Structure of Factor H-binding Protein B (FhbB) of the Periopathogen, Treponema denticola

INSIGHTS INTO PROGRESSION OF PERIODONTAL DISEASE

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Background: The Treponema denticola FhbB protein binds FH, a complement regulator.

Results: The structure of FhbB was solved, and its interaction with FH was further defined.

Conclusion: The structurally unique FhbB protein interacts with CCP7 of FH through electrostatic interactions.

Significance: The T. denticola/FH interaction may perturb complement regulation resulting in conditions that favor the development of periodontal disease.

Periodontitis is the most common disease of microbial etiology in humans. Periopathogen survival is dependent upon evasion of complement-mediated destruction. Treponema denticola, an important contributor to periodontitis, evades killing by the alternative complement cascade by binding factor H (FH) to its surface. Bound FH is rapidly cleaved by the T. denticola protease, dentilisin. In this report, the structure of the T. denticola FH-binding protein, FhbB, was solved to 1.7 Å resolution. FhbB possesses a unique fold that imparts high thermostability. The kinetics of the FH/FhbB interaction were assessed using surface plasmon resonance. A K_D value in the micromolar range (low affinity) was demonstrated, and rapid off kinetics were observed. Site-directed mutagenesis and sucrose octasulfate competition assays collectively indicate that the negatively charged face of FhbB binds within FH complement control protein module 7. This study provides significant new insight into the molecular basis of FH/FhbB interaction and advances our understanding of the role that T. denticola plays in the development and progression of periodontal disease.

Complement evasion is a critical aspect of the molecular pathogenesis of bacteria that cause periodontal disease (1), the most common infection of middle-aged adults (2). The etiology of periodontal disease can be traced to several host-determined susceptibility factors and the population dynamics of the oral microflora community (3, 4). More than 70 species of anaerobic spirochetes (Treponemes) are found in the oral cavity. In the healthy subgingival crevice, they account for ~1% of the total bacteria (5). With the progression of periodontitis, the abundance of oral treponemes increases dramatically and can reach 40% of the total bacterial population (6, 7). Disease severity correlates specifically with the outgrowth of Treponema denticola and other bacterial species of the red microbial complex (6, 8).

To survive in the subgingival crevice, bacteria must be able to evade immune-mediated destruction. Crevicular fluid and periodontal lesion exudate are rich in active complement (9–12). Pathogens or other “non-self” surfaces trigger a proteolytic cascade that results in the amplification of complement activation. Complement activity must be carefully regulated or significant damage to host cells and tissues can occur. Factor H (FH), a 155-kDa glycoprotein (400–800 µg/ml serum), regulates complement activation in serum and on host cell surfaces through several mechanisms (13). FH serves as a cofactor for the factor I-mediated cleavage of C3b, competes with factor B for binding to C3b (thereby preventing C3 convertase formation), and accelerates decay of preformed C3 convertase complex (14, 15). FH also contributes to the regulation of complement through its interaction with C-reactive protein (CRP), a positive regulator of the classical pathway that is negatively regulated by FH (16). FH consists of 20 imperfect, ~60-amino acid repeat units, referred to as complement control protein (CCP) domains. Specific CCPs have been demonstrated to mediate intermolecular interactions and regulatory functions (17, 18). CCP1–4 are involved in fluid phase complement regulation, whereas CCP19–20 interact with cell surfaces facilitating self-discrimination. As detailed below, CCP6–8 and CCP19–20 have also been demonstrated to interact with surface proteins produced by numerous pathogens. Heritable polymorphisms in FH that disrupt its complement regulatory activity are the...
underlying basis for several important human diseases, including age-related macular degenerative disease (the most common cause of blindness in the elderly), atypical hemolytic uremic syndrome, and dense deposit disease (19).

*T. denticola* binds FH to its surface (20) via the FhbB protein (TDE0108). FhbB is the smallest (11.4 kDa) bacterially produced FH-binding protein identified to date (20, 21). FhbB binds and positions FH on the cell surface thus allowing FH cleavage by the *T. denticola* protease, dentilisin (22–24). It is our hypothesis that in vivo, FH cleavage leads to its depletion in the subgingival crevice resulting in local dysregulation of complement and conditions that favor the development and progression of periodontal disease.

