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Interactions of the streptococcal C5a peptidase with human fibronectin

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Abstract

Group B Streptococci (GBS) is a leading cause of sepsis and meningitis in neonates and immunocompromised adults in western countries. GBS do not bind to fibronectin (Fn) in solution, but will bind to Fn adsorbed onto a solid surface. The reason for the specificity of this binding is unknown. Single molecule force spectroscopy was used to test the hypothesis that GBS, through streptococcal C5a peptidase (ScpB) molecules present on the surface of the bacteria, binds to a motif created by the juxtaposition of multiple adjacent Fn molecules. Atomic force microscopy (AFM) topographical images of adsorbed Fn deposited from various Fn coating concentrations were used to determine the Fn surface concentration. ScpB was tethered to an AFM tip with all surface modifications characterized by X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry. At the lowest Fn coverages the probability of observing a ScpB–Fn binding event increased linearly with Fn surface coverage. As an Fn monolayer was reached the probability of a ScpB–Fn binding event occurring increased markedly (~50 fold), with a concomitant increase in the rupture force from 17 pN to 33 pN. These results are consistent with the hypothesis that ScpB binds to a motif created by the juxtaposition of multiple Fn molecules.

Keywords: Single molecule force spectroscopy; Fibronectin; Streptococcal C5a peptidase; Poly(ethylene glycol)

1. Introduction

Group B Streptococci (GBS or *Streptococcus aglactae*) is a leading cause of bacterial infections among newborns and immunocompromised adults in industrialized countries [1,2]. Early-onset GBS (infants < 7 days old) accounts for 80% of the occurrence in newborns, and late-onset GBS is characterized by infection at 8 days to 3 months [3,4]. Clinical features of GBS in infants include sepsis, meningitis, and pneumonia. While GBS is less common in adults, skin and soft tissue infection are the most common manifestations of the disease. Common to both infants and adults is the occurrence of sight or hearing loss, cerebral palsy, and death [2,3,5–7]. Interactions of GBS with biomaterials include infections of ventriculoperitoneal shunts [8], prosthetic heart valves [9], IV catheters [6], and artificial bone joints [7].

Absorbed fibronectin (Fn) is targeted by bacteria as an anchoring point for adhesion and invasion of epithelial cells [3,10–12]. Hydrogen bonding [13], electrostatic interactions [14], specific interactions between surface proteins and extracellular matrix proteins [11,15,16], and shear forces [17] all have been shown to mediate bacterial adhesion. GBS will only adhere to adsorbed Fn, not soluble Fn [1,18,19]; this behavior helps GBS evade the host immune system since soluble Fn acts as an opsonin [20]. The mechanism for specific binding of GBS to immobilized Fn is unknown, but one hypothesis is that the adsorbed Fn undergoes conformational changes to reveal cryptic binding sites [19]. Another hypothesis is that GBS binds to a cluster of adsorbed Fn molecules.

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The serine protease C5a peptidase (ScpB), which is present on the surface of GBS, has been identified as an Fn adhesin [21]. ScpB has been proposed to interact with adsorbed Fn through two binding sites [22]. One binding site has a high affinity of 4 nM and is expected to be biologically significant. The other binding site has a low affinity in the mM range and is unlikely to be of biological significance. Recent studies have shown that Scp does not bind Fn in solution [19], suggesting a conformation change in Fn induced by a surface is necessary for binding.

Soluble Fn is a large dimeric glycoprotein with a radius of approximately 20 nm, while adsorbed Fn can take on a number of different conformations ranging from globular to elongated and cross-linked [23–25]. The binding sites on Fn are distributed along the length of the molecule. The common anchoring point for various bacterial adhesins and the collagen receptor are located towards the N-terminus of the molecule [26].

