Biotechnology Letters **23:** 437–441, 2001.

Bacterial adhesion to a model surface with self-generated protection coating of mucin via jacalin

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Received 2 November 2000; Revisions requested 30 November 2000; Revisions received 8 January 2001; Accepted 8 January 2001

Key words: bacterial adhesion, jacalin, mucin, *Staphylococcus epidermidis*, tear proteins

Abstract

Using a mechanism of 'self-generation', polymer surfaces were coated with ocular mucin-type glycoproteins that were extracted from tear fluid and immobilized through specific interaction with a lectin, jacalin. Separately, jacalin affinity chromatography of tear fluid showed the main retained components had molecular weights higher than 200 kDa. In evaluations of bacterial adhesion, a model surface with jacalin-immobilized ocular mucins took up a significantly smaller number of adhered *Staphylococcus epidermidis* (0.041×10^6 cells cm^{-2}) than a bare surface of the same polymer (1.202×10^6 cells cm^{-2}). The lectin-mediated ocular mucin coating reduced the bacteria uptake by about 95% showing that the presence of mucin on surfaces may afford a general protection against bacterial colonization.

Introduction

Protein adsorption is an important issue in the evaluation of synthetic materials for their potential to serve as implants and prosthetic devices in contact with blood, tears, and tissues. During the past several years our laboratory has investigated processes for protecting surfaces from fouling associated with adsorbing proteins. Mucin is superior in surfactant behavior to several of the Pluronic surfactants that have been the focus of our previous studies (Shi *et al.* 1999). In a separate investigation of bacterial adhesion, we compared bovine submaxillary mucin (BSM) and Pluronic F108 coated surfaces in terms of their respective uptakes of *Staphylococcus epidermidis* and *S. aureus*, and found that adhesion of each of the two types of bacteria was greatly suppressed on the mucin coated surfaces (Shi *et al.* 2000). The bacterial adhesion results further demonstrated a strong correlation between adhesion suppression and the surface concentration of BSM, which led us to conclude that mucin coatings could profitably be employed to reduce the risk of microbial infections on polymeric biomaterials.

In the present work, jacalin was used to extract mucin from tear fluid as a result of its specific binding to *O*-linked glycans in mucin molecules (Glen & Beverly 1990). The model surfaces were created by first adsorbing streptavidin to polystyrene substrates, and then loading biotinylated jacalin onto these constructs. These surfaces were then exposed, *in vitro*, to tear fluid for the potential extraction of ocular mucin. Since jacalin is known to interact strongly also with IgA, which in its secretory form (sIgA) represents another significant component in human tear fluid, it was necessary to eliminate this protein from the tear fluid prior to any attempt at specific affinity trapping of its mucin content. For this purpose an anti-IgA affinity column was used to remove sIgA from the tear fluid before loading it onto a jacalin-containing surface. Coagulase-negative staphylococci (CNS) of type *S. epidermidis*, one of the most frequently encountered biomaterial-associated pathogens, was used as a bacterial source (Clark & Ruffin 1990, Wadström *et al.*

1990). CNS *epidermidis* was exposed to the jacalin bound model surfaces, as well as to the various control surfaces, in order to evaluate their respective affinities for the differently treated surfaces. To further examine the specific lectin-mucin interaction, the jacalin bound mucin-type glycoproteins from tears were also characterized by gel electrophoresis.

Materials and methods

Model surface preparation

Sterile polystyrene tissue culture plates, containing 6 flat bottom wells (Becton Dickinson), were used for coating experiments. Streptavidin (Sigma) and biotinylated jacalin (Pierce) were prepared at 1 mg ml^{-1} in 175 mM Tris/HCl buffer (pH 7.4). Surfaces were cleaned with compressed N_2, and 3 ml streptavidin solution was then loaded on the surface. After 3 h on a rotary shaker at room temperature, the protein containing supernatant was decanted and the surfaces were rinsed three times with buffer. Biotinylated jacalin solution was then added to the streptavidin coated surfaces for an additional 3 h of shaking, followed by similar washing steps. These surfaces were then ready for further use.