Most characterized bacterial FH-binding proteins bind within CCP6–8 or CCP19–20 (17). Molecular structures have been determined for only two microbially produced FH-binding proteins, *Borrelia burgdorferi* CspA and *Neisseria meningitidis* Hbp (25, 26). These proteins do not share sequence or structural homology. The molecular or structural “signature” for bacterial recognition of FH remains unknown. The determination of additional FH-binding protein structures and the elucidation of the molecular basis of their interaction with FH will have significant implications for the development of preventive and therapeutic strategies for both infectious and inheritable diseases. Here, we report the atomic structure of the FhbB protein of the periodontal pathogen, *T. denticola*, and further define the molecular basis of its interaction with FH. FhbB possesses a previously undescribed protein fold that imparts high stability to the protein. Kinetic analyses of the FH/FhbB interaction revealed a *K_D* value in the micromolar range with rapid on-off-rates. Site-directed mutagenesis led to the identification of FhbB and FH residues required for the FH/FhbB interaction. The binding site for FhbB on FH was localized to CCP7 and found to overlap with the binding site for glycosaminoglycans (GAG) and the GAG analog, sucrose octasulfate (SOS). These analyses represent a significant advancement in our understanding of the molecular interactions between FH and microbially produced FH-binding proteins (specifically those that bind to CCP6–8). In addition, the data allow for the development of a refined hypothesis regarding the biological role of FH binding and cleavage in *T. denticola* biology and the pathogenesis of periodontal disease.

**EXPERIMENTAL PROCEDURES**

*Generation of Recombinant Proteins*—Recombinant FhbB was generated as described previously (27). Site-directed mutations were introduced into the gene using mutagenic primers. All FhbB proteins were purified by immobilized metal affinity chromatography (HisTrap, GE Healthcare) according to the manufacturer’s protocol. Proteins consisting of FH CCP6–8 and CCP19–20 were generated by amplification of the corresponding segment from human FH cDNA (Source BioScience imaGenes). The amplicons were annealed with pET46 Ek/LIC (Novagen). The proteins were expressed in *Escherichia coli* NovaBlue(DE3) cells overnight. The FH-derived proteins were purified from inclusion bodies by resuspension of the insoluble cell lysate in resolubilization buffer (0.1 M NaH$_2$PO$_4$, 0.5 M NaCl, 6 M guanidine hydrochloride, 5 mM β-mercaptoethanol, pH 8.0) for 1 h at room temperature. The proteins were purified by immobilized metal affinity chromatography (HisBind Resin, Novagen) and refolded using standard approaches.

*Structure Determination, Data Collection, and Processing*—Conditions for crystallization were as described previously (27). Native and selenomethionine single wavelength anomalous diffraction datasets were collected on an ADSC Q315 CCD x-ray detector at the X25 beamline of the National Synchrotron Light Source, Brookhaven National Laboratory. The diffraction images were processed using the HKL2000 software package (28). The data processing statistics are summarized in Table 1.

*Structure Determination and Refinement*—Phases were determined from the 1.77-Å single wavelength anomalous diffraction dataset using the AutoSol module of PHENIX (29). The resultant model was further manually built in COOT (30, 31) and refined in PHENIX (29). The native structure was solved by the molecular replacement method using the PHASER (32) program as part of the CCP4 Software Suite (33) and the selenomethionine model as the search probe. Refinement statistics are given in Table 1. Models were evaluated by MolProbity (34, 35). Structural figures were made using PyMOL (Schrödinger Scientific). Electrostatic surface representations of FhbB were generated with APBS plugin in PyMOL. Structural similarity searches were completed using DALI (36).

*Circular Dichroism (CD)*—Recombinant wild type FhbB and lysozyme were used for thermal denaturation and renaturation studies. Proteins were extensively dialyzed into 10 mM NaH$_2$PO$_4$, 50 mM NaCl, pH 7.4, buffer and diluted to 20 mg ml$^{-1}$ for analysis of CD spectra. Spectra (190–300 nm) were acquired on a Jasco J-720 spectropolarimeter at 4 °C in a 10-mm quartz cuvette. Replicate scans (5) were collected, averaged, and base-line subtracted. α-Helical content was monitored at 222 nm as the temperature was increased from 4 to 90 °C and returned to 4 °C (rate of 1 °C per minute). CD spectra were acquired prior to heating the protein and after cooling the protein.

