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BINDING OF *Staphylococcus aureus* ONTO BOVINE INTESTINAL MUCIN

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Abstract

Mucins act as protection for the gastrointestinal tract against various invading organisms. They are also crucial in developing drugs against these organisms as well as other therapeutic purposes. This study was carried out to investigate the binding of *Staphylococcus aureus* onto bovine intestinal mucin in vitro. The isolate of *S. aureus* was added into graded concentration of mucin ranging from 100 - 15000 mg. The mixture was agitated in test tubes for 5 min. The viable count of the bacteria in the mixture was taken at the end of the agitation period using the surface viable method and incubated along with control containing the same quantity of bacterial suspension at 37°C for 24 h. After 24 h incubation, the count was taken so as to determine the cells adsorbed by the mucin. The result of the study indicates that bovine intestinal mucin had binding capacity and hence, a reduction in the total cell count of *S. aureus in vitro*. Thus mucins may enhance the effectiveness of antibiotics when used in combination as they may immobilize the organisms for ready destruction by the antibiotics.

Keywords: *Binding*; *Staphylococcus aureus*; *bovine mucin*

INTRODUCTION

Mucins are the major macromolecular constituents of the mucous secretions that coat the oral cavity and the respiratory, gastrointestinal and urinogenital tracts of animals (Colina *et al.*, 1996; De Repentigny *et al.*, 1993). They are responsible for the viscoelastic properties of the secretions, providing protection for the exposed delicate epithelial surfaces from microbial and physical injuries. Secretory mucins are typically of very high molecular mass (over 1 mDa) and have hundreds of *O*-linked saccharine constituting between 50% and 80% of the molecule by weight (Allen *et al*., 1984). The saccharides are based, at present, on seven core structures and can vary in length from disaccharides to oligosaccharides of approximated 20 monosacharides and exhibit astonishing diversity (Adikwu and Nnamani, 2005; Berry *et al*., 2000). The biological relevance of this diversity is not fully understood, but one possibility is that they act as 'decoy' receptors for the prevention from binding of pathogens to epithelial cell (Corfield *et al*., 2002; Mantle and Husar, 1994). It has been shown for a long time that the saccharides are linked to serine and threonine residues of the protein scaffold. However, owing to the technical problems associated with deglycosylation of mucins, the biochemical characterization of the protein backbone of the large discrete mucins have been fraught with difficulties (Forstner *et al*., 1979; Montagne *et al*., 2000).

Mucins are vital in the host intestine, acting as protective physical barriers. They also produce certain protective enzymes responsible for the host defense mechanism. The defensins and magainins that protect the host in the intestine are largely responsible for the prevention of many microbial diseases.

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The integrity of the intestinal mucin may also help as a physical barrier to the entrance of bacteria into the underlying tissue. Thus organisms that produce enzymes capable of hydrolyzing mucins can easily establish infections. Microorganisms that produce sialidases are capable of hydrolyzing cervical mucin. Such organisms have been implicated in the pathogenesis of sexually transmitted infections in the female genital tract (Wiggins *et al*., 2001). The detection of these enzymes may be indicative of the presence of invading organisms and may be used as a diagnostic tool (Myziuk *et al*., 2003; Cauci *et al*., 2003). In this study, the binding of *Staphylococcus aureus* to purified intestinal mucin is presented.

MATERIALS AND METHODS

Materials

Acetone (BDH) and nutrient agar (Oxoid) were the main materials purchased. They were used without further purification. Mucin was obtained from a batch processed in our laboratory.

Methods

Processing of the mucin

Cow intestine was bought from the abattoir after fresh slaughtering and was prepared in our laboratory. The mucoid layer was scraped from the intestine into chilled water. The material was washed to remove blood. The crude mucin so obtained was transferred into chilled acetone in a plastic container. The chilled acetone caused the precipitation of the mucin and whitish wooly material was washed several times with fresh chilled acetone and collected on a Buchner funnel by means of suction from a vacuum pump. The mucin was allowed to dry under a vacuum desiccator containing calcium chloride for 3 weeks, then pulverized using an end runner mill and stored in airtight containers until used.

Standardization of the bacterial cells

Twenty-hour culture of *S. aureus* on nutrient agar slant was harvested with 2.5 ml of sterile distilled water to get an approximate concentration of $1x10^9$ CFU/ml. The standardization was done using the surface spread viable method by counting on the surface of an over-dried nutrient agar using Miles and Misra method. This served as the standard concentration. From which dilutions were made to a suspension containing 10^3 CFU/ml and the count taken.