Normal human reflex tear fluid from a 37-year old male was obtained by stimulation through the cutting of an onion. It was immediately diluted 1:1 (v/v) in 175 mM Tris/HCl (pH 7.4) containing 0.8 mM imidazole, centrifuged at 15 000 *g* for 10 min and the supernatant was kept at 4 °C for further use. The tear fraction to be loaded on this jacalin-containing surface had been run through an anti-IgA affinity column, and was therefore free from IgA (confirmed by SDS-PAGE). A 3 ml portion of the fraction, which contained all the remaining components from the original 0.5 ml of tear fluid, was added to each surface. After incubation for 2 h, the surface was washed twice with Tris/HCl buffer. Surfaces coated with streptavidin only, and with streptavidin followed by biotinylated jacalin, were used as controls.

Preparation of other pre-coated surfaces

For comparison, other surfaces were prepared by coating with (a) BSM, (b) unfractionated tear fluid, (c) human lysozyme, and (d) Pluronic F108. BSM (M-3895 from Sigma) was purified by affinity chromatography following the same procedure as described above for ocular mucin. The final sample was diluted with a 175 mM Tris/HCl (pH 7.4). Unfractionated tear fluid was centrifuged to remove precipitates and was then diluted 2-fold prior to coating. A solution of human lysozyme (Sigma) was likewise prepared in the same Tris/HCl buffer. A solution of the synthetic surfactant Pluronic F108 (BASF) in the same Tris/HCl buffer was also included. All concentrations were 1 mg ml^{-1}, and 3 ml of each solution was added to the wells. The multiwell plates were then placed on a rotary shaker for 24 h at room temperature. They were then gently rinsed three times with the Tris/HCl buffer to remove unbound materials.

Bacterial adhesion assay

Staphylococcus epidermidis (ATCC 12228) was incubated on a PS microtiter plate for 24 h in tryptic soy broth (TBS) at 37 °C, and the wells were then washed three times with phosphate buffered saline (PBS) (0.014 M Na_2HPO_4, 0.003 M NaH_2PO_4, 0.15 M NaCl, pH 7.4). Aliquots of 100 μl of the bacterial suspension in PBS (density: 5×10^7 c.f.u. ml^{-1}) were added to the test surfaces. All of the pre-coated polystyrene surfaces and one uncoated surface (treated with Tris/HCl only) were incubated with the bacterial suspension by placing the multiwell dish on an incubator shaker at 37 °C and 80 rpm. Following 2 h incubation, all surfaces were rinsed twice with PBS to remove non-adherent bacteria and were fixed in Karnovsky's fixative solution.

Characterization of jacalin bound tear glycoproteins

In order to examine which tear components bind to jacalin, a parallel two-step experiment using affinity chromatography was performed. The first step was to remove sIgA, a known jacalin binding component, from the tear sample. This was done with a column containing polyclonal anti-human IgA antibody. The antibody in a biotin-conjugated form (Pierce) was immobilized on a column packed with NeutrAvidin agarose (Pierce) via the specific avidin-biotin coupling. The second step was to extract mucin from tears by jacalin affinity chromatography. Jacalin agarose (ICN) was packed in a 5 ml column and equilibrated with 50 ml Tris/HCl buffer; the above unbound tear fraction (2 ml) was then added to the column. Proteins specifically bound by jacalin were then eluted using 100 mM melibiose in the same buffer.

