Urol Res (1983) 11:93–95

Urological Research

Rabbit Bladder-Surface Mucin: A Thermodynamic Mechanism for Inhibiting Bacterial Adhesion

J. F. Boyce, R. B. Podesta, S. Schürch and Margot R. Roach

Departments of Biophysics, Zoology and Medicine, University of Western Ontario, London, Ontario, Canada

Accepted: November 3, 1982

Summary. A possible thermodynamic mechanism for the inhibition of bacterial adhesion to the epithelial bladder surface was investigated in rabbits. Contact angles of aqueous polymeric droplets were measured to assess the relative hydration and surface-free energy, of normal and mucin-free bladder surfaces. We measured an angle of $91.2 \pm 1.2^\circ$ (SEM), $n = 37$ for the intact mucin surface and an angle of $120.5 \pm 1.2^\circ$, $n = 46$ for the epithelium after the mucin was removed with acid. These results indicate that mucin makes the epithelial surface significantly more hydrophilic and so produces a very low free energy interface with the urine environment. Such a low energy surface would inhibit bacterial adhesion because the surface already exists at its free energy minimum.

Key words: Mucin, Bacterial adhesion, Surface energy, Surface hydration.

Introduction

Epithelial surface-mucin appears to play an important role in preventing bladder infections. Edebo et al. [2] suggest that mucin acts by excluding antigen from the underlying host tissue, rather than by destroying microbes which have penetrated into the tissue. The mechanism involved remains unknown. While a number of authors have searched for specific antiadherence factors such as immunoglobulin A and glycoproteins [11, 4], Parsons and Mulholland [5] have shown that the mucin surface of the bladder is a nonspecific inhibitor of adhesion for a wide variety of bacterial species, whether they are alive or dead. They proposed that the strongly hydrophilic glycosaminoglycans in mucin create a "hydration barrier" to macromolecular interactions on the cell surface and so inhibit the adhesion of microbes [7, 6]. A new contact-angle technique recently developed in our laboratory [8] allows us to test if this hypothesis is thermodynamically valid.

The hydration of a tissue directly affects its surface-free energy, and free-energy changes determine whether physical adhesion will or will not occur [3]. In this report we present evidence that mucin may prevent microbe attachment by forming a low free-energy interface with the urine environment, making bacterial adhesion thermodynamically unfavourable.

Materials and Methods

Polymeric Test System

A solution of aqueous polymers consisting of 4% polyethylene glycol (PEG) MW: 20,000 (Fisher Scientific)/4% dextran MW: 2,000,000 (Pharmacia) in Hepes' buffered physiological saline solution (pH = 7.4) was prepared and allowed to equilibrate overnight. The solution separates into two distinct equilibrium phases: a PEG-rich upper phase which was decanted off and used as the tissue-bathing medium, and a dextran-rich bottom phase which provided the test-fluid droplets.

Animals and Tissue Preparation

Five male New Zealand White rabbits (2–3 kg) were killed with an overdose of intravenous sodium pentobarbital. The bladder of each animal was distended by injecting 10 ml of physiological saline solution and the outer surface was glued in situ to a small brass mounting bar with cyanoacrylate adhesive (Loctite Adhesives). After removing the bladder from the animal, we split the tissue open and washed it by rinsing it gently in physiological saline for 5 min. The entire preparation was transferred to a small viewing chamber filled with the PEG bathing medium.

Testing Procedure

We used a micromanipulator fitted with a micropipette to place small droplets (diameter $\cong$ 0.1–0.2 mm) of the dextran phase at random locations over the tissue surface. By illuminating the preparation from behind with polarised light, the droplet profiles could be observed through a dissecting microscope and photographed. Advancing contact angles of droplets resting on the mucosal surface were measured from these photographs. After testing the intact sur-