*Factor H Binding Overlay Assays*—Overlay assays were performed as described previously (37). In brief, recombinant proteins were fractionated by SDS-PAGE, transferred to PVDF membrane by electroblotting, and the membranes incubated with purified FH (Complement Tech) or recombinant FH fragments consisting of CCP domains 6–8 or 19–20 (10 μg ml$^{-1}$ in phosphate-buffered saline with 0.2% Tween 20 (PBST)). Binding was detected with goat anti-human FH (1:800 dilution; Complement Tech) with rabbit anti-goat IgG serving as the secondary (1:40,000 dilution; Calbiochem). Signal was detected by chemiluminescence with the Pierce ECL Western blotting substrate (Thermo).

*FH-binding ELISAs*—Recombinant proteins were immobilized in wells of ELISA plates (in triplicate; 1 μg per well in 100 mM NaHCO$_3$, pH 9.6, overnight at 4 °C). Non-specific binding was blocked for 1 h with 5% nonfat dry milk in PBST (PBSTM). FH (10 μg ml$^{-1}$ in PBST) was added for 1 h, and the wells washed three times with PBST. Goat anti-human FH (1:800 in PBSTM; Complement Tech) was added for 1 h, and the plates were washed three times. Rabbit anti-goat IgG (1:20,000 in PBSTM; Calbiochem) was added for 1 h, washed, and signal was detected using 1-step ultra 3,3′,5,5′-tetramethylbenzidine sub-
strate (Bio-Rad) as detailed by the supplier at 450 nm. The data were averaged across three plates. To determine whether SOS can inhibit FH binding, 1 μg of FhbB was immobilized in each well and non-specific binding was blocked with PBST for 1 h. FH (5 μg ml⁻¹) was incubated with increasing concentrations of SOS (0–50 mM) in PBST for 1 h with constant gentle agitation. FhbB was overlaid with the FH/SOS solution for 1 h at RT. After 1 h, the FH was removed and binding was detected as described above. Data were averaged and normalized to FH binding with no SOS added.

Surface Plasmon Resonance—Surface plasmon resonance analyses were performed on a Biacore T100 and data evaluated using BIAEvaluation version 1.1 (Biacore, Uppsala, Sweden). Recombinant FhbB proteins (ligand) were immobilized to NiCl₂-charged NTA chips via their N-terminal His₆ tag. Low levels of immobilized protein (~40 response units) were used to eliminate protein leaching and bulk contributions. Increasing concentrations of FH were passed over the chips in the fluid phase. Kinetic analyses were performed in NTA running buffer (0.01 m HEPES, 0.15 m NaCl, 50 μM EDTA, and 0.05% Tween 20 adjusted to pH 7.4). Nickel was immobilized to the sensor chip with NTA running buffer supplemented with 0.5 mM NiCl₂ for 60 s at a rate of 10 μl/min. FH was extensively diazylized in running buffer and diluted from 2.5 to 0.3125 μM. FH was passed over the immobilized FhbB protein for 30 s at 60 μl/min and allowed 600 s for dissociation. Flow cells were regenerated with running buffer supplemented with 0.35 M EDTA at pH 8.3 for 180 s at 30 μl/min and washed with running buffer under the same conditions. Data were fit to a Langmuir 1:1 binding model and averaged from three replicate experiments.

Analytical Ultracentrifugation—Sedimentation velocity experiments were performed in an XL-I analytical ultracentrifuge (Beckman Coulter), using adsorption optics at 280 nm and Rayleigh interference optics. Recombinant FhbB (3–821) was incubated with increasing concentrations of SOS (0–50 mM) in PBST for 1 h with constant gentle agitation. FH was overlaid with the FH/SOS solution for 1 h at RT. After 1 h, the FH was removed and binding was detected as described above. Data were averaged and normalized to FH binding with no SOS added.

