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Outer Membrane Protein DsrA Is the Major Fibronectin-Binding Determinant of *Haemophilus ducreyi*▽

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The ability to bind extracellular matrix proteins is a critical virulence determinant for skin pathogens. *Haemophilus ducreyi*, the etiological agent of the genital ulcer disease chancroid, binds extracellular matrix components, including fibronectin (FN). We investigated *H. ducreyi* FN binding and report several important findings about this interaction. First, FN binding by *H. ducreyi* was greatly increased in bacteria grown on heme and almost completely inhibited by hemoglobin. Second, wild-type strain 35000HP bound significantly more FN than did a *dsrA* mutant in two different FN binding assays. Third, the expression of *dsrA* in the *dsrA* mutant restored FN binding and conferred the ability to bind FN to a non-FN-binding *Haemophilus influenzae* strain. Fourth, an anti-DsrA monoclonal antibody partially blocked FN binding by *H. ducreyi*. The hemoglobin receptor, the collagen-binding protein, the *H. ducreyi* lectin, the fine-tangle pili, and the outer membrane protein OmpA2 were not involved in *H. ducreyi* FN binding, since single mutants bound FN as well as the parent strain did. However, the major outer membrane protein may have a minor role in FN binding by *H. ducreyi*, since a double *dsrA momp* mutant bound less FN than did the single *dsrA* mutant. Finally, despite major sequence differences, DsrA proteins from both class I and class II *H. ducreyi* strains mediated FN and vitronectin binding. We concluded that DsrA is the major factor involved in FN binding by both classes of *H. ducreyi* strains.

Haemophilus ducreyi is the cause of the sexually transmitted ulcer disease chancroid (3, 32, 33, 39, 46). *H. ducreyi* is thought to initiate infection by entering the skin through small abrasions acquired during sexual intercourse (3, 33, 41). Thus, attachment of *H. ducreyi* to extracellular matrix (ECM) proteins of the skin may be an important initial step in the infection process, as shown for other bacterial skin infections (8, 15, 37).

Little is known about the mechanisms employed by *H. ducreyi* in the first critical steps of attaching to host skin. In vitro, *H. ducreyi* binds to fibrinogen, fibronectin (FN), type I and III collagen, gelatin, and laminin, but not elastin (2, 10). FN may have a role in the interaction of *H. ducreyi* with foreskin fibroblasts (4). During the later pustule stages of human experimental chancroid infection, *H. ducreyi* localizes with inflammatory infiltrates present in pustules and colocalizes with collagen and fibrin (9, 11). In naturally acquired chancroid, where patients are seen mostly at the last ulcerative stage, *H. ducreyi* colocalizes with neutrophils and fibrin (12). These data suggest that ECM binding by *H. ducreyi* may contribute to chancroid pathogenesis.

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Bacterial Oca (*o*ligomeric *c*oiled *a*dhesion) proteins (25), also referred to as trimeric autotransporters (17), comprise a multifunctional family with demonstrated roles in binding to ECM proteins, binding to various eukaryotic cells, mediating invasion of cells, and resistance to killing by serum complement (21). Oca proteins are expressed on the bacterial surface of the cell as homotrimers exhibiting a lollipop shape, with head, neck, and stalk structures (21, 25). Different Oca family members share similarity in the C-terminal domain, the region that is believed to traverse and anchor the protein in the bacterial outer membrane (25). However, Oca N-terminal domains vary widely, even among family members expressed by closely related species or within a species (25).

H. ducreyi expresses two Oca proteins, namely, DsrA (*d*ucreyi *s*erum *r*esistance *A*) and NcaA (*n*ecessary for *c*ollagen *a*dhesion *A*). Both were identified by their C-terminal similarity to the prototypical Oca family protein YadA. DsrA was first described as critical for high-level resistance to killing by normal human serum (20) and was later shown to be an adhesin for HaCat keratinocytes and to bind to vitronectin (VN) (16). DsrA is also essential for *H. ducreyi* infection in the human challenge model of chancroid (13). NcaA mediates type I collagen binding by *H. ducreyi* and is also important for virulence of *H. ducreyi* in both the porcine and human models of chancroid (22).

