Analysis of plasminogen binding to Treponema denticola, a key periopathogen

Britney Tegels
Virginia Commonwealth University

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ANALYSIS OF PLASMINOGEN BINDING TO TREPONEMA DENTICOLA, A KEY PERIOPATHOGEN

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

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Virginia Commonwealth University
Richmond, Virginia
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I would first like to thank my advisor, Dr. Richard Marconi, for trusting me and giving me the perfect amount of guidance and freedom to develop into an independent, successful scientist. Thank you to my graduate committee, Dr. Jason Carlyon, Dr. Daniel Conrad, and Dr. Jessica Bell, for taking the time to listen and provide helpful insight into the development of my project. I would also like to give a special thanks to Dan Miller who became not only my mentor but one of my closest friends. He pushed me to be the best scientist I could be and always expected more out of me. I owe a great deal of my scientific success to him. Thank you to Katie Mallory for providing moral support and encouragement through the ups and downs of graduate school. Thank you to my family, especially my parents, Barry and Denise, for providing constant support and never letting me give up. Finally, I would like to thank my rock, Harwood. Thank you for always being there with unconditional love and patience, and for bringing a smile to my face when I need it most.
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# Abbreviations

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<tr>
<td>Δ</td>
<td>Deletion</td>
</tr>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>A405</td>
<td>Absorbance measured at 405 nm</td>
</tr>
<tr>
<td>ABTS</td>
<td>2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
</tr>
<tr>
<td>B.</td>
<td><em>Borrelia</em></td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>CCP</td>
<td>Complement control protein</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CTLP</td>
<td>Chymotrypsin-like protease</td>
</tr>
<tr>
<td>D</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>E</td>
<td>Glutamic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>ε-ACA</td>
<td>ε-aminocaproic acid</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>FH</td>
<td>Factor H</td>
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<tr>
<td>FhbB</td>
<td>Factor H-binding protein B</td>
</tr>
<tr>
<td>FhbA</td>
<td>Factor H-binding protein A</td>
</tr>
<tr>
<td>FI</td>
<td>Factor I</td>
</tr>
<tr>
<td>GCF</td>
<td>Gingival crevicular fluid</td>
</tr>
<tr>
<td>Glu-Plg</td>
<td>Glutamic acid plasminogen</td>
</tr>
<tr>
<td>Glu-Pln</td>
<td>Glutamic acid plasmin</td>
</tr>
<tr>
<td>H</td>
<td>Histidine</td>
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<td>His</td>
<td>Histidine</td>
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<td>hr</td>
<td>Hour</td>
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<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<td>HRP</td>
<td>Horseradish peroxidase</td>
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<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IPTG</td>
<td>isopropyl β-D-1-thiogalactopyranoside</td>
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<tr>
<td>K</td>
<td>Lysine</td>
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<td>K1-K5</td>
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<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>Monopotassium phosphate</td>
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<tr>
<td>LBS</td>
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<td>Lys</td>
<td>Lysine</td>
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<td>Lys-Plg</td>
<td>Lysine plasminogen</td>
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Lys-PIn  Lysine plasmin
MAC  Membrane attack complex
MMP  Metalloproteinase
μg  Microgram
μl  Microliter
μM  Micromolar
mg  Milligrams
mL  Milliliter
mM  Millimolar
min  Minute
M  Molar
NaCl  Sodium chloride
NaHCO₃  Sodium bicarbonate
NaH₂PO₄  Monosodium phosphate
Ni-NTA  Nickel-nitrilotriacetic acid
nm  Nanometer
nM  Nanomolar
NOS  New oral spirochete
OD₆₀₀  Absorbance measured at 600 nm
OspA  Outer surface protein A
OspE  Outer surface protein E
PAM  Group A streptococcal M-like protein
PA  Plasminogen activator
PAP  N-terminal activation peptide of plasminogen
PAGE  Polyacrylamide gel electrophoresis
PBS  Phosphate buffered saline
PBST  0.2% Tween 20 in PBS
PBSTM  5% nonfat dry milk in PBST
Plg  Plasminogen
PVDF  Polyvinylidene difluoride
R  Arginine
r-  Recombinant
rpm  Revolutions per minute
SAAPFNA  succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-p-nitroanilide
SDS  Sodium dodecyl sulfate
SPD  Serine protease active site of plasminogen
T.  Treponema
TBS  Tris buffered saline
TBST  0.2% Tween 20 in TBS
TBSTB  5% BSA in TBST
Tris  Tris(hydroxymethyl)aminomethane
uPA  urokinase plasminogen activator
WHO  World Health Organization
WT  Wild type
x g  Gravitational force
Periodontitis is a chronic inflammatory disease that affects over 116 million adults in the United States. A shift in the normal microflora occurs as periodontal disease develops resulting in a larger number of Gram-negative anaerobes and spirochetes. An increase in the oral spirochete, *Treponema denticola*, is highly correlated with periodontal disease progression and severity. The ability of this periopathogen to thrive in the subgingival crevice is dependent on complement evasion mechanisms. Earlier analyses demonstrated that the primary mechanism of *T. denticola* serum resistance is binding of the human complement regulatory protein, Factor H (FH), to the factor H-binding protein (FhbB). FH serves as cofactor in the Factor-I mediated cleavage of C3b and accelerates the decay of the C3 convertase complex, leading to downregulation of C3b production. Several pathogens bind FH, and a number of these bacterial binding
proteins have been shown to bind plasminogen. Plasminogen is a plasma glycoprotein that circulates as a zymogen. Its active form, plasmin, degrades components of the extracellular matrix and cleaves complement proteins C3b and C5 inhibiting complement pathway progression. Through molecular and biochemical analyses, this study demonstrates that FhbB simultaneously binds plasminogen and FH to residues located on its positively and negatively charged surfaces, respectively, and that the two ligands do not compete for binding. This study also shows that the surface-bound plasminogen is available for proteolytic cleavage into the active serine protease plasmin. The activated plasmin could break down components of the periodontal tissue leading to increased nutrient availability and creation of a larger anaerobic environment where the bacteria can flourish, thereby promoting periodontal disease.
Chapter 1: Introduction

Periodontal Disease

Periodontitis, the most common infection among middle-aged adults (12), is a broad clinical term used to describe the inflammation and infection of the ligaments and tissue that support the teeth (Figure 1-1). The common clinical outcome of periodontitis is deepening of periodontal pockets and destruction of the tissue that supports the tooth (periodontium), progressively leading to alveolar bone resorption and eventual tooth loss. As periodontal disease progresses, the pocket depth increases significantly and the physiochemical properties of the subgingival crevice are altered. The early stages of periodontal inflammation (gingivitis) can be corrected through treatment and improved oral hygiene. Although the progression of advanced periodontal disease can be stopped, complete restoration of the damaged tissue is impossible (76).