**RESULTS**

**FhbB Crystal Structure at 1.7 Å Resolution**—To determine the structure of the *T. denticola* FhbB protein (GenBank™ accession number EF032156), recombinant protein was generated. The leader peptide (residues 1–23) was replaced with a 1.7-kDa hexahistidine tag and enterokinase cleavage site. The crystallization conditions have been previously reported (27). Primary phasing was obtained by selenomethionyl single wavelength anomalous diffraction using a single derivatized crystal. The native structure was solved at 1.7 Å resolution by molecular replacement using the selenomethionine model as the search probe. Two molecules were present in the asymmetric unit with a root mean square deviation of 1.47 Å. Exclusion of the N-terminal six residues of FhbB, which exist in two conformations, resulted in a root mean square deviation of 0.347 Å. The structural model for residues 24–101 is in good agreement with the experimental data and conforms to expected geometric parameters (Table 1). The FhbB structure is highly ordered throughout the temperature range of 4–90 °C (Fig. 2, A and B). The α1-α2 and β1-α3-β2 faces of FhbB are connected by turns 1 and 2 that span residues 47–52 and 77–88, respectively. The atomic interactions that occur between these turns are indicated in Fig. 1C. The core of the protein is largely defined by hydrophobic interactions (Fig. 1D) with a single hydrogen bond between residues His-34 and Tyr-72. Several C-terminal residues of FhbB are constituents of the core of the protein. DALI structural alignment analyses confirmed that the protein fold of FhbB is unique. The *Thermoplasma acidophilum* trehalose-6-phosphate phosphatase-related protein, a functionally unrelated protein, shared the highest homology (Z score = 4.2). To assess the stability of this unique fold, thermal denaturation-renaturation circular dichroism studies were performed. The CD spectrum of FhbB was measured at 4 °C before and after heating to 90 °C (Fig. 2A). Lysozyme served as a control (Fig. 2C). α-Helical content was measured at 222 nm over a temperature range of 4–90 °C (Fig. 2, B and D). The helical content of FhbB decreased only slightly at 90 °C and was fully restored upon cooling. In contrast, a large and irreversible loss of helical content was observed for lysozyme. These analyses demonstrate that FhbB is a highly thermostable protein.

**FhbB Forms a Weak Dimer**—The FhbB asymmetric unit contains two subunits with an interface occurring along the α2-helices in an anti-parallel alignment (Fig. 3A). The dimer contains an extensive water network at its dimeric interface with no direct hydrogen bonding between the subunits (Fig. 3, A–C). To determine the subunit affinity, analytical ultracentrifugation

### TABLE 1

| Data collection, phasing, and refinement statistics | Native | Selenomethionine |
|---------------------------------------------------|--------|-----------------|
| Space group                                       | P4₃₂₂ | P4₂₂₂           |
| Cell dimensions                                   | a, b, and c | 46.98, 46.98, 168.95 Å | 46.99, 46.99, 168.96 Å |
| a, β, and γ                                       | 90°    | 90°             |
| Wavelength                                        | 1.5    | 0.9793          |
| Resolution                                        | 40 to 1.7 Å | 50 to 1.77      |
| Rmerge (%)                                        | 0.06% (0.41) | 0.07 (0.28)    |
| I/σ(I)                                           | 44 (3) | 20.8 (7.4)      |
| Completeness                                      | 99.6% (100%) | 99.4% (99.9%)  |
| Redundancy                                        | 9.8% (7%) | 9.3 (8.7%)      |
| Refinement                                        | 36 to 1.7 Å | 45.2 to 1.77 Å |
| No. of reflections                                | 21,797 | 19,035          |
| Rmerge/Fmerge                                     | 21/23.6 | 18.9/20.6       |
| No. of atoms                                      | 1,222  | 1,252           |
| Protein                                           | 6      | 12              |
| Water                                             | 117    | 152             |
| B-factors                                         | 21.4   | 23.1            |
| Protein                                           | 19.7   | 31.3            |
| Water                                             | 28.69  | 33.2            |
| Root mean square deviation                        | 0.007 Å | 0.006 Å        |
| Bond angles                                       | 0.984° | 1.038°         |

A single crystal was used to collect each dataset.

* Values in parentheses are for highest resolution shell: native, 1.73 to 1.7; selenomethionine, 1.83 to 1.77.
was performed (Fig. 3D). As FhbB concentration was increased, the sedimentation coefficient shifted from ~1.31 S to ~2.15 S (monomer to dimer transition). The data conform well to a monomer-homodimer model (root mean square deviation of 0.005 fringes). The dissociation constant for the dimer was calculated to be 2×10^{-4} M indicative of a relatively weak interaction.