Recently, we identified two classes of *H. ducreyi* strains, classes I and II (47), based on the presence of variant forms of several outer membrane components, including DsrA, NcaA, DltA (*d*ucreyi *l*ec*t*in *A*), and lipooligosaccharide (LOS). The DsrA proteins from the two classes of *H. ducreyi* strains have almost identical C-terminal domains, sharing 88.5% identity in the last 86 amino acids. However, these proteins have diver-

TABLE 1. Bacterial strains used in this study

Strain	Description or genotype	Source or reference
H. ducreyi strains		
35000HP	wt; human-passaged (HP) variant of strain 35000HP	7, 23
FX504	35000HP *hgbA*::CAT	19
FX517	35000HP *dsrA*::CAT	20
FX521	35000HP *ncaA*::Kan	This study
FX533	35000HP *dltA*::Kan	31
FX534	35000HP *dsrA*::CAT *dltA*::Kan	31
FX536	35000HP *ncaA*::Kan *dsrA*::CAT	This study
FX538	35000HP*dsrA*::Kan	This study
35000HP-SMS1	35000HP *ftpA*::mTn*3* (Cm)	5
FX541	35000HP *ftpA*::mTn*3* (Cm) *dsrA*::CAT	This study
35000HP-SMS2	35000HP *momp*::ΩKan2	44
FX544	35000HP *momp*::ΩKan2 *dsrA*::CAT	This study
35000HP-SMS3	35000HP *ompA2*::ΩKan2	This study
FX545	35000HP *ompA2*::ΩKan2 *dsrA*::CAT	This study
HMC21 (V-1168)	wt strain from Seattle, WA	18
FX528	HMC21 *dsrA*::CAT	This study
HMC50 (010-2)	wt strain from Jackson, MS	18
FX530	HMC50 *dsrA*::CAT	1
HMC54 (425)	wt strain from Dominican Republic	18
FX529	HMC54 *dsrA*::CAT	1
H. influenzae strain		
KW20 Rd	Host for *H. ducreyi* clones	American Type Culture Collection

TABLE 2. Plasmids used in this study

Plasmid	Description or relevant genotype and/or phenotype	Source or reference
pCR2.1-TOPO	PCR cloning vector; Kan^r Amp^r	Invitrogen
pUC4K	Source of Kan^r cassette	42, 43
pNC40	Source of CAT cassette	19
pLSKS	*H. ducreyi* shuttle vector; Sm^r	48
pRSM1791	Mutagenesis plasmid; β-galactosidase positive; Amp^r	14
pUNCH1256	*dsrA* class I from 35000HP with CAT cassette in pRSM1791	20
pUNCH1260	Complete *dsrA* class I gene from 35000HP in pLSKS	20
pUNCH1272	*ncaA* PCR clone in pCR2.1-TOPO	This study
pUNCH1274	*ncaA* with CAT cassette in PCR2.1-TOPO	This study
pUNCH1275	*ncaA* with CAT cassette in pRSM1791	This study
pUNCH1286	Complete *dltA* class I gene from 35000HP in pLSKS	31
pUNCH1296	Complete *dsrA* class II gene from CIP 542 in pLSKS	47
pUNCH1297	*ncaA* with Kan cassette in pCR2.1-TOPO	This study
pUNCH1298	*ncaA* with Kan cassette in pRSM1791	This study
pUNCH1299	*dsrA* with Kan cassette insert in pLSKS	This study
pUNCH1401	*dsrA* with Kan cassette insert in pRSM1791	This study
pUNCH1405	*dsrA* with Kan cassette insert in pRSM1791	This study

gent N-terminal domains, sharing little amino acid sequence similarity (47).