Preparation of bovine mucin suspension

Bovine mucin was prepared according to an earlier established method (Adikwu and Okafor, 2006). Various quantities of mucin, ranging from 100-1500 mg, were weighed using an analytical weighing balance. The weighed samples were suspended in 10 ml distilled water and were sterilized at 121°C for 15 min using an autoclave. This was to eliminate foreign organisms.

Binding studies

Known volumes (20 ml) of the suspension of *S. aureus* containing 10^3 cfu/ml (determined using the surface spread viable method) were aseptically transferred into the containers containing the mucin suspensions. The mixtures were then subjected to shaking in an Endecotts flask shaker for 5 min. At the end of the 5 min the bacterial count was repeated using the surface viable method. Both mucin and bacterial suspension were individually subjected to similar treatment and the viable count carried out.

The surface viable count method

Sterile nutrient agar of 20 ml volume was aseptically poured into a sterile Petri dish and allowed to solidify. The dish was turned upside down and allowed to stand in an incubator for 30 min to obtain an over-dried nutrient agar medium. The back of the Petri dish containing the medium was marked into 8 parts using a marker. Sterile Miles and Misra pipettes were used to place drops of the treated materials on the marked sections and incubated at 37°C for 24 h. At the end of the 24 h incubation period, the viable counts were taken with the help of a magnifying glass.

RESULTS AND DISCUSSION

The results of the binding studies of *Staphylococcus aureus* onto the bovine mucin are shown in Figure 1. At mucin concentration above 800 mg all the cells were bound; that is no cell count could be taken. From the graph, it is clear that the extent of binding is

concentration dependent. This result, therefore, shows that mucin has the ability to prevent microbial pathogens such as *S. aureus* from penetrating mucosal surface of the intestinal tract. This demonstrates the usefulness of the mucin as a barrier agent against many organisms.

Among the specific adhesion-receptor interactions described for *S. aureus,* different domains of the protein component of mannoprotein have been shown to specifically recognized *L*-frucose of the disaccharide component. Such a mechanism could be postulated since mucin contains frucose and sialic acid and other monosaccharides as sugar moieties of the *O*-linked glycan (Shuter and Hatcher, 1996). Interestingly, the terminal glycopeptides of intestinal mucin which bind *S. aureus* is rich in hydrophobic amino acids.

Previous attempts to characterize the interactions of *S. aureus* with mucin have been focused on human nasal mucin (Shuter and Hatcher, 1996). Scanning electron microscopy of *S. aureus* incubated with human nasal mucosal tissue showed minimal binding to ciliated respiratory epithelium. Binding of pathogenic micro organisms to mucin is recognized as a critical initial step in successful colonization of host mucosal surfaces (Corfield *et al*., 1992). The study reported, describes the first example of binding of a microbial pathogen to the protein backbone of the 118-kDa C- terminal glycopetide mucin.

Escherichia coli also binds to the glycopeptide, but to the *N*-linked oligomannoside side chain. *O*-Glycans in the tandem repeats of mucin represent a far more ubiquitous recognition site for pathogenic bacteria, either through specific receptor-ligand interactions or by hydrophobic-bonding (Crowther *et al*., 1987). The production of mucin-degrading proteases, mucinsase, sialidase have been implicated as a virulence factor in the breakdown of mucus and penetration of the mucin barrier by a number of enteropathogenic bacteria (Wiggins *et al*., 2001).

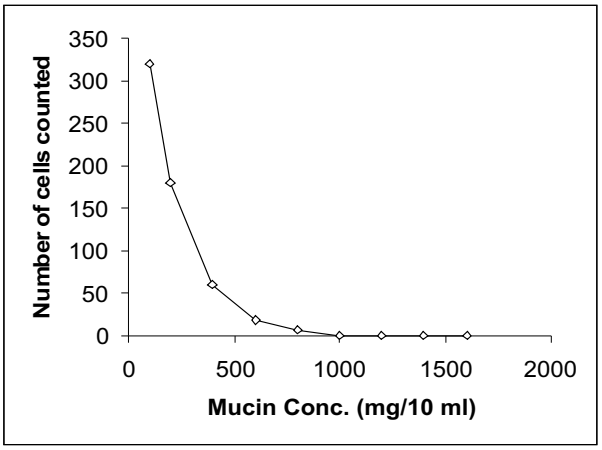

Fig. 1: Effect of concentration of mucin on the binding of *Staphylococcus aureus*

Conclusion

The results demonstrate that pathogenic fungus and bacteria may both adhere to, and degrade mucins in the small intestine and both properties may act to modulate microbial populations in the gastrointestinal tract thereby explaining the major role mucins play in protecting mucosal or intestinal surfaces and decrease its susceptibility to infective microorganisms.

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