Adhesion of Staphylococcus epidermidis and Staphylococcus saprophyticus to a Hydrophobic Biomaterial

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The relative surface charge and hydrophobicity of 16 strains of Staphylococcus epidermidis showed large variations. For this species no relationship between the two surface parameters was found. A highly negative surface charge was observed in all seven encapsulated strains (one S. epidermidis and six Staphylococcus saprophyticus strains). The adhesion of the staphylococci to fluorinated polyethylene-propylene films was not related to the relative surface charge and the hydrophobicity of the bacteria. On films pre-exposed to human plasma, the bacterial adhesion was substantially reduced. Mechanisms involved in the adhesion of coagulase-negative staphylococci to this biomaterial are discussed.

INTRODUCTION

Infections associated with prosthetic implants and medical devices are mostly caused by coagulase-negative staphylococci (CNS) (Garvey, 1980). Adhesion of bacteria to tissue surfaces of the host and to implanted artificial surfaces is considered to be an important step in the pathogenesis of infections (Ofek & Beachey, 1980). The mechanism of the adhesion of CNS to artificial surfaces has been studied (Locci et al., 1981; Christensen et al., 1983) but remains unclear. In many adhesion studies bacteria were applied to various surfaces in (physiological) salt solutions. However, artificial surfaces in contact with body fluids are usually rapidly coated with proteins (Feijen et al., 1979), which means that the adhesion also has to be studied using protein-coated surfaces. Electrostatic and hydrophobic interactions between bacteria and protein-coated surfaces may be involved in bacterial adhesion (Rutter & Vincent, 1980; Doyle et al., 1982). The adhesion of various oral bacteria to hydroxyapatite coated with salivary protein is related to the hydrophobic character of the bacteria (Gibbons & Etherden, 1983). The adhesion of Streptococcus mutans to such a surface appeared to be mediated by short range hydrophobic and ionic interactions (Reynolds & Wong, 1983). These findings indicate that the surface charge and hydrophobicity of bacteria used in adhesion studies might be important.

This paper describes the relative surface charge and hydrophobicity of various encapsulated and non-encapsulated strains of Staphylococcus epidermidis and Staphylococcus saprophyticus. The adhesion of these strains to fluorinated polyethylene-propylene (FEP) films and to FEP films pre-exposed to citrated human plasma was investigated.

METHODS

Coagulase-negative staphylococci. A total of 23 CNS strains were used. Six Staphylococcus epidermidis strains (NCTC) were a gift of Dr R. R. Marples, Central Public Health Laboratory, Colindale Avenue, London, UK. These strains were derived from patients with prosthetic endocarditis. The remaining 17 strains were isolated from the skin of open-heart surgery patients and laboratory staff members. CNS were identified according to the scheme of Kloos & Schleifer (1975) using the API Staph gallery.

Abbreviations: CNS, coagulase-negative staphylococci; FEP, fluorinated polyethylene-propylene; TSB, trypticase soy broth.

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Bacteria were cultured for 4-5 h in Trypticase Soy Broth (TSS; BBL) as previously described (Hogt et al., 1983a). Bacteria grown on sheep blood agar plates (Oxoid) and in TSB were stained using the India ink wet-film method to demonstrate the presence of capsules (Duguid, 1951). In all experiments latex exponential phase cells were used. Bacteria were washed three times with 0.14 M NaCl/phosphate buffer (0.14 M NaCl, 3 mM KCl, 8.4 mM Na₂HPO₄, 1.6 mM KH₂PO₄; pH 7.2).

**Relative bacterial surface charge.** The relative surface charge of bacteria was tested by their adhesion to an anion-exchange resin according to a modified procedure of Pedersen (1981). The bacteria were not labelled and a 0.33 M NaCl/phosphate buffer (0.33 M NaCl, 3 mM KCl, 8.4 mM Na₂HPO₄, 1.6 mM KH₂PO₄; pH 7.2) was used for suspension and elution of the bacteria. The bacterial cell concentration in suspension was determined by measuring the optical density at 540 nm (Beckman model 24 spectrophotometer). Suspensions with an OD₅₄₀ of 1.0 containing approximately 10⁸ c.f.u. ml⁻¹, as determined by colony count, were used in all experiments. Pasteur pipettes (inner diameter 5 mm) containing 0.5 g anion-exchange resin (Dowex 1 x 8 mesh size 100/200, 80–150 μm; Serva, Heidelberg, FRG) were first rinsed with 0.33 M NaCl/phosphate buffer and then 1 ml of the bacterial suspension in 0.33 M NaCl/phosphate buffer was applied to the columns. The columns were eluted with 8 ml 0.33 M NaCl/phosphate buffer and the OD of the eluate was measured. The relative surface charge of the bacteria was expressed as the percentage of the bacteria bound to the anion-exchange resin.