FNs are high-molecular-weight disulfide-linked glycoprotein dimers found in both soluble and insoluble forms in human blood and skin. The modular structure of FN promotes its involvement in cell adhesion by binding to host cell integrins and a plethora of other molecules present in the ECM and in the blood (27, 34). Bacteria express MSCRAMMs (*m*icrobial *s*urface *c*omponents *r*ecognizing *a*dhesive *m*atrix *m*olecules), adhesins that bind ECM proteins of the host, such as FN (29). FN binding by MSCRAMMS has been studied widely in the gram-positive genera *Staphylococcus* and *Streptococcus* (38), and recent reports document the presence of such adhesins in a broad variety of diverse genera, including gram-negative bacteria and mycobacterial species (29). FN binding by bacteria occurs via different bacterial components (38) and is redundant in many microorganisms, which may indicate the importance of this function in bacterial pathogenesis.

Given the importance of FN binding in other bacterial systems, the aim of this study was to investigate the role of DsrA in FN binding by *H. ducreyi*.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Bacterial strains and plasmids used in this study are shown in Tables 1 and 2, respectively. *H. ducreyi* strains were routinely maintained by minimal subculture (no more than five passages) on heme agar containing 50 μg/μl of hemin and 1× GGC (0.1% glucose, 0.01% glutamine, 0.026% cysteine) (45) or 1% IsoVitaleX (Becton Dickinson, NJ) and incubated at 34.5°C in 5% CO_2. Preliminary FN binding experiments (Fig. 1) and construction of mutants were conducted with bacteria grown on chocolate agar (CA) plates supplemented with 5% FetalPlex (a fetal bovine serum substitute; Gemini, CA) and 1× GGC. The following antibiotics were used when appropriate: 1 μg/ml chloramphenicol (Cm), 100 μg/ml kanamycin (Kan), and 100 μg/ml streptomycin (Sm). *Haemophilus influenzae* strain KW20 Rd clones expressing *H. ducreyi* genes from plasmid pLSKS were routinely maintained on heme plates containing 50 μg/μl of hemin, 1% IsoVitaleX, and 100 μg/ml of Sm.

Construction of *H. ducreyi* mutants. (i) Construction of 35000HP *ncaA* (FX521). To construct the single *ncaA* mutant FX521, PCR primers *ncaA*.01 (GAATTATTTTAAGCAATTTTTTTGC) and *ncaA*.05 (TTATTGAAAATTA TATACAAAGCCTACACC) were used to amplify the *ncaA* locus, using crude chromosomal DNA from 35000HP as a template and the following amplification parameters: a single denaturation step of 1 min at 95°C and 30 amplification cycles, each consisting of a 1-min denaturation at 95°C, annealing at 52°C for 1 min, and extension at 72°C for 2 min. The approximately 1-kb amplified product was ligated into the pCR 2.1 TOPO vector (Invitrogen, Carlsbad, CA) to form pUNCH1272. pUNCH1272 was digested with ClaI, made blunt-ended with T4 DNA polymerase, and ligated to a BglII- and Klenow-treated chloramphenicol acetyltransferase (CAT) cassette from pNC40 (19) to form pUNCH1274. pUNCH1274 was digested with SacI and EcoRV to obtain a 2.0-kb insert containing the mutagenized *ncaA* locus. The latter insert was treated with Klenow and ligated to NotI- and Klenow-treated mutagenesis plasmid pRSM1791 (14) to form pUNCH1275. Finally, *H. ducreyi* strain 35000HP was electroporated with 1 μg of pUNCH1275, with selection on CA containing 1 μg/ml Cm (14). Cointegrate colonies were resolved by being streaked heavily onto CA containing 1 μg/ml Cm and 40 μg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and white colonies were isolated and confirmed (see below).

