INFECTION AND IMMUNITY, Nov. 1983, p. 831–834
0019-9567/83/110831-04$02.00/0

Vol. 42, No. 2

NOTES

Cell Surface Hydrophobicity of Dental Plaque Microorganisms In Situ

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Received 23 May 1983/Accepted 9 August 1983

The cell surface hydrophobicity of bacteria obtained directly from human tooth surfaces was assayed by measuring their adherence to liquid hydrocarbons. Fresh samples of supragingival dental plaque were washed and dispersed in buffer. Adherence of the plaque microorganisms to hexadecane, octane, and xylene was tested turbidimetrically and by direct microscopic observation. The results clearly show that the vast majority of bacteria comprising dental plaque exhibit pronounced cell surface hydrophobicity. These data support the hypothesis that hydrophobic interactions play a major role in mediating bacterial adherence on tooth surfaces.

The role of cell surface hydrophobicity in mediating bacterial adherence to mammalian cells was conceived of over half a century ago in the pioneering studies of Mudd and Mudd (11, 12). During the past decade, the development of quantitative techniques for the study of bacterial hydrophobicity (2–5, 10, 16, 20, 25, 26) has facilitated studies on the role of hydrophobic interactions in the adherence and subsequent proliferation of bacteria on solid surfaces and at liquid-liquid and liquid-air interfaces. Bacterial hydrophobicity has been implicated in phagocytosis (26), partitioning of bacteria at the air-water interface (2), adherence and growth on hydrocarbon (10, 19, 22), attachment of bacteria to buccal epithelial and intestinal mucosal cells (16, 17, 21, 25), adherence of bacteria to nonwettable plastics (4, 5, 18), and adherence of benthic cyanobacteria to sediment (24).

In a recent study (27), the hydrophobic properties of 102 adherent bacterial isolates obtained from the surfaces of freshly extracted teeth were determined on the basis of their affinity for liquid hydrocarbon (hexadecane). An extremely high proportion (80%) of the isolates tested adhered with high affinity at the aqueous-hydrocarbon interface. Similarly, 58% of the isolates obtained from the surfaces of stainless steel dental matrices incubated briefly in the oral cavity were hydrophobic. This investigation presented initial experimental data in support of the premise that the majority of plaque microorganisms are hydrophobic and that hydrophobic interactions are involved in mediating adherence of oral bacteria to human tooth surfaces. However, two objections could be raised in this regard: (i) the hydrophobic surface properties observed after growth of the isolates in vitro do not necessarily reflect the surface characteristics of the same bacteria on tooth surfaces (6); it is known that cell surface hydrophobicity may change as a function of the physiological state of the bacteria (21); and (ii) since the great majority of bacteria on tooth surfaces are not cultivable (14), the isolates tested represent a small, disproportionate sample of the microbial population extant on human teeth.

In the present investigation we attempted to overcome these possible reservations by investigating the hydrophobic surface properties of microorganisms obtained directly from tooth surfaces in the form of supragingival dental plaque. Supragingival dental plaque is a macroscopic biofilm (1), consisting of a dense, heterogeneous array of bacteria directly or indirectly attached to tooth surfaces (14), which has been directly linked to oral disease in human volunteers (9). In the experiments described here, supragingival dental plaque was scraped with a sterile curette from the mouths of healthy adult volunteers and dispersed in PUM buffer (22.2 g of $K_2HPO_4 \cdot 3\ H_2O$, 7.26 g of KH_2PO_4, 1.8 g of urea, 0.2 g of $MgSO_4 \cdot 7\ H_2O$, and distilled water to 1,000 ml [pH 7.1]) by mild shearing for 2 min in an Ultra-turrax homogenizer (Janke and Kunkel Ika-Werk, Staufe, Federal Republic of

TABLE 1. Adherence of dispersed plaque to hydrocarbons[a]

Test hydrocarbon	% Adherence in sample[b]:							% Mean adherence
	1	2	3	4	5	6	7	
n-Hexadecane	62	76	85	68	61	60	62	68
n-Octane	80	94	100	100	83	91	100	93
p-Xylene	87	99	100	99	100	100	95	97

[a] Supragingival dental plaque was collected from seven individuals, washed, and dispersed in PUM buffer to yield an initial absorbance of 0.8 to 1.0 at 400 nm. To 1.2 ml of dispersed plaque, 0.3 ml of hydrocarbon was added, and the solution was mixed uniformly for 120 s. After phase separation, the lower aqueous phase was transferred to a fresh test tube, an additional 0.3 ml of hydrocarbon was added, and the solution was mixed as described above. After this second extraction, the lower aqueous phase was transferred to a semi-micro cuvette, and its absorbance was recorded.