In order to study the effect of ionic strength of the buffer on binding of bacteria to the resin, bacteria were eluted with NaCl/phosphate buffers containing 0.03 M KCl and NaCl concentrations ranging from 0.14 to 0.66 M or with phosphate buffer without NaCl and KCl. The effect of ionic strength of the buffer on the recovery of bacteria from the resin was studied as follows. Bacteria, suspended in 0.14 M NaCl/phosphate buffer, were applied to the resin and eluted with the same buffer. The resin was then collected and resuspended in 3 ml NaCl/phosphate buffer with NaCl concentrations ranging from 0.14 to 1.20 M in round-bottomed test tubes which were gently shaken four times with intervals of 3 min. After an additional 10 min in which the resin settled, the OD of the supernate was measured and the number of c.f.u. was determined using the pour plate method.

**Bacterial surface hydrophobicity.** Bacterial cell-surface hydrophobicity was determined by measuring the bacterial adhesion to xylene in a xylene–water system according to Rosenberg et al. (1980). Washed bacteria were suspended in 3 ml 0.14 M NaCl/phosphate buffer to an OD₅₄₀ of 1.0. A volume of 0.25 ml p-xylene (Merck) was added to disposable glass test tubes (10 mm inner diameter) containing the bacterial suspensions. The tube contents were mixed on a vortex mixer for 60 s and after phase separation occurred, the OD of the aqueous phase was measured. The hydrophobicity of the bacteria was expressed as the percentage of bacteria that adhered to xylene.

**Polymer films and pre-exposure to plasma.** The polymer films used for the bacterial adhesion experiments were fluorinated polyethylene-propylene (FEP) films (type ‘500 A’; DuPont). FEP is a hydrophobic biomaterial used in various biomedical devices. The preparation of the polymer film test specimens was described previously in detail (Hogt et al., 1983a). In order to investigate bacterial adhesion to protein-coated films, polymer films were pre-exposed to citrated human plasma. Citrated fresh plasma (CPDA-1; Bloedbank, Groningen, The Netherlands) was stored at −20°C. FEP films were rinsed with 0.14 M NaCl/phosphate buffer and exposed to 3 ml plasma for 1 h at 37°C. The films were then rinsed eight times with 3 ml amounts of NaCl/phosphate buffer. Control films were exposed only to NaCl/phosphate buffer.

**Contact angle measurements.** Water contact angles of FEP films and FEP films pre-exposed to plasma were measured by the captive bubble method (Adamson, 1967). Polymer films were attached to a panel which was submerged in hyperfiltrated water at 21°C in a Perspex chamber. Hyperfiltrated water was prepared by ultrafiltration of demineralized water through polysulfon membranes (Wavilin, Hardenberg, The Netherlands) and then hyperfiltration using a hollow fibre configuration RO module (Hollosep, type HR 8650; Toyobo, Japan). An air bubble was released from a microlitre syringe tip beneath the polymer films and allowed to rise to a film surface. The air bubble was then photographed within 30 s after contact with the film. The height (h) of the bubble and the width (b) of the bubble interface with the film were measured. The contact angle θ = 180° − 2 arctg (2h/b). Measurements were made in triplicate.

**Bacterial adhesion to polymer films.** Bacterial suspensions in 0.14 M NaCl/phosphate buffer were filtered through membrane filters (‘Unipore’, pore size 3 μm; Bio-Rad) to remove clusters. Details of the procedure for bacterial adhesion experiments were previously described (Hogt et al., 1983a). The polymer films were exposed to bacterial suspensions with an OD₅₄₀ of 0.5 containing 5 x 10⁶ cells ml⁻¹ for 2.5 h at 37°C in a rotary shaker/incubator (New Brunswick Scientific; 90 r.p.m.). Films were then rinsed eight times with 3 ml amounts of NaCl/phosphate buffer and treated with 2% (v/w) glutaraldehyde in NaCl/phosphate buffer for 5 min. Subsequently, the films were rinsed with distilled water and dried at room temperature. All adhesion experiments were done at least twice. The number of adhering cells per mm² of film was determined using a light microscope by examining six 0.05 mm² areas on each film.
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Fig. 1

Fig. 2

Fig. 1. Percentage of bacteria adhering to an anion-exchange column as a function of the NaCl concentration in the elution buffer. Washed bacteria (1 x 10^9) of two non-encapsulated S. epidermidis strains (SEP 2, ○; SL 58, □) and one encapsulated S. saprophyticus strain (SAP 1, △) were suspended in 1-0 ml NaCl/phosphate buffer (pH 7.2) with different NaCl concentrations and then applied to the column.