**Mutagenesis Studies of the FhbB/FH Binding Interface**—To identify FhbB residues involved in FH binding, solvent-accessible charged amino acids were substituted to yield recombinant proteins with single or double amino acid substitutions. The spatial placement of the targeted residues is indicated in Fig. 4A. Circular dichroism analyses revealed that the substitutions did not significantly alter secondary structure as no change in percent \( \alpha \)-helical content was observed (data not shown). FH binding to the substituted proteins was assessed using ELISA-based and membrane overlay assay approaches (Fig. 4, B and C). Alanine substitution of Glu-45, Lys-47, His-50, Asp-58, Glu-62,
values for select FhbB mutants that displayed attenuated FH binding were also assessed. With the E66A FhbB mutant, the $K_D$ increased to 2.97 ± 0.24 μM. No measurable binding of the E45A, D58A, and E62A substitution mutants to FH was detected, and therefore binding constants could not be determined. The association ($k_+$) and dissociation ($k_−$) constants of the FH/FhbB interaction were determined to be 7.56 ± 0.26 × 10^4 M^{-1}s^{-1} and 0.11 ± 0.01 s^{-1}, respectively. The off-rate is significantly faster than that calculated for the interaction of FH with other bacterially produced FH-binding proteins (25, 26, 42). As discussed in detail below, the biological role that FH binding plays in T. denticola pathogenesis may be facilitated by the rapid off kinetics of the FhbB/FH interaction.

**FhbB Binds to a Glycosaminoglycan-binding (GAG) Site within FH CCP7**—In a previous study (20), FhbB bound to a FH fragment consisting of CCP1–7 (designated as the H7 fragment) but not to the H7AB construct that has four amino acid substitutions within CCP7. These data and competitive binding studies with heparin, a GAG that binds within CCP7 and CCP19–20 (43), implicated CCP7 as the FhbB interaction site (20). To test this, recombinant FH proteins consisting of CCP6–8 or CCP19–20 (negative control) were generated. Binding was assessed using membrane overlay assays. Immobilized FhbB bound to the CCP6–8 construct but did not bind to the CCP19–20 construct (Fig. 5A). The Borrelia hermsii FhBA protein, which binds to CCP19–20 (44), and the B. burgdorferi BBK32 protein, a fibronectin-binding protein that does not bind to FH, served as controls (45). To further validate the FhbB/CCP6–8 interaction, competitive binding analyses were conducted using SOS. SOS is a GAG analog that interacts with a large positively charged groove within CCP6–8 (46). SOS inhibited FhbB binding to FH in a dose-dependent manner (Fig. 5B) suggesting overlapping binding sites within the CCP6–8 GAG-binding groove of FH.

**DISCUSSION**

Some bacterial pathogens evade complement-mediated destruction by binding FH, a negative regulator of the complement cascade (47). Although several bacterial binding proteins have been identified (17), the molecular basis of their interaction with FH is poorly defined. Identification of the minimal common determinants required for FH binding requires that structures of a diverse set of FH-binding proteins be solved. Prior to this report, atomic structures have been determined for only two bacterial FH-binding proteins: CspA (BBA68 or BbCRASP-1) of B. burgdorferi (25) and fHbp of N. meningitidis (26). Here, we report the structure of the FhbB protein of the periopathogen, T. denticola, and investigate the molecular basis of its interaction with FH.

The atomic structure of FhbB was determined at high resolution (1.7 Å). The protein has an α-α-α-β-α-β organization with well defined negatively (α1-α2) and positively charged (β1-α3-β2) faces. The tertiary fold, defined by extensive hydrophobic interactions within the protein core, imparts high thermostability to FhbB. Only minimal denaturation of FhbB, as assessed by circular dichroism, was observed after heating to 90 °C. DALI analyses revealed that the FhbB fold is unique, lacking structural homology with all other known FH-binding protein structures.
The asymmetric unit of the FhbB crystal consisted of a dimer. The surface area at the dimeric interface is low (~1000 Å²), and the interface is filled with solvent molecules. In addition, there are no hydrogen bonds between the monomers and minimal van der Waals interactions. The dissociation constant of the dimer is high (~217 μM) with dimerization occurring only at nonphysiological protein concentrations (~100 μM). Collectively, these properties are indicative of a weak dimer. It can be concluded that FhbB is a structurally novel, highly stable protein that likely functions in vivo as a monomer.