According to recent findings from the Centers for Disease Control and Prevention (CDC), roughly one out of every two American adults aged 30 and over has periodontal disease. Of that population, 38% aged 30 years and older and 64% aged 65 years and older have severe or moderate periodontitis. The study also highlights the underestimation of periodontal disease in the US population; previous studies estimated periodontal disease in adults aged 65 years or older at only 26% (15). The public health burden of periodontal disease extends well beyond North America; a recent report from
Figure 1-1. Physiological changes of periodontium associated with the development of periodontal disease. Healthy periodontal tissue (left) contains connective tissue and alveolar bone, which support the tooth. The space between the gingival epithelium and the tooth surface is filled with gingival crevicular fluid. In cases of periodontal disease (right), a dental-plaque biofilm accumulates on the tooth surface leading to destruction of connective tissue and alveolar bone, resulting in eventual tooth loss.
the World Health Organization (WHO) showed that severe periodontitis exists in 5-20% of adult populations worldwide (75).

Some factors may increase the likelihood of an individual developing periodontal disease. Some of these risk factors include poor oral hygiene, socio-economic status, malnutrition, age, hormonal state, smoking, and alcohol consumption (25, 39, 42, 88). Among adults, smoking is responsible for more than half of the cases of periodontitis (95). Severe periodontitis’ health consequences can reach beyond the oral cavity. Patients with severe periodontitis have an increased risk for heart disease, diabetes, adverse pregnancy outcomes, and rheumatoid arthritis (reviewed in 76). In patients with diabetes mellitus, the prevalence of periodontal symptoms is greater and the progression of periodontal disease is more aggressive (93, 94). The economic cost of treating periodontal disease and/or maintaining dental/oral health is staggering. Dental visits and oral health problems result in roughly 164 million lost work hours each year at a cost of 100 billion dollars (61).

Periodontitis is often described as a “polymicrobial disruption of host homeostasis” (12). Under healthy conditions, the oral cavity consists of hundreds of bacterial species that exist in a symbiotic relationship with their host. A shift in the normal microflora occurs as periodontal disease develops, resulting in a larger number of Gram-negative anaerobes and spirochetes present in the plaque (74). The subgingival plaque is complex with over 500 species represented, 57 of which belong to the genus Treponema (14, 72, 73). Of the oral treponemes, Treponema denticola is the dominant spirochete species, and a clear correlation between T. denticola and the occurrence and severity of periodontal disease has been demonstrated (13, 16, 17).
Treponema denticola, serum resistance, and complement

Treponema denticola is a Gram-negative, obligate anaerobic bacterium. In the healthy subgingival crevice, T. denticola represents <1% of the total bacterial population; however, in periodontal pockets this number can exceed 40% (14). The plaque biofilm builds from the tooth outwards with the superficial layers in close association with the gingival epithelium. T. denticola is located in the superficial layer as a part of the red complex with Prophyromonas gingivalis and Tannerella forsythia, and is subsequently exposed to soft tissue and gingival crevicular fluid (GCF) (89). GCF is a serum and local tissue exudate that collects locally around each tooth surface. GCF contains a variety of host and microorganism-derived substances, including markers of inflammation. GCF recovered from periodontal disease patients is rich in immune effectors including activated complement (12). T. denticola is resistant up to 40% serum (Marconi RT unpublished data). This level of serum resistance is sufficient for survival in the levels of complement encountered in the GCF. Thus, the ability of T. denticola to thrive in the subgingival crevice is dependent on evading complement mediated destruction.

The complement system is the oldest branch of the immune system that recognizes bacterial pathogens, marks them for destruction, and clears the infection from the host (Figure 1-2). Specific complement proteins bind to the surface of bacteria and activate the complement cascade resulting in formation of the membrane attack complex (MAC), deposition of opsonins (C3b and iC3b) on the surface of pathogens, production of anaphylatoxins (C3a and C5a), and stimulation of active immunity.
Figure 1-2. Complement activation pathways and the negative regulatory role of Factor H. Complement activation is initiated by the classical, lectin, or alternative pathway. All three activation cascades lead to production of opsonins (C3b, iC3b) and anaphylatoxins (C3a, C5a), and the formation of the membrane attack complex (C5b-9). Factor H inhibits alternative complement activation through cofactor activity in the Factor-I mediated cleavage of C3b, by facilitating the disassembly of preformed C3 (C3bBb) and C5 (C3bBbC3b) convertase, and by blocking formation of new C3 convertase by competing with Factor B for binding to C3b.
However, complement activity must be regulated to prevent damage to host cells or tissue. Factor H (FH) is a highly abundant (400-800 mg ml$^{-1}$ serum) complement regulator that healthy cells bind to their surface to protect themselves from complement mediated destruction. FH regulates the alternative complement pathway at the level of C3b by: (1) serving as a cofactor for Factor I-mediated cleavage of C3b, (2) accelerating decay of preformed C3 convertase, and (3) competing with Factor B for binding to C3b to block formation of new C3 convertase (84, 85, 105).

**Mechanisms of *T. denticola* complement evasion**

The presence of subgingival plaque and subsequent biofilm formation offers passive protection to *T. denticola* by providing a barrier against immune effector cells, complement, and antibody. *T. denticola* also employs active mechanisms of complement evasion, one of which is production of dentilisin. Dentilisin is a multi-subunit chymotrypsin-like protease (CTLP) complex that has a wide range of cytopathic effects on cells and tissue. These various effects facilitate penetration and translocation of *T. denticola* through cell monolayers and extracellular matrix (ECM) models (9, 18, 97). Dentilisin is also a complement degrading protease that has been demonstrated to cleave C3, C3b, and FH (60, 108). Cleavage of these key complement proteins likely favors dysregulation of tissue homeostasis of the subgingival crevice.