Fig. 2. Recovery of bacteria from anion-exchange resin in NaCl/phosphate buffer with different NaCl concentrations. Washed bacteria (1 x 10^9) of a non-encapsulated S. epidermidis strain (SEP 2, ○) and an encapsulated S. saprophyticus strain (SAP 1, △) both suspended in 1-0 ml 0·14 M-NaCl/phosphate buffer were applied to the resin and eluted with the same buffer. Resins with adsorbed bacteria were then suspended in NaCl/phosphate buffer with different NaCl concentrations.

RESULTS

Surface character of S. epidermidis and S. saprophyticus

Sixteen S. epidermidis strains and seven S. saprophyticus strains were studied. Capsules were demonstrated in only one S. epidermidis strain and in six S. saprophyticus strains. Capsules were present both around late exponential phase and stationary phase cells grown on blood agar or TSB.

The relative bacterial surface charge, as measured by bacterial adhesion to anion-exchange resin, was dependent on the salt concentration of the buffer used. The retention of bacteria in the anion-exchange column decreased with increasing salt concentration of the buffer solution (Fig. 1). At salt concentrations between 0·14 and 0·42 M-NaCl there was a significant difference between the adhesion of two non-encapsulated S. epidermidis strains and one encapsulated S. saprophyticus strain. Bacteria suspended in the phosphate buffer containing 0·66 M-NaCl showed a very low adhesion to the resin.

Recovery of bacteria from the anion-exchange resin was tested for an encapsulated S. saprophyticus strain and one non-encapsulated S. epidermidis strain (Fig. 2). The recovery of bacteria of both strains increased when phosphate buffers with increasing salt concentrations were used. A plateau value was reached using buffer containing 0·60 M-NaCl.

In order to determine the relative bacterial surface charge of CNS, anion-exchange resin columns were eluted with 0·33 M-NaCl/phosphate buffer. Bacteria of the different strains of S. epidermidis and S. saprophyticus showed a wide range in adhesion to the resin varying from 17 to 100% (Table 1).

The relative surface hydrophobicity of different strains was determined by bacterial adhesion to xylene. The adhesion of encapsulated as well as non-encapsulated strains to xylene showed a wide range (3–89%), indicating that different strains varied from hydrophilic to hydrophobic (Table 1). The hydrophobicity of stationary phase cells was similar to that of late exponential phase cells (results not shown).
Table 1. Relative surface charge and hydrophobicity of strains of *S. epidermidis* and *S. saprophyticus*

The relative surface charge was determined by bacterial adhesion to anion-exchange resin. Bacterial suspensions in 0.33 M-NaCl/phosphate buffer (1 ml) containing \(10^9\) cells were applied to columns containing 0.5 g Dowex 1 × 8 resin and eluted with 0.33 M-NaCl/phosphate buffer. The bacterial hydrophobicity was measured by the adhesion to xylene. Bacterial suspensions in 0.14 M-NaCl/phosphate buffer (3 ml) containing \(10^9\) cells ml\(^{-1}\) were vortexed for 60 s with 0.25 ml xylene. Further details are given in Methods. The results shown are mean values of duplicate measurements.

<table>
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<th>Species and strain no.</th>
<th>Capsule</th>
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<th>Xylene</th>
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<tr>
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<tr>
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Bacterial adhesion to polymer films

The number of bacteria of the 15 non-encapsulated strains of *S. epidermidis* and the non-encapsulated *S. saprophyticus* strain that adhered to the FEP films ranged from 64 to 99 × 10³ cells mm⁻², while that of the five encapsulated strains of *S. saprophyticus* and the encapsulated *S. epidermidis* strain ranged widely (Fig. 3a, b). The difference between the adhesion values of the encapsulated and those of the non-encapsulated strains was significant (\(P<0.001\) in Student's \(t\)-test; Fisher & Yates, 1957). The encapsulated cells, in contrast to the non-encapsulated cells, always adhered in clusters. The adhesion did not correlate with either the bacterial relative surface charge (Fig. 3a), or with the bacterial hydrophobicity (Fig. 3b).

