Physical and biochemical surface properties of Gram-positive bacteria in relation to adhesion to bovine mammary cells and tissues

A review of the literature

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Summary: Functional properties of cell-wall components of Gram-positive bacteria and outer cell-wall structures (such as extracellular capsule and "slime") are reviewed in relation to pathogenicity. The possession of an extracellular polysaccharide in the form of capsule and/or slime layer by some Gram-positive bacteria is associated with increased virulence and resistance to phagocytosis. Moreover, the influence of non-specific factors such as cell-surface hydrophobicity and surface charge mediates interactions between bacteria and host tissue cells. The role of specific interactions between bacterial adhesins and the host epithelium in bovine mastitis is also reviewed.

KEYWORDS: Bacterial adhesion - Bovine mastitis - Collagen - Extracellular polysaccharide capsule/slime - Fibrinogen - Fibronectin - Hydrophobicity - Staphylococcus - Streptococcus - Surface charge.

INTRODUCTION

Bacterial adhesion is important in various areas of environmental, medical and industrial research (33). Apart from the essential role of selective bacterial adhesion in establishing many natural microbial communities, it is also crucial to such diverse phenomena as the colonisation associated with plant and animal diseases (13, 21) and host phagocytic defence (100). Adhesion of bacteria to animal cells, mediated by adhesin-receptor interaction, is an initial step in the infection process. Thus, bacterial adhesion may be exploited beneficially, e.g. to prevent pathogen colonisation (13).

This paper reviews the literature on physical and biochemical surface properties of Gram-positive bacteria and certain aspects of bacterial adhesion, including non-specific macroscopic and specific molecular interactions.

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Gram-positive bacteria have a relatively thick cell wall consisting mainly of peptidoglycan (74). This is a rigid layer containing a variety of polymers, such as teichoic and teichuronic acids (74, 107) together with polysaccharides and various proteins, such as protein A (87), M protein (99), fibronectin (102, 81), fibrinogen (Clumping Factor, CF) (82, 32) and collagen-binding proteins (91). Some of these molecules are present at, and probably extend from the outer surface of the organism, whereas others are concealed inside the cell wall. Peptidoglycan is also exposed at the bacterial surface. Some species or strains also possess cell wall-associated polymers in the form of capsules, or they form an extracellular slime layer (94, 16).

**Hydrophobicity/hydrophilicity**

The cell surface of staphylococci and streptococci has been studied for a long time. The molecular nature of the bacterial cell surface is crucial in the interaction between the micro-organisms and the host (71).

Bacterial hydrophobicity/hydrophilicity is a major physico-chemical property of their surface and is associated with adhesion to host tissue cells and to inert surfaces (93, 68, 90, 76, 23, 105, 75). Hydrophobic (water-insoluble) molecules tend to aggregate and expel water molecules, or they may associate with water-immiscible solvents, while hydrophilic (charged) molecules form hydrogen bonds with water and thus associate with water molecules (98).

Factors determining staphylococcal and streptococcal cell-surface hydrophobicity have not yet been defined. Several reports have provided evidence that certain proteins associated with cell walls and cell membranes are responsible for bacterial cell-surface hydrophobicity (76, 36, 42, 60, 75, 71).

The presence of M protein (105) and lipoteichoic acid (60) in the cell wall of group A streptococci or protein A in *Staph. aureus* may contribute to cell-surface hydrophobicity. Hogt *et al.* (37) reported that protein constituents at the cell surface of coagulase-negative staphylococci affect hydrophobicity. Proteins sensitive to pepsin appear to be the major hydrophobic sites at the cell-wall surface. These structures might contribute to the non-specific adhesion of bacteria by means of hydrophobic interaction (37, 20).

Bacterial surface hydrophobicity is dependent on growth conditions and upon growth phase of the organism (53, 111, 71, 56). Several methods for quantifying the hydrophobic surface properties of bacteria have been developed, including precipitation of bacteria by salts such as ammonium sulphate (51), where highly hydrophobic cells precipitate at low ammonium sulphate concentration; hydrophobic interaction between non-polar regions on the bacterial cell surface (90, 52), based on adherence to liquid hydrocarbons (76); an aqueous polymer biphasic system consisting of solutions of dextran and polyethylene glycol (93, 60); and the association of adherence of bacteria onto plastic surfaces with hydrophobicity (36).

Of 72 strains of *Staph. aureus* isolated from cases of bovine mastitis, 76% of the strains possessed hydrophobic surface properties after *in vitro* cultivation on blood agar plates (43) and hydrophobicity was correlated with protein A production. It was
reported recently that cultivation of *Staph. aureus* in a media containing bovine milk renders the bacterial cell surface hydrophilic (57).