*T. denticola* also actively evades complement mediated destruction by utilizing the negative regulatory effects of FH. It accomplishes this through binding of FH to its surface via the factor H-binding protein B (FhbB) (58). FhbB is the smallest bacterially produced FH-binding protein identified to date with a molecular weight of 11.4 kDa. It is
the only FH binding protein produced by *T. denticola* and it has no discernible sequence homology with other bacterial FH binding proteins (58). Phylogenetic sequence analyses of *fhbB* from over 30 *T. denticola* isolates revealed the existence of three distinct major FhbB phyletic types (Marconi RT unpublished data, Figure 1-3). The structure of FhbB was recently solved and shown to consist of an αβαβ fold with well-defined negatively (α1-α2) and positively (β1-α3-β2) charged surfaces (66). The FH binding site was characterized to the negatively charged face of FhbB. Residues E45, D58, and E62 located on the negatively charged face are required for FH binding as alanine substitution mutagenesis abolished FH binding to the mutated residues (66).

FH consists of 20 imperfect repeat units referred to as complement control protein (CCP) domains (23). FhbB was demonstrated to bind CCP7 of FH (66). CCP7 serves as an interaction site for glycosaminoglycans presented on host cell surfaces and within the extracellular matrix thereby preventing targeting of host tissues by complement (27). Recent data suggests that the CCP7 binding sites for glycosaminoglycans and FhbB partially overlap (66).

Serum resistance analyses of *T. denticola* mutant strains lacking *fhbB* (35405Δ*fhbB*) and dentilisin (CCE) elucidated their contribution to evading complement encountered in the gingival crevicular fluid. When incubated with 25% serum, the serum sensitivity of 35405Δ*fhbB* was markedly increased compared to the wild type strain 35405 with cell survival at 15% and 85%, respectively (57). However, the serum sensitivity of CCE was at wild-type serum resistance levels demonstrating that dentilisin is not required for complement evasion. It was concluded that FhbB FH binding is the primary mechanism of *T. denticola* serum resistance.
Figure 1-3. Phylogenetic tree of mature FhbB from *Treponema denticola* isolates.

Mature FhbB sequences were aligned and the phylogenetic analyses were performed using MEGA5. The phylogenetic tree is rooted with FhbA, a factor H-binding protein of *Borrelia hermsii*. Phyletic types 1 and 2 are highly conserved while type 3 is more divergent.
A unique feature of dentilisin is its ability to cleave *T. denticola* surface-bound FH (60). Cleavage of FH is somewhat of a paradox as binding of FH serves to protect *T. denticola* against complement mediated attack. If dentilisin cleaves FH to depletion, the expectation would be that *T. denticola* would be more susceptible to complement mediated degradation. It has been proposed that when *T. denticola* establishes infection it is important for the periopathogen to have FH bound to the cell surface to evade complement. However, once the plaque biofilm has formed, there would be a colony of cells protecting the pathogen. At that point, it is thought that *T. denticola* uses dentilisin to cleave FhbB-bound FH as well as the host’s population of FH. This cleavage event would deplete the local population of FH used by healthy cells and lead to immune dysregulation as the host’s complement would begin to attack healthy cells. The resultant tissue destruction would provide nutrients to the periopathogen and create a larger periodontal pocket where the bacteria can thrive (61).

**Plasminogen**

Plasminogen (Plg) binding to bacterial cell surfaces is a common mechanism pathogens use when interacting with hosts. Plasminogen is synthesized in the liver as a 90 kDa polypeptide chain that circulates in plasma (200 µg mL$^{-1}$) as the inactive zymogen form of the enzyme plasmin (1, 79). Several forms of plasminogen are present in the plasma as a result of posttranslational processing (Figure 1-4). The mature form of plasminogen is known as Glu-plasminogen (Glu-Plg) due to the presence of the glutamic acid residue at the N-terminus. Glu-Plg consists of the
Figure 1-4. **Structural domains of human plasmin(ogen) forms.** The circulating mature form of plasminogen is known as Glu-plasminogen (Glu-Plg) due to the presence of the glutamic acid residue at the N-terminus. Glu-Plg consists of the N-terminal activation peptide (PAP) followed by a series of 5 repeating peptide regions termed kringle domains (K1-K5), followed by the serine protease active site (SPD). The activation loop (R561-V562) of Glu-Plg is cleaved by specific plasminogen activators like urokinase forming Glu-plasmin (Glu-Pln). Glu-Pln can cleave the PAP from Glu-Plg or Glu-Pln via the Lys77-Lys78 peptide bond forming Lys-plasminogen (Lys-Plg) or Lys-plasmin (Lys-Pln), respectively. The resulting two-chain Glu- or Lys-plasmin molecule consist of heavy chain A and light chain B, which remain covalently associated by interchain disulfide bonds.
N-terminal activation peptide (PAP) followed by a series of 5 repeating peptide regions termed kringle domains (K1-K5). The function of the kringles is to mediate protein-protein interactions, typically through binding of C-terminal and internal receptor lysine residues to the lysine binding sites (LBS) within the kringle domains. The kringle domains display varying affinities for free lysine or lysine analogues (K1 > K4 > K5 > K2) (54); K3 does not bind plasminogen (91). The C-terminal region of Glu-Plg contains the serine protease active site (SPD) comprised of amino acid residues His603, Asp646, and Ser741, which make up the catalytic triad of the active domain (79). This domain can recruit adaptor molecules, or cofactors, such as bacterial plasminogen activators (PAs) streptokinase and staphylokinase, which modify the substrate presentation to the enzyme and modulate its specificity (71).

Glu-Plg can adopt two different conformations, T and R. The T state (tight formation) is seen in full length human plasminogen. In this state, plasminogen is poorly activated due to intramolecular forces between lysine residues and the LBS of the kringle domains (46, 79, 98, 99). Competitive binding interactions with plasminogen receptors at residues K77-K78 change the conformation of Glu-Plg to the R state (relaxed formation), exposing the activation loop (Arg561-Val562) that can be cleaved by specific PAs thus forming Glu-plasmin (Glu-Pln), the active serine protease (79, 80). Once formed, Glu-Pln can cleave the activation peptide from Glu-Plg or Glu-Pln via the Lys77-Lys78 peptide bond thus forming Lys-plasminogen or Lys-plasmin, respectively. The Lys-plasminogen formation is more readily activated to Lys-plasmin by the PAs due to its open conformation (35, 79, 102). The resulting two-chain Glu- or Lys-plasmin
molecule consists of a heavy and light chain in the N and C-terminal region, respectively, and is held together by interchain disulfide bonds.