FEP films pre-exposed to plasma showed a strongly reduced water contact angle (20°) with respect to that of untreated FEP films (103°). Adhesion of all strains to FEP films pre-exposed to plasma was substantially reduced except for the encapsulated *S. epidermidis* (NCTC 100892) (Fig. 3a, b). The mean adhesion values of hydrophilic (\(<50\%\) adhesion to xylene) and hydrophobic (\(>50\%\) adhesion to xylene) non-encapsulated strains to plasma-treated FEP were not significantly different (\(P>0.05\)).
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DISCUSSION

The relative surface charge of various strains of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* showed a wide range, as indicated by adhesion to anion-exchange resin. The electrostatic bonding of CNS to the resin was reversible. Wood (1980) studied the desorption of *Staphylococcus aureus* from an anion-exchange resin in buffers containing different salt concentrations, and found that at concentrations higher than 0.6 M-NaCl the desorption was minimal due to a salting out effect. CNS were recovered from the resin in buffers with salt concentrations higher than 0.6 M without a salting out effect. This indicates differences between the cell surface constituents of *S. aureus* and CNS.

The relative negative surface charge of encapsulated CNS was high, probably due to the presence of many negatively charged groups within the capsular polysaccharide material (Ichiman & Yoshida, 1981). The relative negative surface charge of non-encapsulated CNS strains ranged widely as was demonstrated also by Pedersen (1981) for various marine bacteria. CNS showed either a hydrophilic or a hydrophobic cell surface, as tested by their adhesion to xylene. No direct correlation was found between the relative surface charge and the hydrophobicity of the cells. Encapsulated CNS, except for one *S. saprophyticus* strain, were hydrophobic. Generally, encapsulated bacteria are more hydrophobic than non-encapsulated bacteria (van Oss & Gillman, 1972). Hyaluronic acid-containing capsules of exponential phase cells of *Streptococcus pyogenes* (Ofek et al., 1983) and polysaccharide capsules of *Acinetobacter calcoaceticus* (Rosenberg et al., 1983) prevented the adhesion to hexadecane, indicating that such encapsulated bacteria are hydrophilic. Stationary phase cells of both species lost their capsules and showed a high affinity to hexadecane (Ofek et al., 1983; Rosenberg et al., 1983). Cells of encapsulated CNS had capsules in both their exponential and stationary growth phase. The hydrophobicity of the cells did not change significantly with the growth phase.

Usually bacterial hydrophobicity as determined by the adhesion to hydrocarbons correlates well with that obtained using octyl sepharose chromatography (Olsson & Westergren, 1982;
Rosenberg, 1984). In general, a high bacterial surface charge is accompanied by a more hydrophilic character of the cells. However, some strains of marine bacteria with a high relative surface charge possess exposed hydrophobic sites, as determined with octyl sepharose chromatography (Pedersen, 1981). Some CNS strains also showed a high relative surface charge combined with a high hydrophobicity. Little is known about the localization and distribution of surface components which mediate bacterial adhesion to hydrocarbons (Rosenberg, 1984). Yoshida & Minegishi (1979) reported that an encapsulated S. epidermidis strain had protein-containing spike-like structures protruding from the cell wall. Such protein structures might contribute to the expression of hydrophobic sites at the capsular surface. The differences in hydrophobicity between CNS strains, encapsulated or non-encapsulated, must be ascribed to differences in the exposure of hydrophobic residues at the surface. These may be proteins or glycolipids complexed to proteins because treatment of the bacteria with pepsin decreases their hydrophobicity (Hogt et al., 1983b).

The number of encapsulated and non-encapsulated S. epidermidis and S. saprophyticus that adhered to FEP and FEP pre-exposed to plasma appeared to be unrelated to the relative bacterial surface charge or to the bacterial hydrophobicity. These findings are in contrast to those reported by Gibbons & Etherden (1983). They showed that the adhesion of various oral bacterial species to saliva-coated hydroxyapatite was related to the hydrophobicity of the bacterial strains.

The number of encapsulated CNS adhering in clusters to FEP films was significantly less than that of non-encapsulated CNS. This decreased adhesion may be due to bacterial aggregation of these strains in suspension. However, bacterial aggregation of encapsulated strains was similar to or less than that of non-encapsulated strains (Hogt et al., 1983a).

The adhesion of CNS to FEP may be mediated by protein-containing residues at the cell wall probably by hydrophobic bonding, since proteolytic enzyme pretreatment decreased the bacterial hydrophobicity and the adhesion to FEP (Hogt et al., 1983a). The reduced adhesion of CNS to FEP pre-exposed to plasma may be due to the decreased hydrophobicity of the FEP films by the adsorbed protein layer, as measured by determining contact angles. Fletcher & Marshall (1982) studying the adhesion of Pseudomonas species to polystyrene surfaces also reported that adsorbed proteins lowered the hydrophobicity of polymer surfaces and decreased bacterial adhesion. In addition, electrostatic repulsion between bacteria and the FEP surface caused by the adsorbed protein may reduce bacterial adhesion (Reynolds & Wong, 1983).

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REFERENCES


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