[b] Results are presented as the percent decrease in absorbance compared with that of the corresponding control mixed without added hydrocarbon.

Germany). The dispersed plaque suspension was washed by centrifugation and suspended in PUM buffer by dispersion (as above) to yield an absorbance at 400 nm of 0.8 to 1.0, as measured with a Uvikon 710 spectrophotometer (Kontron, Zurich, Switzerland). Phase microscopic observation verified that the dispersed, washed plaque consisted mainly of single, intact bacterial cells.

The basic technique for measuring bacterial adherence to liquid hydrocarbons (19–23) was employed in this study with the following modifications. To 1.2 ml of dispersed plaque in round-bottom test tubes (12 by 75 mm) 0.3 ml of test hydrocarbon (*n*-hexadecane, *n*-octane, or *p*-xylene) was added, and the solutions were mixed uniformly for 120 s. After 10 min for phase separation, the lower aqueous phase was transferred to fresh test tubes, each containing 0.3 ml of the same test hydrocarbon. They were mixed as described above. After this second extraction, the lower aqueous phase was removed into semi-micro cuvettes (Greinier, Nurtingen, Federal Republic of Germany), and absorbance at 400 nm was measured. The results are expressed as the percent decrease in turbidity as compared with that of the corresponding sample mixed without added hydrocarbon. The turbidimetric results were corroborated by phase microscopic examination of the aqueous phases before and after mixing, as well as observation of the hydrocarbon droplets after the assay. In all cases, the drop in turbidity corresponded with the disappearance of bacteria from the lower aqueous phase and their appearance on the surface of hydrocarbon droplets which rose after the mixing procedure (20).

The results of a typical experiment, in which adherence of dispersed plaque obtained from seven volunteers was measured, are shown in Table 1. All three test hydrocarbons were able to bind the majority of the dispersed plaque bacteria in all seven samples. Optimal adherence was observed in the presence of xylene; on the average, a 97% decrease in cell-mediated turbidity was observed. Cells also bound to octane with extremely high affinity. Adherence to hexadecane was lower than that observed for the other two hydrocarbons. This sequence of affinity for the test hydrocarbons has been observed with nonoral laboratory strains (23) and may be related to the relatively high viscosity of hexadecane. Results similar to those presented in Table 1 were obtained in experiments in which the plaque was dispersed by sonic oscillation and then suspended and washed in saline or phosphate-buffered saline. In experiments employing plaque which was dispersed in PUM buffer but not washed before the adherence assay, the percentage of adherent cells was ca. 20% lower than that shown in Table 1. This is probably due to the presence of amphipathic molecules in the unwashed samples which interfere with the adherence assay (17).

Figure 1 illustrates the adherence of plaque bacteria to octane droplets after the assay procedure. Various morphological forms, including diplococci, filaments, fusiforms, spiral forms, and large rods, all typical of plaque microbiota (14), were readily observed adhering at the oil-water interface, whereas the bulk aqueous phase was almost completely devoid of bacteria.

The data presented here demonstrate that the large majority of bacteria comprising supragingival plaque exhibit pronounced cell-surface hydrophobicity in situ, as evidenced by their avid affinity for liquid hydrocarbons (20). The relatively small differences observed among the plaque samples obtained from different individuals attest to the general nature of this phenomenon. The results support the hypothesis that hydrophobic interactions play a major role in bacterial adherence on human tooth surfaces and subsequent plaque accumulation. Other observations which support this premise include the low surface energy found for the acquired