(ii) Construction of 35000HP *ompA2* (35000HP-SMS3). A promoterless *ompA2* open reading frame (ORF) was amplified by PCR, using DNA from *H. ducreyi* strain 35000HP and the primers OmpA2-for-5 (5′-TGGATTAGCGCG TAACGATTATAGTG-3′) and OmpA2-rev-5 (5′-GCTCGTCCATCATCATT TGCG-3′). A Ω-Km2 cassette (44) was blunt end ligated into a BssHII site located 775 bp downstream of the start codon of the *ompA2* ORF. The insert was ligated into pRSM1791 and electroporated into 35000HP. Cointegrate colonies were resolved as described above, and an *ompA2* mutant, designated 35000HP-SMS3, was recovered. Southern blot and PCR analyses confirmed that the Ω-Km2 cassette had been inserted into the *ompA2* ORF (data not shown).

(iii) Construction of 35000HP *dsrA ncaA* (FX536). pUNCH1272 was digested with ClaI, treated with Klenow, and ligated to an EcoRI- and Klenow-treated Kan cassette from pUC4K (42, 43) to form pUNCH1297. The latter plasmid was thereafter digested with SpeI and NotI, treated with Klenow, and ligated into pRSM1791 to form pUNCH1298. To obtain FX536, strain 35000HP *dsrA* (FX517) was electroporated with 1 μg of pUNCH1298, and colonies were screened as described above for strain 35000HP *ncaA*.

(iv) Construction of a *dsrA* mutant of *H. ducreyi* strain 35000HP with a Kan cassette insertion (FX538). The strain 35000HP *dsrA* (FX517) contains the Cm cassette inserted into the *dsrA* gene (20). However, the single mutant 35000HP *ftpA* (35000HP-SMS1) already had this antibiotic marker inserted into the *ftpA* (fine-tangle pilus) (5) gene. Thus, in order to construct a double mutant by using strain 35000HP *ftpA* as a recipient, a single *dsrA* mutant with a different antibiotic cassette insertion in the *dsrA* gene was constructed in *H. ducreyi* strain 35000HP. To do so, pUNCH1260 (20), containing the complete *dsrA* ORF, was digested with NdeI, Klenow treated, and ligated to an EcoRI- and Klenow-treated Kan cassette from pUC4K to form pUNCH1299. The latter plasmid was thereafter