**Bacterial surface charge**

This reflects the complex chemistry of the cell surface. In Gram-positive bacteria, teichoic and teichuronic acids of the cell wall and acidic polypeptides and polysaccharides of the glyocalyx (capsule/slime) evidently contribute to a negative surface charge (12). Destruction of the cell-wall ribitol teichoic acid in *Staph. aureus* may reduce the surface negative charge (73). Bacterial surface charge can be estimated simply by electrophoretic mobility of the bacteria (72), isoelectric-focusing (49) and by using fluorescent dyes (1).

Generally, the presence of hydrophilic properties implies that charged groups are present at the bacterial cell surface (85). Nevertheless, hydrophobic bacteria may also have a high surface charge following exposure of hydrophobic domains (35). The surface charge of encapsulated bacteria is generally higher than that of unencapsulated bacteria (34) since the capsule usually consists of polysaccharides containing negatively-charged uronic acid (95).

It has been demonstrated that bacterial surface charge affects the adhesion of bacteria to surfaces (117, 35). Furthermore, both hydrophobic and electrostatic interactions may be involved in bacterial adhesion. However, bacteria and most mammalian cells have a negative charge under physiological conditions, which creates repulsive forces between the cells (41). Hydrophobic interactions might serve to overcome the electrostatic repulsion and thereby facilitate interaction between specific binding molecules on bacteria and host cells.

**Extracellular capsule and slime**

Bacterial extracellular capsule is a polysaccharide-containing component which covers bacterial cells. In contrast, slime is an extracellular bacterial product which dissociates from bacteria placed in a liquid medium (22).

Bacterial extracellular capsule contains abundant polysaccharides and other polymers entrapped or bound by lectin-like interactions to the polysaccharide chains (35). Most bacterial exopolysaccharides are polymers of more than one type of sugar residue, and they often contain uronic acids and/or pyruvyl ketal groups which confer a negative charge on the polymers (35). The capsule of certain bacteria contains protein (66), polyglutamic acids (92), hyaluronic acid and polymers composed of N-acetyl glucosamine and glucuronic acids (95).

The extracellular capsule may be:

1. *rigid* — sufficiently organised to exclude particles (e.g. capsule delineated by India ink staining)
2. *flexible* — does not exclude particles
3. *integral* — under normal circumstances, intimately associated with the cell surface
4. *peripheral* — whose association with the cell surface is dependent on variable factors and which may, therefore, be partly shed into the menstruum (16). It is known that staphylococcal cells grown at elevated temperatures in high salt concentration will form aggregates, show irregular septation and produce capsules (38, 12).
Functional properties of the extracellular capsule and slime in pathogenicity

Pathogenicity, defined as damage to the host, depends on the proliferation of the putative pathogen in a suitable niche (83) to a point where its aggressive activities impinge on host functions. In bacterial pathogenesis, persistence is the key, because most pathogens must persist and proliferate to produce enough toxin to be effective, or to develop a reservoir population sufficient for tissue invasion. Bacterial persistence usually involves adhesion as an initial step. Production of glycocalyx (capsule/slime) inhibits clearance of bacteria by macrophages (115) and provides protection from antibacterial agents such as surfactants and chemicals (29, 80), specific antibodies (6), phagocytes and leukocytes (84). The possession of an extracellular capsule by organisms of different bacterial species has been correlated with increased virulence as compared with the unencapsulated counterparts of the same organisms (11).

Bacterial extracellular slime may interfere with phagocytosis by polymorphonuclear leukocytes through masking of the peptidoglycans (69) and teichoic acid. These peptidoglycans have been shown to enhance opsonisation (115, 116) and hinder diffusion of certain antibiotics (88). Moreover, phagocytosis by macrophages as well as by polymorphonuclear leukocytes would be physically inhibited by an aggregated clump of staphylococci joined by a complex matrix of slime fibres (12), thus forming microcolonies which may be important for the survival of Gram-positive bacteria. Bacterial extracellular slime is also believed to be important in bacterial adhesion and in establishing an infection (14). It is not yet known whether extracellular slime functions as a glue in the initial adhesion of bacteria, or whether it is produced only after the bacteria have adhered and have been subjected to metabolic "stress" (16).

Studies on pathogenicity of Staph. aureus in the ruminant mammary gland suggest that capsular polysaccharides are important in impeding neutrophil-mediated phagocytosis (108). Furthermore, inclusion of Staph. aureus capsular polysaccharides ("pseudocapsule antigens") in a vaccine results in considerable protection from experimental staphylococcal mastitis in ewes (109).

Methods for demonstrating extracellular capsule/slime

The chemistry and biosynthesis of staphylococcal capsular polymers and slime layers have been reviewed in detail elsewhere (22, 89, 114). Several methods have been developed to identify encapsulated bacteria. These include wet and dry India ink staining (64); patent blue staining (77); demonstration of diffuse growth in Serum-Soft-Agar (26, 67, 65); antiserum agar method (110); and electron micrography using fluorescent antibodies (119).