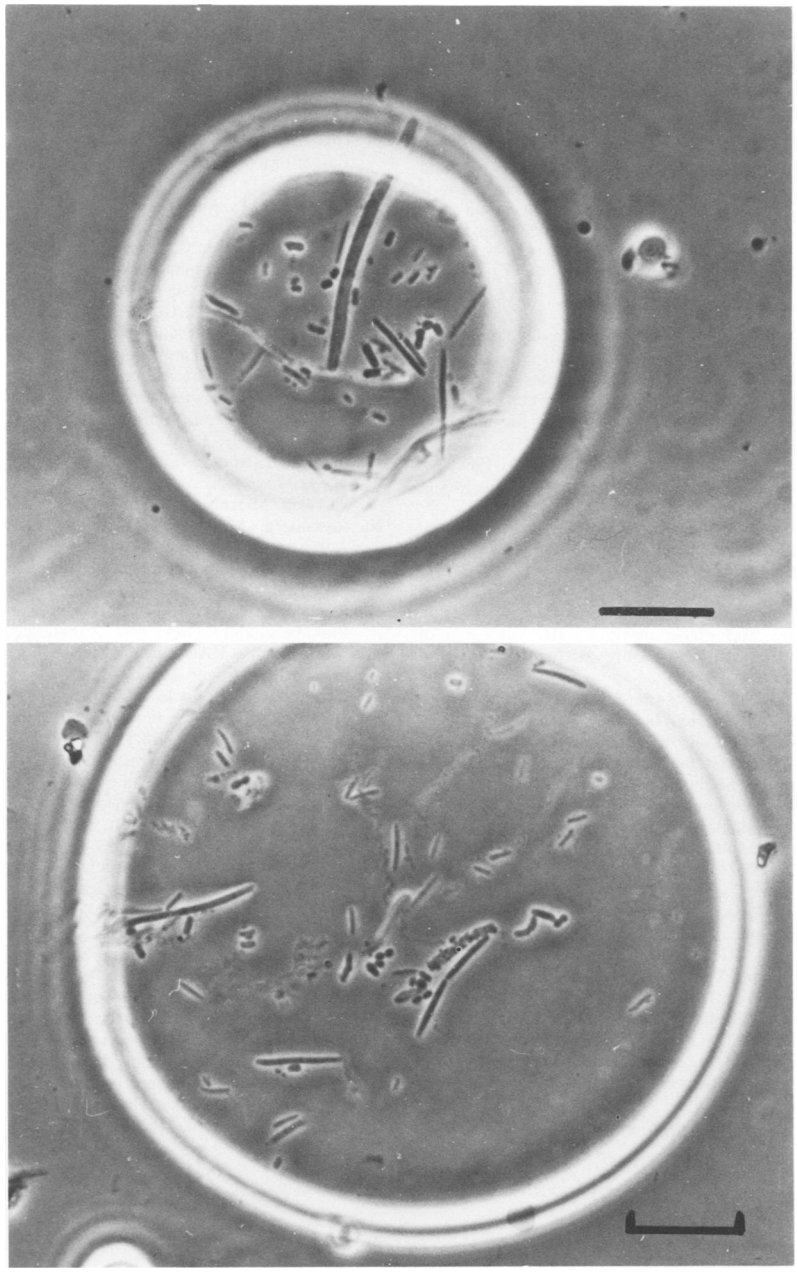

FIG. 1. Adherence of dispersed dental plaque to octane droplets. Supragingival dental plaque obtained from one individual was dispersed by shearing and washed as described in the text. After being mixed in the presence of octane as described in footnote *a* of Table 1, plaque bacteria became concentrated at the oil-water interface. Phase microscopic observation of droplets revealed adherence of various plaque microorganisms, with the surrounding aqueous phase almost completely free of bacteria. Bars, 10 μm.

pellicle which covers tooth enamel in situ (7, 8), the hydrophobic nature of many cultivable oral strains, and correlations between bacterial hydrophobicity and adherence to saliva-coated hydroxyapatite (13, 15, 27; R. J. Gibbons and I. Etherden, Infect. Immun., in press). The high cell surface hydrophobicity of plaque microorganisms, as presented here, may conceivably be due in part to salivary components which are adsorbed on the bacterial cell surfaces in situ. However, preliminary data (I. Eli and M. Rosenberg, unpublished results) indicate that saliva

acts to inhibit, rather than promote, bacterial adherence to hydrophobic surfaces. Experiments are under way to further investigate the relationship between bacterial hydrophobicity and adherence within the oral cavity.

We thank Eugene Rosenberg, Eliora Z. Ron, and Nachum Kaplan for many stimulating discussions and critical review of the manuscript, Zinaida Zosim for helpful advice, and Yardena Mazor and Irit Baram for excellent technical assistance.

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