Using site-directed mutagenesis, residues of FhbB involved in the FH/FhbB interaction were identified. Alanine substitution mutagenesis of surface-exposed residues (Glu-45, Asp-58, Glu-62, and Glu-66) on the α1-α2 face of FhbB eliminated or attenuated FH binding, whereas substitution of residues on the opposite face of the protein (Lys-77, Lys-78, Arg-93, and Lys-94) had no effect on binding. Although mutational analyses of FH were not conducted as part of this study, in an earlier analysis we demonstrated that specific residues within CCP7 contributed to FhbB binding. A recombinant CCP1–7 construct harboring alanine substitutions at residues Arg-387, Lys-388, Arg-404, and Lys-405, which are contained within CCP7(43), lost FhbB binding ability (20). Additional support for the involvement of Lys–405 in FhbB binding comes from FhbB-SOS competitive binding analyses. SOS inhibits the FH/FhbB interaction in a dose-dependent manner. Although SOS binds to at least three sites on FH, co-crystallization of SOS with a...
CCP6–8 construct revealed that SOS hydrogen bonds with K405FH \( \text{[46]} \).

The site-directed mutational analyses presented here, and in an earlier report \( \text{[20]} \), suggest that presentation of an electrostatic environment on the \( \alpha_1-\alpha_2 \) face of FhbB that is conducive to FH binding is dependent on intramolecular interactions within FhbB. Substitution analyses indicate that intramolecular hydrogen bonds between Asn-44–Lys-47 \( \{\alpha_1\text{-turn} \ 1\} \), Glu-45–Arg-49 \( \{\alpha_1\text{-turn} \ 1\} \), and His-50–Gln-85 \( \{\text{turn} \ 1\text{-turn} \ 2\} \) are important for FH binding. Other FhbB residues that have been demonstrated to influence FH binding include Ile-63, Leu-68, Phe-96, and the C-terminal seven amino acids \( \{23\} \). The placement of these residues on the FhbB structure suggests that their influence on FH binding is indirect as they are not surface-exposed. Loss of FH binding by an I63T substitution mutant most likely results from aberrant hydrophobic packing within \( \alpha_2 \). A Phe-96 substitution mutant and C-terminal 7-amino acid truncation mutant have also been shown to lack FH-binding ability \( \{23\} \). The structure of FhbB indicates that several C-terminal residues, including Phe-96, reside within the hydrophobic core of the protein forming a two-stranded \( \beta \)-sheet. C-terminal deletions or substitutions may disrupt FH binding by altering the core structure of the protein. An intact C-terminal domain has been demonstrated to be required for FH binding by other spirochetal FH-binding proteins \( \{44, 48, 49\} \).

As discussed above, a unique aspect of the FH/T. denticola interaction is the cleavage of FH by dentilisin. As the \( T. \ denticola \) population expands in periodontal pockets, FhbB-dentilisin-dependent cleavage of FH may deplete FH present in gingival crevicular fluid. FH depletion would dysregulate complement activation in the subgingival crevice. Decay of the C3bBb convertase complex and factor I-mediated cleavage of C3b would be inhibited resulting in C3b accumulation and deposition on host cell surfaces. This would disrupt normal self-recognition mechanisms resulting in destruction of the periodontium. Complement regulation mediated by CRP, which is elevated in crevicular fluid of periodontal disease patients \( \{50\} \), would also be affected. Unchecked complement activation by CRP would lead to activation of the C5 convertase complex and membrane attack complex formation. The FH-CRP complex also plays an important role in regulating pro-inflammatory cytokine production and in facilitating clearance of apoptotic and damaged cells by macrophages \( \{51\} \). In a FH limited microenvironment, clearance of damaged cells would be impaired, and pro-inflammatory cytokine production would be elevated resulting in inflammation and tissue destruction.

In summary, determination of the FhbB structure identified a unique fold that imparts high stability to the protein and presents distinct positive and negatively charged surfaces. Mutagenesis analyses demonstrated the negatively charged face of FhbB interacts with FH. It can be concluded that the basis of the FH/FhbB interaction is electrostatic. The inhibition of the FH/FhbB interaction by the GAG analog, SOS, is consistent with the primary interaction site for FhbB being within CCP7 of FH. Binding and kinetic analysis revealed that the FH/FhbB interaction is low affinity with a rapid off-rate. The kinetics of the interaction would favor rapid turnover and are consistent with the hypothesis that \( T. \ denticola \) may dysregulate complement control by depleting FH present in crevicular fluid leading to conditions that promote the progression of periodontal disease.