BACTERIAL BIOCHEMICAL SURFACE PROPERTIES

The normal microbial colonisation of sites in the body tissues by certain bacteria requires that the bacteria first bind to extracellular secreted constituents, cell membranes or cell matrices (9, 104).

On the cell surface of Gram-positive organisms, proteins, lipoteichoic acid (8) and polysaccharides are exposed, and some of these are believed to mediate cell attachment. They also possess specific receptors for various mammalian plasma
proteins and, by binding large amounts of such proteins, the surface characteristics of the bacteria can be changed considerably (61, 113, 2).

Bacterial pathogens use several mechanisms to adhere to the host tissue, usually by binding to particular protein or other structures on the surface of the host cell. Pathogens such as *E. coli*, *Staph. aureus*, coagulase-negative staphylococci and groups A, B, C and G of streptococci from human and animal sources can bind to whole eukaryotic cells, as well as to isolated extracellular matrix, and plasma proteins such as fibronectin, laminin, collagen and fibrinogen (46, 96, 27, 97, 104, 91, 47, 106, 55, 54, 58). These binding capacities could play a role in the early stage of tissue invasion and colonisation (106).

**Interaction with subepithelial connective tissue components and plasma protein**

A number of subepithelial connective tissue components can interact specifically with eukaryotic cells and support cell adhesion (18). They are also able to influence cell behaviour *in vitro* (24). Some of these components bind cells by a reaction involving receptors (81, 118). Interactions of fibronectin, fibrinogen and collagen with eukaryotic cells are good examples.

**Fibronectin**

This is a large multifunctional adhesive glycoprotein having an apparent molecular weight of 440,000. It is found mostly on the cell surface (79, 101, 25) and is distributed in such a wide variety of mammalian and avian tissue and cell types that it must be a ubiquitous structural and adhesive protein (3). Fibronectin is found in a range of basement membrane types, and there are at least three major forms of the protein: plasma fibronectin found in blood; cellular fibronectin (cell-surface protein) found on the surface of cultured cells; and fibronectin found in amniotic fluid (3).

Several studies report that fibronectin functions as an adhesive protein mediating the adhesion of bacteria to eukaryotic cells (2, 86).

Recent reports suggest that a number of bacteria, as well as tissue-invading parasites, possess specific surface molecules which bind to fibronectin. In addition, fibronectin mediates adherence of staphylococci to endothelial cells (103) and acts as an opsonin for the phagocytosis of *Staph. aureus* by polymorphonuclear cells (17, 48, 50). Binding to Gram-positive bacteria occurs preferentially at a site located in the amino-terminal domain of fibronectin (62) whereas the eukaryotic cell binding site is located in the centre of the polypeptide chain (27).

**Fibrinogen**

This is a serum protein which induces clumping of staphylococci (40) and streptococci (44, 45). This clumping reaction depends upon a surface protein (fibrinogen-binding protein or clumping factor, CF) (63), which interacts with fibrinogen fragments (fragment D), (78, 31). Fibrinogen can also bind staphylococci and streptococci, and can mediate binding to virus-infected cell cultures (19, 82). Whitnack and Beachey (112) have demonstrated a reduced uptake of virulent streptococci by neutrophils in the presence of fibrinogen. However, it is not clear why fibrinogen should bind to different eukaryotic cell receptors in the process of linking the eukaryotic cell to staphylococci and streptococci. The role of fibrinogen-binding protein in virulence is not yet known.
Collagen

This protein is found in the basement membrane which underlies epithelium (104). Collagen molecules are representatives of a large family of proteins which have a wide range of chemical and structural features (59). Nevertheless, they appear to have an important common function. Thus, it has been reported that collagen can bind and aggregate certain staphylococci and streptococci from human and animal infections (91, 104, 55, 54, 58).

**BACTERIAL ADHESION**
**TO MAMMARY CELLS AND TISSUE COMPONENTS AS A POSSIBLE VIRULENCE FACTOR IN BOVINE MASTITIS**

Udder pathogens like *Staph. aureus*, *Str. agalactiae* and *Str. dysgalactiae*, enter the udder through the teat. After entry, factors governing the development of infectious process and inflammation (mastitis) include the organism’s adherence to the internal epithelial surfaces (28), the number of neutrophils in the mammary secretion, the presence of antibodies to extracellular virulence determinants and ability of the bacteria to utilise nutrients within the udder and to multiply (4). Generally, the development of infection and inflammation depends on the balance between the natural defence mechanisms of the gland and the number and virulence of infective organisms. Adhesion of pathogenic micro-organisms to living cells and tissues may also be an important determinant of pathogenicity (89, 28, 5, 7, 15).