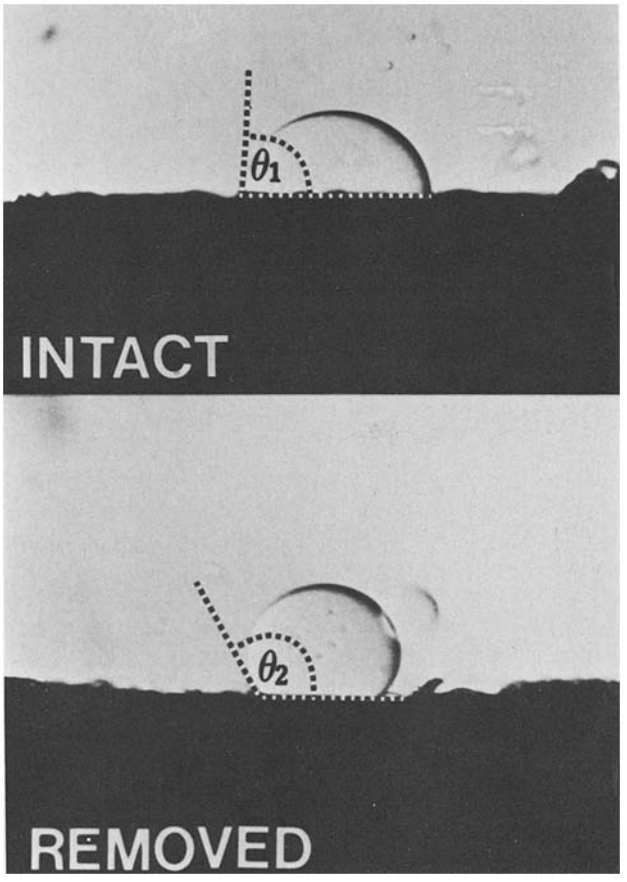

Fig. 1. Dextran droplets resting on the bladder surface before (*top*) and after (*bottom*) mucin removal. θ_1 is 89° and θ_2 = 119°. Both droplets are approximately 0.1 mm in diameter

Table 1. Summary of contact angle measurements on bladder surfaces

	Normal (Mucin intact)	Treated (Mucin removed)
Mean ± SEM (in degrees)	91.2 ± 1.2	120.5 ± 1.2
Number of observations	37	46
Number of animals	5	5

face of the fresh tissue, the mucin layer was removed with 0.3 M. HCl solution for 60 s, as originally described by Parsons and Mulholland [5]. Following this treatment the tissue was tested again in the same manner as described previously.

Results

Figure 1 shows typical examples of our observations for the mucin surface, and the epithelial surface denuded of mucin. The contact angle measurements are summarised in Table 1. For reasons described elsewhere [3] we have chosen Young's equation to study bladder/bathing medium interfacial-free energies:

$$\cos\theta = \frac{\gamma_{\text{bladder/droplet}} - \gamma_{\text{bladder/bathing medium}}}{\gamma_{\text{droplet/bathing medium}}}$$

where γ is the free energy per unit area at the given interface and θ is the angle of contact the droplet makes at the surface. Although the absolute values of the substrate (bladder) interfacial-free energies are not obtainable experimentally, this equation does establish a direct relationship with the cosine of the contact angle. An unpaired Student's t-test performed on the cosines of the measured contact angles indicated that a significant difference existed between the mean angles measured for the two test groups ($p < 0.001$).

Discussion

Since dextran is a long polymer of sugar it is more hydrophilic than the adjacent PEG bathing phase [1]. An average contact angle of 120.5 ± 1.2° (SEM) or beading of the dextran droplet indicates that the mucin-free surface repels the hydrophilic test fluid. This implies that the mucin-free epithelial surface is hydrophobic relative to the dextran droplet. However, when the mucin layer is intact, the droplet spreads to a greater extent over the surface (contact angle: 91.2 ± 1.2° (SEM)) indicating that the mucin layer is more highly hydrated. These results establish that the mucin layer is more hydrophilic than the epithelial surface denuded of mucin. This may explain how mucin might act as a non-specific inhibitor of bacterial adhesion.