Utilization of the host plasminogen activation system during infection is a common mechanism various pathogens employ (10, 44, 56, 103). Bacteria interact with the plasminogen system by expressing plasminogen receptors on their surface and secreting PAs like streptokinase and staphylokinase to activate surface-bound Plg to plasmin (77). The plasminogen receptors direct plasmin activity to locations where proteolytic activity may be required. Plasmin can degrade fibrin clots through a process called fibrinolysis as well as various ECM components. This offers a potential proteolytic system that could be utilized by pathogenic bacteria to enter and disseminate into the host’s system (48, 49).

In addition to degradation of ECM components, plasminogen is involved in the immune system at multiple levels. In the complement pathway, plasminogen functions as a C3- and C5-binding protein, and activated plasmin degrades and inactivates the two major complement proteins C3b and C5 (3). Degradation of C3b by plasmin blocks C3b effector functions like cascade progression to C3 and C5 convertase. Without the C3 and C5 convertase the C3b opsonin is not deposited on bacterial cell surfaces, the anaphylotoxins C5a and C3a are not produced, and the MAC is not formed. Plasminogen binding has also been shown to enhance the cofactor activity of FH in the inactivation of C3b by the complement protease Factor I (FI) (3). Activated plasmin is also capable of degrading immunoglobulin (Ig) molecules (11, 33, 41, 101). Ig molecules function as opsonins, and the Fc portions of Ig are recognized by specialized Fc receptors expressed by phagocytic cells such as macrophages and neutrophils. The
Fc portions can also bind to the classical complement C1q complex and initiate the complement cascade.

Several pathogenic microbes bind plasminogen to their surface and utilize its effector functions. Multiple plasminogen-binding proteins have been identified, and the binding of plasminogen to these proteins is typically mediated by lysine residues. Some examples include FhbA from *B. hermsii* (83), OspE, CspZ, and CspA from *B. burgdorferi* (6, 32), and HcpA from *B. recurrentis* (31). Interestingly, all of these plasminogen-binding proteins bind the complement regulator FH and exploit its negative regulatory function also (31, 34, 36, 37, 40, 62-64, 81, 83, 87).

**Research objective**

It is evident that plasminogen binding to bacterial cell surfaces is an important dissemination and immune evasion mechanism. Multiple bacterial plasminogen-binding proteins have been demonstrated to bind additional complement regulators such as FH to evade complement-mediated destruction. As FhbB is the only FH-binding protein produced by *T. denticola* and complement evasion is essential for the periopathogen’s survival in the periodontal pocket, we sought to characterize plasminogen binding to FhbB. Through biochemical methods such as plasminogen binding assays, inhibition and competition assays, amino acid substitution, and activation and degradation assays, our lab has demonstrated that FhbB binds plasminogen to its surface, and that the surface bound plasminogen is available for proteolytic cleavage into the active serine protease plasmin.
Chapter 2: Materials and Methods

Bacterial cultures and growth conditions

*T. denticola* strains 35405, 33521, SP46, SP54, SP72, SP61, and CCE were grown anaerobically in New Oral Spirochete (NOS) medium as described previously (59). CCE is a derivative strain of *T. denticola* 35405 in which specific regions of the operon that code for dentilisin were inactivated by allelic exchange and insertion of an erythromycin cassette (22).

Protein expression and purification

All recombinant proteins in this study were expressed in LB Broth, Lennox (Fisher BioReagents) supplemented with 100 mg/L ampicillin (Fisher BioReagents). Cultures of the transformed NovaBlue (DE3) cells were grown to exponential phase at 37°C, and protein expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) at a 10 mM concentration for 8 hours at 37°C. Cells were pelleted by centrifugation (5,000 x g, 15 min, 4°C), frozen, resuspended in lysis buffer (50 mM NaH$_2$PO$_4$, 300 mM NaCl, 10 mM Imidazole [pH 8.0], lysozyme [1 mg/mL]), incubated on ice (1 hour), sonicated, and centrifuged (10,000 x g, 30 min, 4°C). Soluble protein was purified from the supernatant using Ni-NTA affinity chromatography with His-trap columns (GE Healthcare) on an AKTA Purifier (GE Healthcare). The purified proteins were dialyzed into phosphate-buffered saline (PBS) using Spectra/Por dialysis
membranes (Spectrum Laboratories). Protein concentrations were determined using a BCA protein assay kit (Thermo Scientific), and visualized via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (BioRad) and stained with Coomassie Brilliant Blue to assess purity. The recombinant FhbB mutants were generated as part of a previous study and were purified as described above (66).

**Plasminogen binding ELISAs**

Microtiter plates (Corning Costar) were coated (in triplicate; 1 µg per well in 100 mM NaHCO₃, pH 9.6, overnight at 4°C) with various recombinant FhbB proteins from *Treponema denticola* strains 35405 (wild type), SP50, 35404, 33521, CF170, and SP46, or with the various FhbB double amino acid mutants. OspA, a known borrelial plasminogen ligand (24), was used as a positive control and bovine serum albumin (BSA) served as a negative control. Plates were washed with tris-buffered saline (TBS) containing 0.2% Tween 20 (TBST) and blocked for 2 hours at room temperature with TBST containing 5% BSA (TBSTB). After washing the wells three times with TBST, plasminogen (1-4 µg/mL in TBSTB; Calbiochem) was added and incubated for 1 hour. Thereafter, the wells were washed and incubated for 1 hour with goat anti-human plasminogen (1:1,000 in TBSTB; Rockland Immunochemicals). Subsequently the wells were washed, rabbit anti-goat IgG (1:20,000 in TBSTB; Calbiochem) was added for 1 hour, the wells washed a final time, and signal was detected using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) at 405 nm.

For experiments examining the role of lysine residues in the plasminogen-FhbB interaction, the lysine analogue ε-aminocaproic acid (ε-ACA) (1-40 µM; Sigma Aldrich)
was added with plasminogen (10 µg/mL; Calbiochem) to recombinant FhbB-coated wells. To determine if plasminogen and FH compete for binding to FhbB, increasing molar ratios of plasminogen (see text for concentrations; Calbiochem) was added with purified FH (5 µg/mL; Complement Tech) to recombinant FhbB-coated wells. Binding was detected with goat anti-human FH (1:1,000 in TBSTB; Complement Tech) and goat anti-human plasminogen (1:1,000 in TBSTB; Rockland Immunochromicals) with rabbit anti-goat IgG serving as the secondary (1:20,000 in TBSTB; CalBiochem). Three independent replicates were performed for each plasminogen binding assay.