The adhesive properties of some udder pathogens roughly parallel their prevalence as a cause of mastitis. For example, in most cases *Staph. aureus* adheres well, and causes a chronic infection with irregular acute incidences, whereas Gram-negative *E. coli* and *Klebsiella* sp. adhere poorly, and it is unlikely that adherence has a significant role in the pathogenesis of mastitis due to these organisms (28). However, these organisms often contaminate the teat orifice and are isolated from mastitis milk. Perhaps their virulence depends on other factors and products. Early reports indicated that *Str. agalactiae*, *Str. dysgalactiae* and *Str. uberis* vary in their adhesive properties (39, 10). *Staph. aureus* does not always invade the tissues, and inflammation (mastitis) may be a consequence of the organism adhering to the internal duct and sinus epithelia (30). Frost *et al.* (28) studied the adherence of bacteria to epithelial cells in different regions of the mammary gland. Adherence increased from the teat sinus to lactiferous sinus and the large milk ducts. Adhesion offers the bacteria the advantage of a firm attachment that can withstand the purging effect of milk flow. Adhesion mechanisms may allow the bacteria to attach and thereafter penetrate target tissues, producing deep-seated foci. In such cases, intra-mammary antibiotic therapy would not be completely successful in eradicating diseases such as staphylococcal mastitis (39).

Most, but not all, of the adhesins reported so far are proteins. In general, bacteria utilise lectin-carbohydrate interactions or non-lectin-receptor interactions. Bacteria utilising the latter mechanisms bind to tissues through the agency of a third substance, which attaches the bacterium to a target cell, forming a bacterium-cell complex. These mediating (bridging) substances may be bacterial products or host-derived molecules (15). Alternatively the bacteria may attach to a tissue surface by binding first to an intermediary cell which in turn binds to the tissue surface. It has been reported (30) that group B streptococci utilise the protein-mediated adhesion mechanism in bovine udder.
The ability of udder pathogens to adhere specifically to host cells by means of specific cell surface adhesins may enable infective foci to become established (70). Moreover, antibodies against adhesion determinants of udder pathogens may prevent the bacteria from attaching to the surface epithelium, thus being retained in the lactating mammary gland and producing mastitis.

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PROPRIÉTÉS PHYSIQUES ET BIOCHIMIQUES DE LA SURFACE DES BACTÉRIES GRAM-POSITIVES EN RAPPORT AVEC L'ADHÉSION AUX CELLULES ET AUX TISSUS MAMMAIRES DE BOVINS. REVUE BIBLIOGRAPHIQUE. - W. Mamo.

Résumé : Les propriétés fonctionnelles des constituants de la paroi cellulaire des bactéries Gram-positives et des structures extérieures de leur paroi cellulaire (telles que capsule et enveloppe «visqueuse» extracellulaires) sont passées en revue dans leur rapport avec le pouvoir pathogène. La possession, par certaines bactéries Gram-positives, d'un poliholoside extracellulaire sous forme de capsule et/ou de couche «visqueuse», est associée à un accroissement de leur virulence et de leur résistance à la phagocytose. En outre, l'influence de facteurs non spécifiques, tels que l'hydrophobicité et la charge de la surface cellulaire, intervient dans les interactions entre les bactéries et les cellules tissulaires de l'hôte. Le rôle des interactions spécifiques entre les facteurs d'attachement bactériens et l' épithélium de l'hôte dans le cas de la mammite bovine est également étudié.

MOTS-CLÉS : Adhésion bactérienne - Capsule/enveloppe visqueuse extracellulaire poliholoside - Charge de surface - Collagène - Fibrinogène - Fibronectine - Hydrophobicité - Mammite bovine - Staphylococcus - Streptococcus.

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PROPIEDADES FÍSICAS Y BIOQUÍMICAS DE LA SUPERFICIE DE LAS BACTERIAS GRAM-POSITIVAS RESPECTO A LA ADHESIÓN A LAS CÉLULAS Y LOS TEJIDOS MAMARIOS DE BOVINOS. REVISTA BIBLIOGRÁFICA. - W. Mamo.

Resumen: Se analizan las propiedades funcionales de los constituyentes de la pared celular de las bacterias gram-positivas y de las estructuras exteriores de ésta (cápsula y envoltura «viscosa» extracelulares) en su relación con la patogenicidad. La posesión, por algunas bacterias gram-positivas, de un polisacárido extracelular en forma de cápsula y/o de capa «viscosa» va asociada con un aumento de su virulencia y su resistencia a la fagocitosis. Además, la influencia de factores no específicos, como la hidrofobicidad y la carga de la superficie celular, intervienen en las interacciones entre las bacterias y las células tisulares
del huésped. También se analiza el papel de las interacciones específicas entre los factores de adhesión de bacterias y el epitelio del huésped en el caso de la mastitis bovina.


**REFERENCES**


