1, 3, 4, 6, 8, 9, 12) with various degrees of success. The structural and molecular complexity of the bacterial cell surface (14) can facilitate adhesion to solid substrata, but it impedes the application of the physicochemical principles of adhesion phenomena that are valid for inert particles.

It is the objective of this study to compare the adhesion of inert polystyrene particles with the adhesion of *Streptococcus mitis*, an oral microorganism with zeta potential and surface free energy almost identical to those of polystyrene, and to determine the extent to which physicochemical principles can be applied to bacterial adhesion. The size of the polystyrene particles was also selected to approximate the diameter of the streptococci (1 μm). However, the exact diameter of bacteria is difficult to express numerically because of the presence of appendages on the cell surface, as recently discussed in detail (3).

To this end, *S. mitis* BMS (dispersion surface free energy $\gamma^d = 36$; polar surface free energy $\gamma^p = 1 \text{ erg} \cdot \text{cm}^{-2} = 1 \text{ mJ} \cdot \text{m}^{-2}$ [13]), grown and cultured as described previously (10, 12), and polystyrene particles ($\gamma^d = 42$; $\gamma^p = 1 \text{ erg} \cdot \text{cm}^{-2}$ [11]), kindly prepared and provided by W. Norde (Agricultural University of Wageningen), were suspended in 0.2, 20, and 500 mM potassium phosphate buffer (pH 7.0). Subsequently, the adhesion of bacteria or polystyrene particles was measured on 1-cm² slabs of various substrata as a function of time (up to 2 h) to yield the number of bacteria or polystyrene particles adhering at equilibrium (n_e) (9). The following substrata with known surface free energies (9) were used: fluorethylenepropylene ($\gamma^d = 20$; $\gamma^p = 0 \text{ erg} \cdot \text{cm}^{-2}$), polymethylmethacrylate ($\gamma^d = 42$; $\gamma^p = 11 \text{ ergs} \cdot \text{cm}^{-2}$), and glass ($\gamma^d = 44$; $\gamma^p = 65 \text{ ergs} \cdot \text{cm}^{-2}$). The surface free energies of the bacteria or polystyrene particles, the solid substrata, and the suspending buffers ($\gamma^d = 21$; $\gamma^p = 50 \text{ ergs} \cdot \text{cm}^{-2}$ for all ionic strengths) were used to calculate the interfacial free energy of adhesion (ΔF_{adh}) (2), denoting whether adhesion is energetically favorable ($\Delta F_{\text{adh}} < 0$) or unfavorable ($\Delta F_{\text{adh}} > 0$). Finally, zeta potentials of

the bacteria and the polystyrene particles as well as of suspended filings (1 to 5 μm) of the solid substrata were determined electrophoretically with a model 500 Lazer Zee meter (PenKem, Bedford Hills, N.Y.).

Table 1 lists the zeta potentials of the solid substrata in the various phosphate buffers. Figure 1 shows the number of adhering bacteria and polystyrene particles as a function of their zeta potentials together with their interfacial free energies of adhesion to the various substrata.

The data in Fig. 1 demonstrate that both the bacteria and the polystyrene particles adhered in highest numbers when the interfacial free energy of adhesion was most negative, in accordance with the laws of thermodynamics. Increased electrostatic repulsion, as in the low-ionic-strength buffer, decreased adhesion as expected, but never to such an extent as to interfere with the dominant influence of interfacial free energies at a specific ionic strength. This was also recently noted by van Loosdrecht et al. (7) and Absolom et al. (2) for ionic strengths above 100 mM NaCl. A surface energy analysis thus seems to be a most fruitful physicochemical approach for predicting both bacterial and particle adhesion in relatively simple systems.

However, this approach cannot explain why bacterial adhesion was much greater than polystyrene particle adhesion despite their identical radii and similar ranges of zeta potentials and interfacial free energies of adhesion (Fig. 1). Surface structures as long as approximately 100 nm in this strain (unpublished results) obviously provided additional possibilities for the bacteria to overcome the potential energy barrier due to electrostatic repulsion. In this respect, it is interesting that the ratio of bacterial to polystyrene particle adhesion was dependent on the ionic strength of the buffer and decreased from 30 in the 0.2 mM buffer to 5 and 4 in the 20 mM buffer and the 500 mM buffer, respectively. This indicates that at high ionic strengths the bacterial cell

TABLE 1. Zeta potentials of filed solid substrata in potassium phosphate buffers (pH 7.0) of various ionic strengths

Substratum	Zeta potential (mV \pm 1) at:		
	0.2 mM	20 mM	500 mM
Fluorethylenepropylene	-47	-15	+4
Polymethylmethacrylate	-60	-22	+2
Glass	-80	-66	+3

* Corresponding author.