Cofactor assay

The ability of T. denticola-bound plasminogen to enhance the cofactor activity of FH in the Factor I-mediated cleavage of C3b was assessed with a cleavage assay. T. denticola CCE cells grown to mid-log phase were recovered by centrifugation (5,000 x g, 15 min, 4°C), washed twice with PBS, and the cell density determined by measuring the absorbance at 600 nm (OD₆₀₀). T. denticola CCE is a dentilisin gene inactivation mutant strain and was used in this assay because dentilisin cleaves C3b (60). Aliquots (0.5 OD₆₀₀) were suspended in PBS (100 µl) with plasminogen (1, 5, 10 µg/mL; CalBiochem) and FH (5 µg/mL; Complement Tech), and incubated for 1 hour at 37°C. The cells were centrifuged (5 min; 5,000 x g) to remove unbound plasminogen/FH, the cell pellet was suspended in PBS (100 µl) with Factor I (1.5 µg/mL; Complement Tech) and C3b (5 µg/mL; Complement Tech), and incubated for 2 hours at 37°C. After incubation, SDS-PAGE sample buffer was added (1:1 ratio), and the samples were fractionated by SDS-PAGE (Bio-Rad) and transferred to a PVDF membrane.
Non-specific binding was blocked by incubating the membrane with PBS containing 0.2% Tween 20 (PBST) and 5% nonfat dry milk (PBSTM) at room temperature for 1 hour. After washing three times with PBST, C3b degradation was detected with goat anti-human C3 (1:1000 in PBSTM; Complement Tech) with rabbit anti-goat IgG serving as the secondary (1:20,000 in PBSTM; CalBiochem). Signal was detected by chemiluminescence with the SuperSignal West Pico Western blotting substrate (Thermo). The controls for this assay consisted of reactions containing CCE cells with purified human C3b, FI, FH, and/or plasminogen. The purpose of this set of controls was to demonstrate the specificity of C3b cleavage by FI and that cleavage is dependent on the presence of FH.

**Dentilisin activity and plasminogen degradation assay**

The ability of dentilisin to degrade plasminogen was examined via a cell cleavage assay. *T. denticola* cells grown to mid-log phase were recovered and quantified as described above. Aliquots (0.1 OD<sub>600</sub>) were suspended in PBS (50 µl) with plasminogen (1 µg/mL; CalBiochem) and incubated for 2 hours at 37°C with rotation (500 rpm). Samples were collected at 0 and 2 hours, SDS-PAGE sample buffer was added (1:1 ratio), and the samples were assessed by SDS-PAGE and transferred to PVDF. To determine if the various *T. denticola* strains degraded plasminogen, the membrane was screened with goat anti-human plasminogen (1:1000 in PBSTM; CalBiochem). The procedure for immunoblot analysis was performed as described above. The dentilisin activity of all strains was measured by monitoring hydrolysis of
succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-p-nitroanilide (SAAPFNA), as previously described (57, 96).

**Plasminogen activation assay**

*T. denticola* cells grown to mid-log phase were recovered and quantified as described above. Aliquots (0.25 OD$_{600}$) were suspended in PBS (100 µl) with plasminogen (50 µg/mL; CalBiochem) and urokinase (uPA) (630 µg/mL; Sigma), and incubated for 2 hours at 37°C with rotation (500 rpm). Following incubation, the cells were centrifuged (5 min; 5,000 x g) and washed with PBS three times, and the OD$_{600}$ determined. Aliquots (0.25 OD$_{600}$) were suspended in PBS (500 µl) and immobilized (100 µl; in triplicate) in a microtiter plate (Corning Costar). The wells were incubated with 100 µl of D-Val-Leu-Lys 4-nitroanilide dihydrochloride (125 µg/mL; Sigma) and plasmin activity was recorded at intervals of 1 hour at 37°C (405 nm; ELx808 Bio-Tek).
Chapter 3: Results

**FhbB binds plasminogen.**

Some bacterially produced Factor H (FH) binding proteins are multi-functional and bind other host-derived ligands including plasminogen (6, 31, 32, 43, 50, 78, 83). To determine if FhbB binds plasminogen, an ELISA-based approach was used where recombinant OspA, FhbB, and BSA were immobilized in a microtiter plate and overlaid with increasing concentrations of plasminogen. Plasminogen bound to immobilized FhbB in a concentration-dependent manner (Figure 3-1). The known borrelial plasminogen ligand OspA (24) was used as a positive control and BSA as a negative control.

**Lysine residues are involved in FhbB plasminogen binding.**

The kringle domains of plasminogen have been demonstrated to interact with lysine residues of bacterial receptors (6, 31, 32, 79, 100, 106). To analyze whether lysine residues are relevant to the plasminogen-FhbB interaction, the lysine analogue ε-aminocaproic acid (ε-ACA) was utilized. The synthetic lysine analogue inhibited plasminogen binding to FhbB in a concentration-dependent manner, strongly suggesting lysine residues of FhbB are involved in binding to the LBS of plasminogen (Figure 3-2).
Figure 3-1. Binding of plasminogen to factor H-binding protein B (FhbB). Binding of plasminogen to immobilized FhbB, analyzed by enzyme-linked immunosorbent assay. Results are representative of 3 independent experiments; error bars indicate standard deviations. A405, absorbance measured at 405 nm.
Figure 3-2. Inhibition of FhbB plasminogen binding by the lysine analogue ε-ACA. r-FhbB was immobilized and overlaid with combinations of plasminogen (10 μg/mL) and increasing μM concentrations of ε-ACA. r-FhbB incubated with buffer alone served as a negative control. Plasminogen binding was detected by sequential addition of goat anti-human plasminogen (1:1,000), HRP-conjugated rabbit anti-goat (1:20,000), and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid). Results are representative of 3 independent experiments; error bars indicate standard deviations.
Positively charged amino acid residues mediate the plasminogen-FhbB binding interaction.

The FH binding site has previously been characterized to the negatively charged face of FhbB (Figure 3-3A) (66). The inhibition of plasminogen binding by ε-ACA suggested that plasminogen may bind to lysine residues located on the positively charged face of FhbB. To determine this, recombinant wild type FhbB and double amino acid substitution mutants were tested for plasminogen binding. Substitution of positively charged amino acids, including lysine residues, significantly reduced plasminogen binding (47/50, 77/78, and 93/94; Figure 3-3B) while substitution of negatively charged amino acids had no effect (58/62 and 62/66, Fig 3-3B). This result demonstrated that plasminogen binds to multiple residues on the positively charged surface of FhbB.