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REFERENCES
1. Krauss, J. L., Potempa, J., Lambiris, J. D., and Hajishengallis, G. (2010) Complement tolls in the periodontium. How periodontal bacteria modify complement and Toll-like receptor responses to prevail in the host. Periodontol. 2000 \textbf{52}, 141–162
2. Darveau, R. P. (2010) Periodontitis. A polymicrobial disruption of host homeostasis. \textit{Nat. Rev. Microbiol.} \textbf{8}, 481–490
3. Van Dyke, T. E., and Serban, C. N. (2003) Resolution of inflammation. A new paradigm for the pathogenesis of periodontal diseases. \textit{J. Dent. Res.} \textbf{82}, 82–90
4. Paster, B. I., Olsen, L, Aas, J. A., and Dewhirst, F. E. (2006) The breadth of bacterial diversity in the human periodontal pocket and other oral sites. \textit{Periodontology} \textbf{2000} \textbf{42}, 80–87
5. Paster, B. I., Boches, S. K., Galvin, J. L., Ericson, R. E., Lau, C. N., Levanos, V. A., Sahasrabudhe, A., and Dewhirst, F. E. (2001) Bacterial diversity in human subgingival plaque. \textit{J. Bacteriol.} \textbf{183}, 3770–3783
6. Ellen, R. P., and Galimanas, V. B. (2005) Spirochetes at the forefront of periodontal infections. \textit{Periodontology} \textbf{2000} \textbf{38}, 13–32
7. Dewhirst, F. E., Tamer, M. A., Ericson, R. E., Lau, C. N., Levanos, V. A., Boches, S. K., Galvin, J. L., and Paster, B. J. (2000) The diversity of periodontal spirochetes by 16S rRNA analysis. \textit{Oral Microbiol. Immunol.} \textbf{15}, 196–202
8. Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., and Kent, R. L., Jr (1998) Microbial complexes in subgingival plaque. J. Clin. Periodontol. \textbf{25}, 134–144
9. Boackle, R. J. (1991) The interaction of salivary secretions with the human complement system. A model for the study of host defense systems on inflamed mucosal surfaces. \textit{Crit. Rev. Oral Biol. Med.} \textbf{2}, 355–367
10. Boackle, R. J., Pruitt, K. M., Silverman, M. S., and Gymph, J. L., Jr. (1978) The effects of human saliva on the hemolytic activity of complement. J. Dent. Res. \textbf{57}, 103–110
11. Schenkein, H. A. (1991) The role of complement in periodontal diseases. \textit{Crit. Rev. Oral. Biol. Med.} \textbf{2}, 65–81
12. Schenkein, H. A., and Genco, R. J. (1977) Gingival fluid and serum in periodontal diseases. I. Quantitative study of immunoglobulins, complement components, and other plasma proteins. \textit{J. Periodontol.} \textbf{48}, 772–777
13. Ruddy, S., and Austen, K. F. (1971) C3b inactivator of man. II. Fragments produced by C3b inactivator cleavage of cell-bound or fluid phase C3b. J. Immunol. \textbf{107}, 742–750
14. Zipfel, P. F., and Skerka, C. (2009) Complement regulators and inhibitory proteins. \textit{Nat. Rev. Immunol.} \textbf{9}, 729–740
15. Zipfel, P. F., Skerka, C., Hellwage, J., Jokiranta, S. T., Meri, S., Brade, V., Kraiczy, P., Noris, M., and Remuzzi, G. (2002) Factor H family proteins. On complement, microbes, and human diseases. \textit{Biochim. Soc. Trans.} \textbf{30}, 971–978
16. Jarvis, H., Jokiranta, T. S., Hellwage, J., Zipfel, P. F., and Meri, S. (1999) Regulation of complement activation by C-reactive protein. Targeting the complement inhibitory activity of factor H by an interaction with short consensus repeat domains 7 and 8–11. \textit{J. Immunol.} \textbf{163}, 3957–3962
17. Ferreira, V. P., Pangburn, M. K., and Cortés, C. (2010) Complement control protein factor H. The good, the bad, and the inadequate. Mol. Immunol. \textbf{47}, 2187–2197
18. Schmidt, C. Q., Herbert, A. P., Hocking, H. G., Uhrin, D., and Barlow, P. N. (2008) Translational mini-review series on complement factor H. Structural and functional correlations for factor H. \textit{Clin. Exp. Immunol.} \textbf{151}, 86–91
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14–24