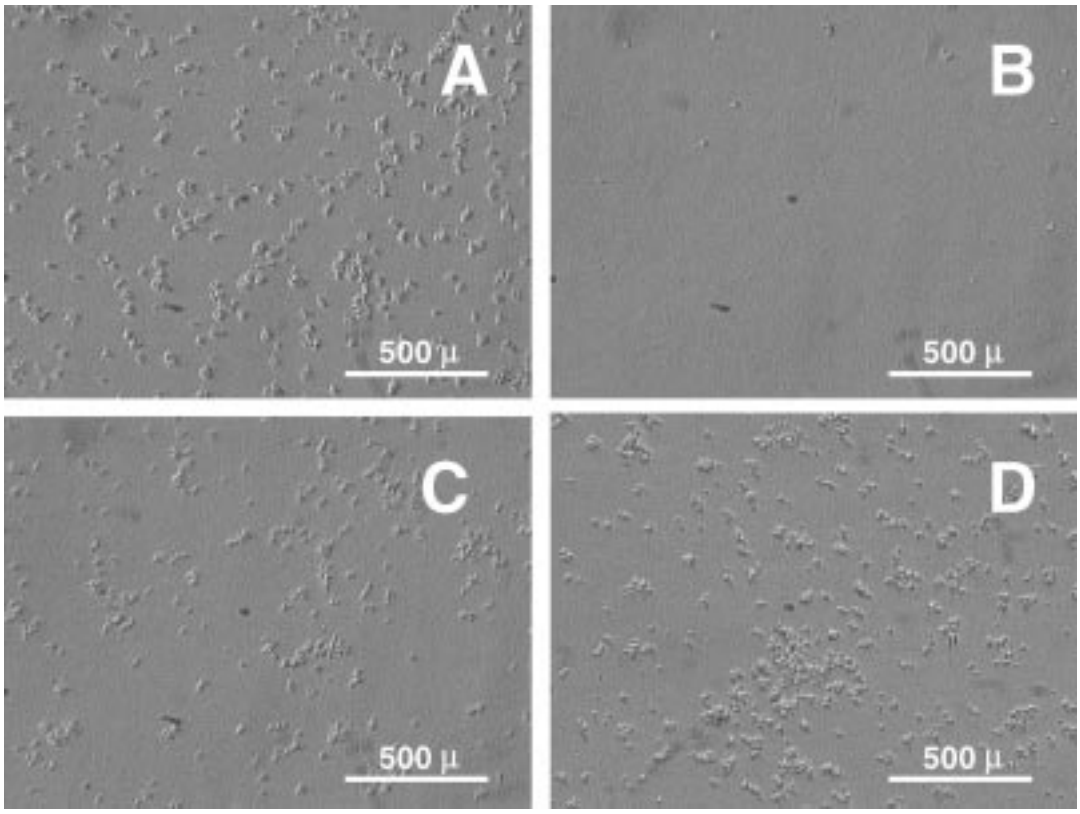

Fig. 1. A: uncoated PS surface; B: pre-coated with streptavidin and biotinylated jacalin, then tear fluid free of sIgA; C: pre-coated with streptavidin, then biotinylated jacalin; D: pre-coated with only streptavidin.

SDS-PAGE

Electrophoresis of the fractions from the anti-IgA column was performed on a 4–20% reduced minigel (Bio-Rad). In addition to the isolated tear sIgA, the electrophoretic analysis involved unfractionated tears and tears freed from sIgA. For electrophoresis of fractions from the jacalin column, two 10% minigels (Bio-Rad) were run in parallel under non-reducing conditions. One of them was stained with silver (silver staining plus, BioRad) and the other with periodic acid-Schiff (PAS) to illuminate the glycoproteins (Clerke 1964, Robert *et al*. 1969).

Results and discussion

Bacterial adhesion to various surfaces

Figure 1 illustrates the adhesion of CNS *epidermidis* to the four surfaces. These micrographs clearly show the surface covered with immobilized ocular mucins to contain by far the smallest number of adhered bacteria. Table 1 summarizes the numbers of adherent bacteria on the ocular mucin-containing surface and the uncoated surfaces respectively, as well as those adhering to the controls and four other pre-coated surfaces. Among these, the uncoated surface shows the largest number (1.202×10^6 cm^{-2}) of bacteria, while the control surfaces containing streptavidin only, or streptavidin in complex with biotinylated jacalin, also show large numbers (1.132×10^6 cm^{-2} and 0.81×10^6 cm^{-2}, respectively) of bacteria. By contrast, the surface with immobilized ocular mucins bound only a small number (0.041×10^6 cm^{-2}) of cells, indicating that adhesion was greatly suppressed. The bacteria were thus to a significant degree inhibited from adhering to the model surface because of its immobilized mucin coating. Similarly, the surface coated with BSM shows a comparably low bacterial colonization (0.061×10^6 cm^{-2}). These observations suggest that the presence of mucin on surfaces may cause a general repulsion of bacteria, which likely derives from the carbohydrate side-chains on the mucin molecule.