Plasminogen and FH bind to separable FhbB binding sites.

The FhbB structure is highly ordered consisting of a negatively charged face and a positively charged face with FH binding characterized to the negatively charged face (66). The inhibition of plasminogen binding by the lysine analogue ε-ACA, and the decrease in plasminogen binding observed for the positively charged double-amino acid mutants, suggested that plasminogen was binding to the positively charged face of FhbB. To determine if FH and plasminogen compete for binding, recombinant FhbB was immobilized in a microtiter plate and binding of FH was assessed with increasing molar concentrations of human plasminogen. Increasing concentrations of plasminogen
Figure 3-3. Mediation of plasminogen binding by positively charged amino acid residues.  A, the electrostatic surface charge maps are presented.  Positive and negative charges are depicted in blue and red, respectively.  The amino acid residues targeted for substitution are labeled on the appropriate face of FhbB.  B, measurement of plasminogen binding to recombinant wild type and double amino acid FhbB substitution mutants by ELISA.  Plasminogen binding was detected as described above and is represented as % binding relative to 35405.  Results are representative of 3 independent experiments; error bars indicate standard deviations.  * $P < 0.01$ compared to 35405 (Student t test).
had no effect on FH binding suggesting the binding sites for the two ligands are on opposite faces of FhbB with little or no overlap (Figure 3-4).

**FhbB phyletic type has no effect on plasminogen binding.**

Phylogenetic analyses of *fhbB* from a panel of *T. denticola* isolates identified three distinct FhbB phyletic types. Sequence variation among these types localizes primarily within the FH binding interface (Marconi RT unpublished data). To determine if sequence differences influence plasminogen binding, recombinant FhbB proteins of each phyletic type were immobilized in ELISA plates and coated with plasminogen. Equivalent binding across types 1 and 2 was observed while binding to type 3 isolates was varied (Figure 3-5). Although plasminogen binding to 33521 and SP46 is significantly lower than 35405, the effect is less drastic compared to FH binding across phyletic types (Marconi RT unpublished data). This observation is consistent with the conserved nature of the positively charged face of FhbB which appears to serve as the plasminogen binding interface (Marconi RT unpublished data).

**Plasminogen binding does not enhance the cofactor activity of FH.**

Plasminogen has been shown to enhance Factor I-mediated C3b degradation in the presence of the cofactor FH (3). As plasminogen and FH bind to FhbB simultaneously, the ability of plasminogen to influence cofactor activity was assessed. Factor H, Factor I, and increasing concentrations of plasminogen were added to C3b and *T. denticola* CCE cells. After incubation, the reaction mixture was separated by SDS-PAGE and cleavage products were identified by Western blotting described above. Intact C3b was detected by the presence of α' and β-chains (Figure 3-6). Factor I in the
Figure 3-4. Plasminogen-Factor H competitive binding analyses. r-FhbB was immobilized and binding of plasminogen and FH were analyzed by ELISA. Upon addition of plasminogen (molar ratios are shown), binding was visualized by sequential addition of goat anti-human plasminogen (1:1,000) or goat anti-human FH (1:1,000), HRP-conjugated rabbit anti-goat (1:20,000), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Plasminogen binding is represented as % binding relative to the 5:1 molar ratio, and FH binding is represented as % binding relative to the 0:1 molar ratio. Error bars indicate standard deviations.
Figure 3-5. FhbB phyletic type does not alter plasminogen binding. Recombinant FhbB proteins isolated from each phyletic type were immobilized in an ELISA plate, coated with plasminogen, and binding detected as previously described. The isolate of origin and phyletic type designation are indicated on the x-axis. Binding is represented as % binding relative to the wild type r-35405 FhbB. Results are representative of 3 independent experiments; error bars indicate standard deviations. **$P < 0.001$ compared to 35405 (Student $t$ test).
The presence of FH cleaved C3b as demonstrated by the appearance of the C3dg cleavage product (Figure 3-6, lane 5). The presence of intact C3b α’ and β-chains confirmed that plasminogen itself cannot serve as a cofactor in the FI-mediated degradation of C3b (Figure 3-6, lane 3). In the presence of plasminogen, FH, and FI, C3b cleavage was not enhanced as seen by C3dg cleavage products at roughly the same intensity as conditions where plasminogen is absent (Figure 3-6, lanes 5, 7-9). Therefore, plasminogen binding to FhbB does not enhance Factor I-mediated cleavage of C3b. It is also of note that increasing concentrations of plasminogen did not diminish C3b degradation, providing further evidence that FH and plasminogen do not compete for binding to FhbB.

The *T. denticola* protease dentilisin degrades plasminogen.

*T. denticola* is highly proteolytic and the most characterized of its proteases is dentilisin. Dentilisin is important in *T. denticola* periodontal pathogenicity as it has been shown to have a variety of functions including cleavage of key complement proteins C3, C3b, and FH (20, 22, 57, 108). To assess dentilisin activity of *T. denticola* strains used in this study, SAAPFNA assays were conducted (Figure 3-7A). The dentilisin positive strain, 35405, and dentilisin deficient mutant strain, CCE, served as positive and negative controls, respectively. Dentilisin activity at or below the level of CCE is considered background and the strain is designated as dentilisin deficient (33521, SP54, and SP72). Dentilisin activity 3 logs above CCE is considered above background and the strain is designated as dentilisin positive (SP61 and SP46).
Figure 3-6. Plasminogen does not enhance cofactor-assisted cleavage of C3b by Factor I. Combinations of plasminogen (1, 5, or 10 µg), Factor H, and Factor I were added to C3b and *T. denticola* CCE (dentilisin mutant) cells. The reaction mixture was assessed by SDS-PAGE and C3b degradation products were visualized by Western blotting using goat anti-human C3 (1:1000) and HRP-conjugated goat antiserum. Additional control reactions with CCE cells incubated with various combinations of purified human plasminogen, C3b, FI, and/or FH were performed and analyzed as described above.
Figure 3-7. *T. denticola* cleavage of plasminogen is dentilisin dependent.  

*A*, dentilisin activity of *T. denticola* strains was quantitated by SAAPFNA hydrolysis measured at 405 nm, as described in methods.  Dentilisin activity is represented as % hydrolysis relative to 35405; (‘) denotes minutes.  Results are representative of 3 independent experiments; error bars indicate standard deviations.  