19. Holers, V. M. (2008) The spectrum of complement alternative pathway-mediated diseases. *Immunol. Rev.* **223**, 300–316

20. McDowell, J. V., Lankford, J., Stamm, L., Sadlon, T., Gordon, D. L., and Marconi, R. T. (2005) Demonstration of factor H-like protein 1 binding to *Treponema denticola*, a pathogen associated with periodontal disease in humans. * Infect. Immun.* **73**, 7126–7132

21. McDowell, J. V., Frederick, J., Stamm, L., and Marconi, R. T. (2007) Identification of the gene encoding the FhbB protein of *Treponema denticola*, a highly unique factor H-like protein 1-binding protein. * Infect. Immun.* **75**, 1050–1054

22. McDowell, J. V., Frederick, J., Miller, D. P., Goetting-Minesky, M. P., Goodman, H., Fenno, J. C., and Marconi, R. T. (2011) Identification of the primary mechanism of complement evasion by the periodontal pathogen, *Treponema denticola*. * Mol. Oral Microbiol.* **26**, 140–149

23. McDowell, J. V., Huang, B., Fenno, J. C., and Marconi, R. T. (2009) Analysis of a unique interaction between the complement regulatory protein factor H and the periodontal pathogen *Treponema denticola*. * Infect. Immun.* **77**, 1417–1425

24. Uitto, V. J., Grenier, D., Chan, E. C., and McBride, B. C. (1988) Isolation of *Treponema denticola* surface protein E coiled coil structural domains and higher order structural elements in the binding of infection-induced antibody and the complement-regulatory protein, factor H. *J. Biol. Chem.* **263**, 21321–21326

25. Emsley, P., and Cowtan, K. (2004) Coot. Model-building tools for molecular graphics. * Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132

26. Hetherington, M., and Minor, W. (1997) Processing of x-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276**, 307–326

27. Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. I., Richardson, D. C., Richardson, J. S., Terwilliger, T. C., and Zwart, P. H. (2010) PHENIX. A comprehensive Python-based system for macromolecular structure solution. * Acta Crystallogr. D Biol. Crystallogr.* **66**, 213–221

28. McDowell, J. V., Wolfgang, J., Senty, L., Sundy, C. M., Noto, M. J., and Marconi, R. T. (2006) Molecular analyses of the interaction of *Treponema denticola* FhbB with the complement regulatory proteins factor H and factor H-like protein 1. * Infect. Immun.* **74**, 2007–2014

29. McDowell, J. V., Lankford, J., Stamm, L., Sadlon, T., Gordon, D. L., and Marconi, R. T. (2005) Demonstration of factor H-binding protein BbCRASP-1 of *Borrelia hermsii*-associated complement regulator-acquiring surface protein for factor H and plasminogen discloses a putative virulence factor of relapsing fever spirochetes. *Nat. Immunol.* **17**, 7292–7310

30. Seshu, J., Esteve-Gassent, M. D., Labandeira-Rey, M., Kim, J. H., Trzacikowski, J. P., Höök, M., and Skare, J. T. (2006) Inactivation of the fibronectin-binding adhesin gene bbb32 significantly attenuates the infectivity potential of *Borrelia burgdorferi*. * Mol. Microbiol.* **59**, 1591–1601

31. McDowell, J. V., Wolfgang, J., Senty, L., Sundy, C. M., Noto, M. J., and Marconi, R. T. (2005) Demonstration of the involvement of outer surface protein E coiled coil structural domains and higher order structural elements in the binding of infection-induced antibody and the complement-regulatory protein, factor H. *J. Immunol.* **173**, 7471–7480

32. Gomes-Filho, I. S., Freitas Coelho, J. M., da Cruz, S. S., Passos, J. S., Teixeira de Freitas, C. O., Aragão Farias, N. S., Amorim da Silva, R., Silva Pereira, M. N., Lima, T. L., and Barreto, M. L. (2011) Chronic periodontitis and C-reactive protein levels. * J. Periodontol.* **82**, 969–978

33. Kang, Y. H., Urban, B. C., Sim, R. B., and Kishore, U. (2011) *Immunobiology* **217**, 455–464