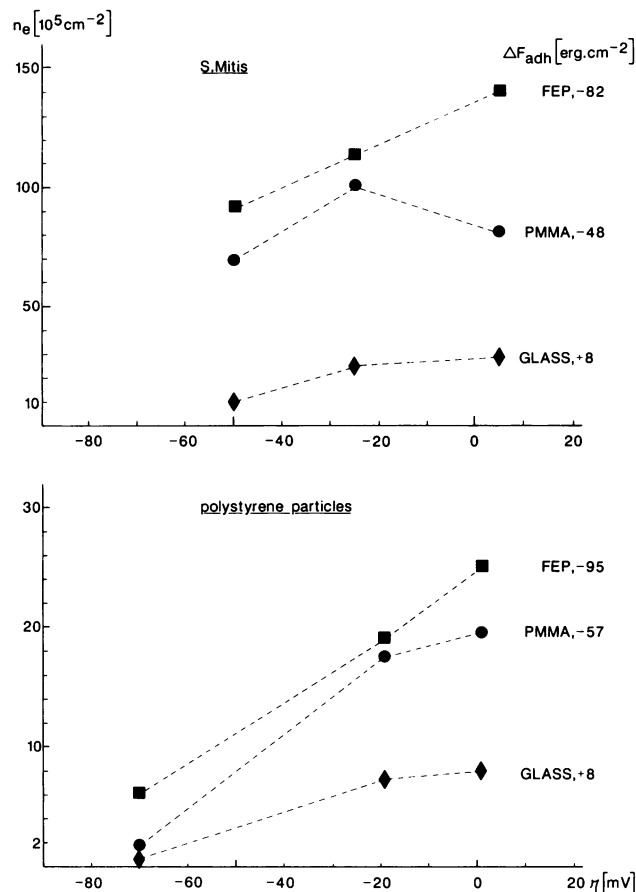


FIG. 1. Number of *S. mitis* cells and polystyrene particles adhering at equilibrium to various solid substrata as a function of the zeta potentials of the bacteria in potassium phosphate buffers of various ionic strengths (pH 7.0). Each datum point represents the average number of bacteria on each of 25 2,500- μm^2 square blocks, randomly distributed over the substratum surface. The standard deviation of each datum point is approximately 25%. FEP, Fluor-ethylenepropylene; PMMA, polymethylmethacrylate.

surface is more inert than at low ionic strengths. This is possibly due to an immobilization or collapse of surface structures in the high-ionic-strength buffer caused by attracting van der Waals forces, whereas in low-ionic-strength buffers surface structures probably extend far into the solu-

tion, thereby stimulating adhesion greater than that of inert particles.

LITERATURE CITED

1. Absolom, D. R., F. V. Lamberti, Z. Policova, W. Zingg, C. J. van Oss, and A. W. Neumann. 1983. Surface thermodynamics of bacterial adhesion. *Appl. Environ. Microbiol.* **46**:90-97.
2. Absolom, D. R., C. Thomson, G. Kruzyk, W. Zingg, and A. W. Neumann. 1987. Adhesion of hydrophilic particles (human erythrocytes) to polymer surfaces: effect of pH and ionic strength. *Colloids Surf.* **21**:446-456.
3. Busscher, H. J., and A. H. Weerkamp. 1987. Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol. Rev.* **46**:165-173.
4. Busscher, H. J., A. H. Weerkamp, H. C. van der Mei, A. W. J. van Pelt, H. P. de Jong, and J. Arends. 1984. Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. *Appl. Environ. Microbiol.* **48**:980-983.
5. Friberg, S. 1977. Colloidal phenomena encountered in bacterial adhesion to the tooth surface. *Swed. Dent. J.* **1**:207-214.
6. Marshall, K. C., R. Stout, and R. Mitchell. 1971. Mechanisms of the initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.* **68**:337-348.
7. Olsson, J., P. O. Glantz, and B. Krasse. 1976. Surface potential and adherence of oral streptococci to solid surfaces. *Scand. J. Dent. Res.* **84**:240-242.
8. Pringle, J. H., and M. Fletcher. 1983. Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. *Appl. Environ. Microbiol.* **45**:811-817.
9. Rutter, P. R., and B. Vincent. 1980. The adhesion of microorganisms to surfaces: physico-chemical aspects, p. 79-91. In R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent (ed.), *Microbial adhesion to surfaces*. Ellis Horwood Ltd., Chichester, England.
10. Uyen, H. M., H. J. Busscher, A. H. Weerkamp, and J. Arends. 1985. Surface free energies of oral streptococci and their adhesion to solids. *FEM Microbiol. Lett.* **30**:103-106.
11. van der Valk, P., A. W. J. van Pelt, H. J. Busscher, H. P. de Jong, C. R. H. Wildevuur, and J. Arends. 1983. Interaction of fibroblasts and polymer surfaces: relationship between surface free energy and fibroblast spreading. *J. Biomed. Mater. Res.* **17**:807-817.
12. van Loosdrecht, M. C. M., J. Lyklema, W. Norde, G. Schraa, and A. J. B. Zehnder. 1987. Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. *Appl. Environ. Microbiol.* **53**:1898-1901.
13. van Pelt, A. W. J., H. C. van der Mei, H. J. Busscher, J. Arends, and A. H. Weerkamp. 1984. Surface free energies of oral streptococci. *FEMS Microbiol. Lett.* **25**:279-282.
14. Wicken, A. J. 1985. Bacterial cell walls and surfaces, p. 45-70. In D. C. Savage and M. Fletcher (ed.), *Bacterial adhesion*. Plenum Publishing Corp., New York.