The mucosal surface of the bladder faces a natural environment that is very hydrophilic because of the high water content of urine. When the mucin layer is intact, the two adjacent hydrophilic phases meet at an interface of extremely low free energy. This low energy surface would inhibit bacterial adhesion because it is unlikely that such adhesion could further reduce the free energy of the system, since it is already small. However, if the mucin layer is removed, a more hydrophobic surface of higher energy is exposed. A surface in this state would favour bacterial adhesion because covering a high energy surface with a lower one (i.e. the bacterial surface) could reduce the system's total free energy, which is most desirable thermodynamically. This mechanism may explain the 10-fold increase in bacterial adhesion observed by Parsons and Mulholland [5] when bladder surface-mucin was removed.

The theory assumes that the interfacial energy between the bacterial surface and urine is smaller than that between the denuded epithelium and urine. Sutherland [9] has reported many bacterial species which are able to produce polysaccharides on the outer surface of the cell wall. Such a polysaccharide coating would produce a hydrophilic, low energy interface with the urine environment. Mucin would, however, fail to inhibit the adhesion of bacteria with a more highly hydrated surface than the epithelial mucin layer. In this situation adhesion would be favoured because an even lower energy state could be achieved. Van Oss's observation that virulent bacteria tend to have more hydro-

philic surfaces than nonvirulent types, supports this theory [10].

While the thermodynamic theory of adhesion may be an oversimplification for biological systems, it agrees well with experimental observations and is consistent with physical theory. Similar results have been found in the vascular system where platelet adhesion is an important event in thrombosis and atherogenesis[1]. The theory is valuable as a predictive model and may be useful in helping our understanding of other forms of pathological cellular adhesion.

Acknowledgements. This work was supported by grants from the Medical Research Council of Canada to Samuel Schürch and the Ontario Heart Foundation to Margot R. Roach.

References

1. Albertsson PA (1971) Partition of cell particles and macromolecules. Wiley Interscience, New York, p 29
2. Edebo L, Kihlström E, Magnusson KE, Stendahl O (1980) The hydrophobic effect and charge effects in the adhesion of enterobacteria to animal cell surfaces and the influences of antibodies of different immunoglobulin classes. In: Curtis ASG, Pitts JD (eds) Cell adhesion and motility. Cambridge University Press, Cambridge, p 65–101
3. Kinloch AJ (1980) The science of adhesion. Part 1: Surface and interfacial aspects. J Mat Res 15:2141–2166
4. Parsons CL, Greenspan C, Mulholland SG (1975) The primary antibacterial defence mechanism of the bladder. Invest Urol 13:72–76
5. Parsons CL, Mulholland SG (1978) Bladder surface mucin: Its antibacterial effect against various bacterial species. Am J Pathol 93:423–432
6. Parsons CL, Pollen JJ, Anwar H, Stauffer C, Schmidt JD (1980) Antibacterial activity of bladder surface mucin duplicated in the rabbit bladder by exogenous glycosaminoglycan (sodium pentosanpolysulfate). Infect Immun 27:876–881
7. Parsons CL, Stauffer C, Schmidt JD (1980) Bladdersurface glycosaminoglycans: An efficient mechanism of environmental adaptation. Science 208:605–607
8. Schürch S, Gerson DF, McIver DJL (1981) Determination of cell/medium interfacial tensions from contact angles in aqueous polymer systems. Biochim Biophys Acta 640:557–571
9. Sutherland IW (1977) Surface carbohydrates of the prokaryotic cell. Academic Press, New York, p 27–97
10. van Oss CJ (1978) Phagocytosis as surface phenomenon. Ann Rev Microbiol 32:19–39
11. Williams RC, Gibbons RJ (1972) Inhibition of bacterial adherence by secretory immunoglobulin A: A mechanism of antigen disposal. Science 177:697–699

J. F. Boyce
Department of Biophysics
Health Science Centre
University of Western Ontario
London, Ontario, N6A 5CI
Canada

1 Boyce, J. F., P. Wong, S. Schürch, and M. R. Roach. Submitted for publication.