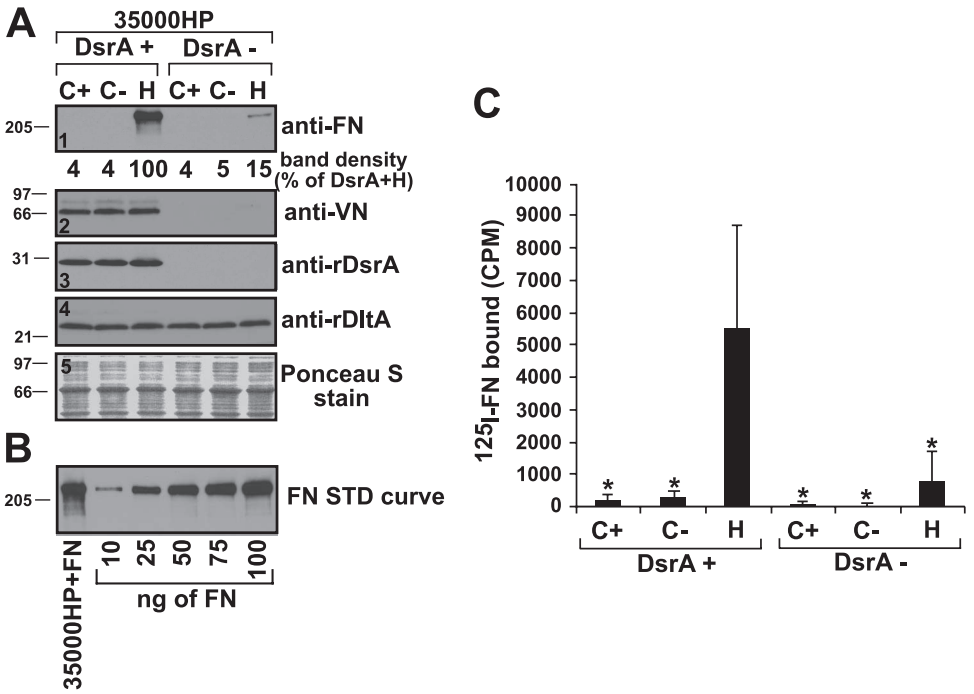

FIG. 1. *H. ducreyi* binding to FN, but not to VN, is increased upon growth on heme agar. (A) Unlabeled FN binding assay. Suspensions of *H. ducreyi* bacterial cells, grown on the indicated media, were mixed with purified FN (panels 1, 3, 4, and 5, first lane) or heat-inactivated normal human serum (source of VN) (panel 2) for 30 min. After being washed to remove unbound ligands, bacterial cells were solubilized in Laemmli sample buffer and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting to detect the bound ligands, as indicated. The experiment presented is representative of at least three other similar experiments. Ponceau S staining (panel 5) of the nitrocellulose membrane indicated approximately equal loading of total proteins for all strains. (B) FN standard curve. Lane 1, strain 35000HP incubated with FN as described for panel A; lanes 2 to 6, FN standard curve using the indicated amounts of purified FN loaded directly into wells. (C) ^{125}I-labeled FN binding assay. Suspensions of *H. ducreyi* were mixed with purified ^{125}I-labeled FN. After incubation and washing, bacterially associated cpm were determined. Asterisks indicate statistically significant ($P < 0.01$) differences in FN binding compared to that obtained for the wt strain *H. ducreyi* 35000HP (DsrA+) grown on heme (H). C+, CA containing 5% FetalPlex; C−, CA without FetalPlex; H, gonococcal medium plates with 50 μg/μl hemin.

digested with ApaI and XbaI, treated with Klenow, and ligated into NotI- and Klenow-treated pRSM1791 to form pUNCH1401. To produce FX538, 1 μg of pUNCH1401 was electroporated into 35000HP. Mutants were obtained as described above for strain 35000HP *ncaA*, except that cointegrate colonies were plated on Kan-containing plates.

(v) Construction of 35000HP *dsrA ftpA* (FX541), 35000HP *dsrA momp* (FX544), and 35000HP *dsrA ompA* (FX545). Mutant strain 35000HP *dsrA ftpA* was obtained by PCR amplification of the mutated *dsrA* gene, using plasmid pUNCH1405 (Kan insertion in the *dsrA* gene in pRSM1791) as the template and the primers 301038-15 (5′-TGGACAGCATTCCACTAACAGTC-3′) and OPA 33 (5′-CATCGTCGAACGCACACTG-3′), using the following PCR conditions: one denaturation cycle at 94°C for 5 min and 30 amplification cycles, each consisting of a 1-min denaturation at 94°C, annealing at 50°C for 1 min, and extension at 72°C for 4 min. The purified PCR product was subsequently electroporated into the 35000HP *ftpA* mutant without prior cloning into the suicide vector pRSM1791. Colonies were thereafter selected on Cm- and Kan-containing plates.

Mutant strains 35000HP *dsrA momp* and 35000HP *dsrA ompA2* were obtained using the same method as that for strain 35000HP *dsrA ftpA*, except that plasmid pUNCH1256 (with the Cm antibiotic cassette in *dsrA*) was used as the template for a PCR with primers 301038-15 and OPA 33. The product obtained from the PCR was also directly electroporated into strains 35000HP *momp* (35000HP-SMS2) and 35000HP *ompA* (35000HP-SMS3) to produce the double mutant strains 35000HP *dsrA momp* and 35000HP *dsrA ompA2*, respectively.

(vi) Construction of HMC21 *dsrA* (FX528). The single *dsrA* mutant FX528 was constructed in *H. ducreyi* strain HMC21 exactly as the *dsrA* mutant strains FX529 and FX530 were, as previously described (1).