Table 1. Bacterial adhesion to different surfaces.

Surface preparation ($n = 4$)	Number of bacteria (10^6 cm^{-2})
Uncoated surfaces	1.20 ± 0.06
Surfaces pre-coated with streptavidin + biotinylated jacalin, then ocular mucin	0.04 ± 0.01
Surfaces pre-coated with streptavidin	1.13 ± 0.02
Surfaces pre-coated with streptavidin + biotinylated jacalin	0.81 ± 0.01
Surfaces pre-coated with tear fluid	0.61 ± 0.05
Surfaces pre-coated with bovine submaxillary mucin	0.06 ± 0.005
Surfaces pre-coated with Pluronic F108	0.15 ± 0.04
Surfaces pre-coated with lysozyme	0.371 ± 0.01

In comparison with the strongly repellant ocular mucin, coatings with the synthetic surfactant Pluronic F108 appeared less effective. This block copolymer, which has previously been found to confer bacteriostatic properties on polymer surfaces, gave rise to a bacterial adhesion between that of the bare and mucin coated surfaces (0.145×10^6 cm^{-2}). Lysozyme is a bactericidal enzyme with lytic activity directed against Gram-positive bacteria (Hara & Matsuchima 1967). With a concentration of around 2 mg ml^{-1} (Struchell *et al.* 1984, Vinding *et al.* 1987), it is the most abundant protein component in tear fluid. A certain suppression effect (0.317×10^6 cm^{-2}) was observed in the bacterial adhesion to the lysozyme coated surface. However, the reduction of bacterial adhesion was not as great as that of either the mucins or the Pluronic F108. Since the sandwich attachment of ocular mucin, via streptavidin and biotinylated jacalin, yielded a surface with much weaker bacterial colonization than that coated with lysozyme, one can conclude that streptavidin remains firmly adsorbed, even in the presence of the high concentration of lysozyme present in tears. Given the previously described 'coating inhibitory activity' (Boonstra *et al.* 1985) of whole tears, it was interesting to note that the surface coated with whole tear fluid still showed a fairly large number of bacteria.

Jacalin bound tear glycoproteins

Electrophoretic analysis of both bound and unbound fractions from a polyclonal human anti-IgA column showed that sIgA had been removed from the tear fluid by binding to its immobilized antibody. The profile of reduced whole tears was shown to contain at least two additional bands compared to the break-through fraction. These missing bands coincided with the 60 and 28 kDa bands observed in the displacement fraction, known to originate from the IgA molecule's heavy chain (γ, ~60 kDa) and light chain (α, ~28 kDa). In addition to these two bands, a third major band (~80 kDa) represented the characteristic of the secretory component of reduced sIgA (Kuizenga *et al.* 1991).

With two companion gels, non-reducing SDS-PAGE in conjunction with protein silver staining and carbohydrate PAS staining was used to characterize the glycoproteins present in the fraction passed through the jacalin column. Heavily stained bands could be found on the PAS stained gel; they corresponded to greatly glycosylated proteins with molecular weights >200 kDa. Yet another heavily stained band (~30 kDa) was visible on the silver stained gel. However, this band was only very lightly stained by PAS. This was evidently also a glycoprotein, although with less carbohydrate than the other two components. Another minor band (~45 kDa) could be visualized on both gels and was thus also a glycoprotein, although again with a very small sugar content.

Conclusion

Surfaces can be prepared which specifically adsorb mucin from biological secretions and thereby confer protection against bacterial colonization and other non-specific fouling of the surface. Through this specifically deposited shield a superior surface protection is obtained than in cases where mucin adsorption takes place directly from the secretion, in competition with its lower molecular weight, adsorption prone constituents.

Acknowledgement

The research was supported by the Bausch and Lomb Company, which is gratefully acknowledged.

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