*B*, dentilisin cleavage of plasminogen was assessed by incubating *T. denticola* cells with plasminogen for 2 hours.  Plasminogen degradation was detected by screening with human plasminogen antiserum.
Dentilisin has been demonstrated to cleave *T. denticola* surface-bound FH (60). As plasminogen and FH have been shown to bind FhbB simultaneously (Figure 3-4), the ability of dentilisin to degrade plasminogen was evaluated. Dentilisin positive or negative *T. denticola* strains were incubated with plasminogen for 2 hours and separated by SDS-PAGE. Plasminogen degradation was detected using human plasminogen antiserum. Complete degradation of plasminogen was observed at 2 hours in dentilisin positive strains while dentilisin negative strains were unable to cleave plasminogen (Figure 3-7B). Therefore, in addition to cleavage of C3, C3b, and FH, dentilisin is also able to degrade the zymogen, plasminogen.

**T. denticola** surface-bound plasminogen is activated to plasmin.

Plasminogen is activated to the serine protease plasmin by human activators such as urokinase (uPA) (7). In order for these activators to proteolytically cleave plasminogen, the appropriate domain of plasminogen must be accessible. Through the use of the chromogenic substrate \( \text{D-Val-Leu-Lys 4-nitroanilide dihydrochloride} \), we demonstrated that *T. denticola*-bound plasminogen is accessible to human uPA and is cleaved to proteolytically active plasmin (Figure 3-8). Plasmin activity of all six *T. denticola* strains incubated with urokinase and plasminogen was significantly higher than plasminogen activation for the control reactions.
Figure 3-8. Analysis of plasminogen activation on the *T. denticola* cell surface. *T. denticola* strains 35405, SP61, and SP46 (*A*) and CCE (dentilisin gene inactivation mutant), 33521 (natural dentilisin deficient strain), and SP54 (*B*) were incubated alone (purple), with the activator urokinase (uPA) (green), with plasminogen (red), or with plasminogen and uPA (blue). After washing, the chromogenic substrate D-Val-Leu-Lys 4-nitroanilide dihydrochloride was added, and conversion of the substrate was determined by measuring absorbance at 405 nm (A405). Plasmin activity at 8 hours for all strains incubated with plasminogen and urokinase was significantly higher compared to control reactions (*P* < 0.0001, Student *t* test).
Chapter 4: Discussion

Due to its location in the periodontal plaque biofilm, *Treponema denticola* is in direct contact with the gingival crevicular fluid produced by the gingival epithelium. Evasion of complement mediated destruction is essential for the periopathogen’s survival as GCF recovered from diseased patients is rich in immune effectors including complement (12). The primary mechanism of *T. denticola* serum resistance is binding of FH to the protein FhbB (57). The binding interaction enables *T. denticola* to regulate the alternative complement pathway at the level of C3b through a variety of mechanisms (84, 85, 105). Numerous pathogenic bacteria have been shown to utilize the negative regulatory function of FH by binding FH to proteins present on the cell surface (23, 109). In addition to FH, many of these surface proteins also bind plasminogen (23).

Plasminogen is a host-derived glycoprotein that circulates in plasma as an inactive zymogen. It is converted to its active serine protease form, plasmin, through cleavage by plasminogen activators such as staphylokinase and urokinase. Most pathogenic bacteria utilize the serine protease function of plasmin, and interact with the plasminogen system by secreting plasminogen activators or expressing plasminogen receptors on their surface (77). Activated plasmin is able to degrade ECM components and regulate the immune response through cleavage of complement proteins and
immunoglobulin molecules. These functions facilitate the invasion and dissemination of pathogenic microbes (48, 49).

In the present study, we show that FhbB is a novel plasminogen-binding protein. The kringle domains, located within the native form of plasminogen, mediate protein-protein interactions through binding of lysine residues to the krings’ LBS. The lysine analogue, ε-ACA, was able to compete out plasminogen binding to FhbB demonstrating that FhbB lysine residues interact with the kringle LBS during the binding interaction. Recently, the FhbB FH binding site was characterized to residues located on the negatively charged surface while the positively charged surface appeared unoccupied. Mutations to negatively charged residues E45, D58, and E62 completely abolished FH binding to FhbB (66). Using site directed mutagenesis, this study indicates that positively charged residues K47, H50, K77, K78, R93, and K94 are important for the interaction of FhbB with plasminogen while mutations to negatively charged residues have little to no effect. Although plasminogen typically mediates binding via lysine residues, other plasminogen binding proteins, such as the group A streptococcal M-like protein (PAM), mediate the interaction through arginine and histidine residues even in the presence of lysine residues (86). Unlike FH binding, alanine substitution of any one of the positively charged residues does not completely eliminate plasminogen binding. This may be due to the localization of the negatively charged electrostatic environment compared to the positively charged electrostatic environment which is less localized on the protein (Figure 3-3A). Also, the negatively charged mutations represent all possible negative amino acids whereas we only mutated 4 of the 10 positive residues on the positively charged surface. The ε-ACA and site directed mutagenesis results suggests
that plasminogen binding is a multi-contact point interaction mediated by positively charged lysine, arginine, and histidine residues present on the positively charged face of FhbB. If true, we would expect that FH and plasminogen do not compete for binding as they bind to negatively and positively charged residues, respectively. Our competition analyses confirm that FH and plasminogen do not compete for binding. Together, these data demonstrate that FhbB uses positively and negatively charged residues on opposite faces to bind plasminogen and FH, respectively.

In order to establish infection, *T. denticola* must evade the destructive effects of complement. The serum resistance of *T. denticola* is dependent on the binding of FH to FhbB (57). Earlier studies with FhbB suggested that it is highly conserved among *T. denticola* isolates, but recently a divergent FhbB protein was identified in strain 33521 (58, 67). The Marconi lab sequenced *fhbB* from over 30 different isolates and revealed three major phyletic types (type 1, 2, and 3) (Marconi RT unpublished data, Figure 1-3). There is ~70% similarity and ~60% identity between types, and sequence variations are localized to the negatively charged FH binding site of FhbB. The positively charged surface is more conserved across types with ~88% similarity and ~70% identity. Also, all lysine and arginine residues present on the positively charged surface are conserved across the three phyletic types (Figure 4-1). To determine if sequence variation across FhbB phyletic types affect plasminogen binding, an ELISA assay was used. The results demonstrate that there are no major differences in plasminogen binding to the three FhbB phyletic types. This is consistent with the high sequence conservation of the positively charged face of FhbB which appears to serve as the plasminogen binding
Figure 4-1. Sequence alignment of mature FhbB from various *T. denticola* isolates. Alignment was generated by MEGA5. Identical amino acids are indicated by (.) and gaps are indicated by (-). The signal peptide sequence is underlined, and the residues present on the negatively and positively charged surface of FhbB are depicted in red and blue, respectively. High conservation of the lysine and arginine residues on the positively charged surface are shown in bold.
interface. This further demonstrates that FhbB uses separate contact sites on opposite faces for plasminogen and FH.