Confirmation of the genotypes and phenotypes of the *H. ducreyi* mutants. The *hgbA* (FX504) (6), *dsrA* (FX517) (13), *dltA* (FX533) (28), *ftpA* (35000HP-SMS1) (5), and *momp* (35000HP-SMS2) (44) mutants have been characterized extensively at the DNA and protein levels. As for the single *ncaA* (FX521) and *dsrA* (FX538 [Km insert]) mutants and all of the double *dsrA* mutants, PCR products obtained using whole-cell lysates as templates and specific primers were appropriately longer due to the presence of an antibiotic cassette inserted in the gene of interest (data not shown). Furthermore, each mutant lacked expression of the appropriate protein in a Western blot assay (Fig. 2, panels 2 to 4). However, outer membrane profiles of 35000HP and the 35000HP *ompA2* mutant were similar (data not shown). We were unable to demonstrate a loss of OmpA2 in the mutant via Western blotting due to the lack of a monoclonal antibody (MAb) that specifically recognizes OmpA2 and the fact that the major outer membrane protein (MOMP), which is expressed more abundantly than OmpA2, and OmpA2 comigrate (30). The serum resistance phenotypes for all double *dsrA* mutants were also evaluated and found to be similar to that of the single *dsrA* mutant (FX517), except for that of 35000HP *dsrA dltA*, which has been shown to be more serum susceptible than FX517 (31). All of the mutants used in this study grew well on heme agar, the medium used for the FN and VN binding assays described below.

Cloning of *momp*. The *momp* gene without its native promoter was PCR amplified using whole cells of strain 35000HP as the template and primers mompF (ATGGCTATCTAGAGGAGTATCAAAA) and mompR (GGGCTT AATCTAGAGACTGAAAAT). XbaI sites (underlined in primers) were included in the design of the primers. PCR was carried out under the following conditions: one denaturation cycle at 95°C for 5 min and 30 amplification cycles, each consisting of a 1-min denaturation at 95°C, annealing at 42°C for 1 min, and extension at 72°C for 2 min, followed by 1 cycle at 72°C for 1 min. The PCR products and the shuttle vector pLSKS (48) were treated with XbaI, and XbaI-treated pLSKS was subsequently treated with shrimp alkaline phosphatase (New England Biolabs, MA) to prevent religation. The restriction enzyme-treated PCR products and plasmid were ligated at room temperature for 30 min, and 1 μl of the ligation reaction mix was transformed into *Escherichia coli* DH5α and

electroporated into *H. influenzae* strain KW20 Rd and the *H. ducreyi dsrA momp* mutant. Clones were selected on Sm-containing plates.

The *momp* gene with its native promoter was obtained the same way as the *momp* gene without a promoter, except that primer mompF2 (TACCGGTTAA TAGGCTCGAGTTT [XhoI site is underlined]) was used instead of mompF, and an annealing temperature of 39°C was used in the PCR. Furthermore, PCR products and plasmid pLSKS were treated with both XbaI and XhoI prior to ligation.

FN and VN binding assays. Two methods were used to evaluate the role of DsrA in FN binding by *H. ducreyi*, including an FN binding assay using unlabeled FN and an FN binding assay using ^{125}I-labeled FN. For the unlabeled FN binding assay, suspensions containing bacterial growth from approximately 2×10^8 CFU/ml were prepared in GC broth (GCB) from 16- to 18-h heme agar plates. One milliliter of each suspension was incubated with 500 ng of purified native FN from human plasma, prepared in distilled water as described by the manufacturer (catalog no. F2006; Sigma, MO), at 34.5°C in 5% CO_2 for 30 min. The bacterial cells were subsequently washed four times (for 1 h each) in GCB. Between the third and fourth washes, the bacterial cells were transferred to a new microcentrifuge tube. After the fourth wash, the cells were resuspended in Laemmli sample buffer. The bacterial cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions and to Western blotting using an anti-FN antibody (Sigma, MO) as previously described (31). The viability of the bacterial cells after the 4-hour wash in GCB was determined using plate counts. All isolates had >50% viability after incubation for 4 h at room temperature. Only three strains had CFU counts after incubation that were statistically different from the CFU at the beginning of the incubation; they were 35000HP *dsrA dlta* FX534 (52%; $P = 0.0023$), 35000HP *dsrA momp* FX544 (62%; $P = 0.014$), and 35000HP *dsrA ompA* FX545 (60%; $P = 0.015$). We used NIH Image (version 1.63) to perform densitometry on the bands obtained in the unlabeled FN binding assay. The numbers obtained with this program were used to compare the density of the FN binding band for the parent strain to those for the mutant strains. The density is expressed as the percentage of the density of the FN binding band for the *dsrA*-positive strain in the experiment.