In this study, we explore the possibility that GBS binds specifically to adsorbed Fn because ScpB binds to a binding site created by the juxtaposition of multiple Fn molecules that is not present on Fn monomers. Atomic force microscopy (AFM) single-molecule force spectroscopy with a ScpB modified tip is used to address this question by measuring the binding force between ScpB and adsorbed Fn as a function of Fn surface concentration. Fn surface coverages from single molecules to a monolayer were investigated. X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF–SIMS) were used to characterize the AFM tip modification process.

2. Experimental

2.1. Atomic force microscopy

The AFM used in this study is a Molecular Imaging PicoScan (Pheonix, AZ). Protein imaging was performed using the magnetic AC (MAC) mode or the Acoustic AC mode (AAC). Imaging in air and liquid was carried out with Molecular Imaging type II MAC tips (MAC) or MikroMacsh (Wilsonville, OR) NSC35 ALBS tips (AAC). Force experiments were carried out with NP–S tips (Veeco, Santa Barbara, CA) with spring constants in the range 0.06–0.52 N m^{-1}. Spring constants were measured by the thermal noise method [27]. Force curves were carried out at a rate of 1 Hz with a force trigger of 0.25 V and a total travel distance of 1 μm. For each experiment between 500 and 5000 force curves were acquired. The tip location was controlled by a script written to raster the tip over the sample area. All forces curves were taken under 1× phosphate buffered saline solution (PBS, EMD Chemicals, Gibbstown, NJ) at a pH of 7.4.

2.2. Force curve analysis

Jump heights were extracted from the force curves by filtering them to exaggerate vertical segments with a filter proposed by Kasas {−2, −5, −8, −10, 0, 5, 20} [28]. Once the peaks were found, 11 points to the left and right of the peak were fit with a second degree polynomial. The difference between the endpoints of the polynomials was taken as the jump height. This method was proposed by Baumgartner et al. [29]. This analysis routine was written in Java as a plugin for ImageJ. Force distributions were calculated as empirical probability distributions as outlined in Ref. [29]. This method of calculating the force distribution is preferred over the histogram method because there is no need to bin the data. The functional form of the distribution given M observations is

$$\Psi(x) = \frac{1}{M}\sum_{i=1}^{M}\frac{1}{\sqrt{2\pi\sigma_i^2}}\exp\left(\frac{(x-h_i)^2}{2\sigma_i^2}\right)$$

where h_i is the binding force and σ_i is the standard deviation of the binding force. Averages (μ) were calculated from this distribution as $\mu = \sum h_i p_i$ where p_i is the probability of the observation. Since the force distributions are broad, no estimate of the variance was calculated.

2.3. X-ray photoelectron spectroscopy

The XPS measurements were performed on a Surface Science Instrument S-probe spectrometer (Mountain View, CA) equipped with monochromatic AlK_α source ($h\nu = 1486.6$ eV), hemispherical analyzer, and multichannel detector. The binding energy (BE) scale was referenced by setting the hydrocarbon C_{1s} BE to 285.0 eV. Elemental compositions were determined from spectra acquired at an analyzer pass energy of 150 eV. High-resolution spectra were obtained using an analyzer pass energy of 50 eV. Further details of the XPS experiments are published elsewhere [30].

2.4. Time-of-flight secondary ion mass spectrometry

A model 7200 physical electronics instrument (PHI, Eden Prairie, MN) was used for ToF–SIMS data acquisition. The instrument has an 8 keV Cs^+ ion source, a reflectron time-of-flight mass analyzer, chevron-type multichannel plates (MCP), and a time-to-digital converter. Data were acquired over a mass range from $m/z =$ 0 to 500 for both positive and negative secondary ions. The area of analysis for each spectrum was 100 μm × 100 μm. The total ion dose used to acquire each spectrum was less than 2×10^{12} ions cm^{-2}. At least six spots on three samples were analyzed for each step in the modification process. Further details of the ToF–SIMS experimental conditions used in this study are published elsewhere [31].

2.5. Protein adsorption

Human plasma fibronectin (Invitrogen, Grand Island, NY) was adsorbed onto freshly cleaved mica (Ted Pella,

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