Plasminogen has been shown to enhance cofactor-mediated inactivation of C3b by the complement protease Factor I (3). To assess the overall contribution of plasminogen to C3b cleavage, CCE cells (dentilisin deficient) and C3b were incubated with increasing concentrations of plasminogen in the presence of FH and FI. Plasminogen binding to FhbB does not enhance cleavage of C3b in the presence of FH and FI. The assay also demonstrates that plasminogen itself cannot degrade C3b, and that it does not act as a cofactor for FI-mediated degradation of C3b. Although plasminogen itself cannot cleave C3b, the activated protease form, plasmin, has been shown to cleave C3b (3). Future analyses will determine whether activation of FhbB-bound plasminogen to plasmin enhances C3b degradation or the cofactor activity of FH. It is important to note that plasminogen binding did not decrease the cofactor activity of FH providing further evidence that plasminogen and FH bind to separable sites and do not compete.

*T. denticola* produces numerous proteases that mediate processes that are important in *T. denticola* pathogenesis (2, 4, 19, 21, 30, 51-53, 65, 70, 82, 96). Dentilisin is the best characterized of this group, and has been shown to cleave several host proteins including surface bound FH (2, 9, 18, 60, 68, 108). The dentilisin activity of the *T. denticola* strains used in this study was determined by a SAA/PFNA assay; strains 35405, SP46, and SP61 are dentilisin positive while strains CCE, 33521, SP54, and SP72 are dentilisin deficient. In the presence of dentilisin positive strains 35405, SP46, and SP61, plasminogen was successfully degraded at 2 hours demonstrating
that dentilisin cleaves plasminogen. In the presence or absence of dentilisin, plasminogen bound to *T. denticola* is accessible to the activator uPA and is converted to active protease plasmin.

Given the many functions of plasmin(ogen) discussed in the introduction, FhbB binding of plasminogen in addition to FH could provide the periopathogen with additional mechanisms to evade both the innate and adaptive immune response. Plasminogen can bind to the complement proteins, C3 and C5, and activated plasmin can cleave C3b and generate unique C3b fragments (3). A paradox in FhbB FH binding is the cleavage of surface-bound FH by the protease dentilisin (60). If dentilisin cleaves FH to depletion, *T. denticola* would presumably be more susceptible to complement mediated destruction. By binding plasminogen to the surface and activating it to plasmin, *T. denticola* could degrade the opsonin C3b deposited on its surface, and plasmin could bind to and degrade C5 thereby preventing cell lysis by blocking the formation of the MAC. Plasmin can also degrade immunoglobulin molecules (11, 33, 41, 101). Thus, in addition to C3b and C5 cleavage, activated plasmin on the surface of *T. denticola* could cleave the Ig opsonins, further reducing opsonization while simultaneously blocking activation of the classical complement pathway. *T. denticola* in concert with *Prophyromonas gingivalis* can keep plasmin activated as both periopathogens are able to degrade the host plasmin inhibitor, antiplasmin (29). This would provide evasion mechanisms independent of FH binding that allow the periopathogen to evade both innate and adaptive immunity to continue to establish infection.
Another role of plasmin is its activation of matrix metalloproteinases (MMPs) (45). MMPs are a group of enzymes responsible for the degradation of various kinds of extracellular matrix proteins including collagen (92). The collagenases are secreted in a proenzyme form and require proteolytic cleavage for activation. Plasmin can activate procollagenases and thus act in concert with other proteinase systems (such as dentilisin) in ECM breakdown (55, 69, 104). Recent studies have demonstrated that the presence of *T. denticola* in the periodontal pocket is associated with increased levels of MMP-9 in gingival crevicular fluid (90, 107). MMP-9 has the ability to break down type I and type III collagen which is critical for periodontal destruction as gingival fibers are primarily composed of type I and type III collagen (5, 8). Gingival fibers are the connective tissue fibers that inhabit the gingival epithelium adjacent to teeth and help hold the tissue firmly against the teeth (38). Gingival fibers cannot be regenerated and when the fibers are destroyed, the periodontal pocket depth increases, allowing bacteria to invade deeper into periodontal tissue. There is evidence that plasmin(ogen) is directly involved in the activation of MMP-9 (26, 28, 47). This suggests that *T. denticola* may bind plasminogen to its surface and activate it to plasmin which can then subsequently activate the high levels of GCF MMP-9 associated with periodontal disease. Activated MMP-9 could break down the gingival fibers creating a deeper, more anaerobic periodontal pocket where *T. denticola* can thrive.

In summary, my research demonstrates that FhbB is a multifunctional protein that binds plasminogen and FH. After binding, plasminogen is cleaved into its protease form, plasmin. The binding and activation of plasminogen is extremely important for understanding periodontal disease progression and severity. Activated plasmin could
evade the host immune response encountered in the gingival crevicular fluid via cleavage of complement components and Ig molecules. Evasion of innate and adaptive immunity may enable *T. denticola* to enter the bloodstream and disseminate to secondary sites of infection such as the heart (reviewed in 76). The activated plasmin could also activate MMP9 to break down components of the periodontal tissue allowing for deeper invasion. The ensuing tissue destruction would benefit *T. denticola* by increasing nutrient availability and by creating a larger anaerobic environment where the bacteria can flourish, thereby promoting periodontal disease.
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Vita

Brittney Kae Tegels was born March 18, 1988, in Storm Lake, IA. She was raised in Cedar Rapids, IA, and graduated from Xavier High School in 2006. In May of 2010, she received her Bachelor of Science in Biochemistry from the University of St. Thomas in St. Paul, MN. Following graduation, she worked at the interface of chemistry and biology to help reduce hospital-acquired infections at 3M Health Care Division of Infection Prevention in Maplewood, MN. In 2011 she entered the Microbiology and Immunology Master’s program at Virginia Commonwealth University School of Medicine. A brief list of accomplishments performed during her graduate career is listed below.

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Abstracts

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