We determined the effect of preincubation of MAbs or purified proteins on the ability of *H. ducreyi* to bind FN. Purified immunoglobulin G (IgG) MAbs or purified proteins, each at 50 μg/ml, were separately incubated for 30 min at 34.5°C with the bacterial suspensions prior to the addition of FN. The unlabeled FN binding assay was subsequently used to determine FN binding by *H. ducreyi*.

The VN binding assay was performed the same way as the unlabeled FN binding assay described above, except that 10% heat-inactivated normal human serum was added to the bacterial suspensions as the source of VN. An anti-VN antibody (Complement Technology, TX) was used for detection in the Western blot.

The second assay used ^{125}I-labeled FN to measure FN binding by *H. ducreyi*. FN was iodinated by preactivation of two mCi of ^{125}I-Na (Perkin-Elmer, MA) in Iodo-Gen tubes (Pierce, IL) and then its addition to 200 μg of purified plasma FN (Sigma). The iodination reaction was allowed to proceed for 9 min on ice and then quenched by the addition of 50 μl of a solution of tyrosine (10 mg/ml). The iodinated product was desalted on a Bio-Gel column (Bio-Rad, CA). The counts per minute (cpm) associated with the iodinated purified FN (^{125}I-FN) was determined using a gamma counter, and the protein concentration was determined using a bicinchoninic acid protein determination kit (Pierce, IL).

After iodination of FN, a dose-response curve was performed to determine the quantity of ^{125}I-FN to use for FN binding experiments. The quantity of ^{125}I-FN at half-saturation was determined to be approximately 15 ng. Based on this information, 15 ng of ^{125}I-FN was added to 200 μl of a bacterial suspension containing 0.5×10^8 CFU in a Multiscreen (Millipore, MA) plate and allowed to incubate at 34.5°C for 45 min. After incubation, the wells were washed six times with 200 μl of phosphate-buffered saline and allowed to dry, and the bacterium-associated cpm was determined using a gamma counter.

Production of MAb 1.82. MAb 1.82, raised against a recombinant DsrA class I protein, was obtained from the same fusion that generated the anti-recombinant DsrA MAb 4.79, which is described in detail in a previous publication (47). MAb 1.82 binds a conformational epitope of *H. ducreyi* strain 35000HP but not those of the *dsrA* mutant FX517. It also immunoprecipitates DsrA from *H. ducreyi* whole cells (data not shown). The isotype for MAb 1.82 was determined to be IgG2A by use of the SBA Clonotyping system (Southern Biotech, AL).

Statistical analysis. Statistical analyses were performed using the SigmaStat program (Systat Software Inc., CA). The normal distribution of the data was first determined using SigmaStat. Once this was established, a *t* test was used to assess the differences between groups of data.

RESULTS

***H. ducreyi* binding to FN, but not VN, is increased upon growth of *H. ducreyi* on heme agar.** Two different methods were used to detect FN binding by intact *H. ducreyi*. In the first assay, hereafter termed the unlabeled FN binding assay, purified plasma FN bound by *H. ducreyi* was detected in Western blots by use of an anti-FN antibody. In the second assay, hereafter termed the ^{125}I-labeled FN binding assay, purified plasma FN was iodinated prior to incubation with intact *H. ducreyi*, and gamma counting was used to assess FN bound by *H. ducreyi*.

When cells were grown on CA, the standard growth medium, *H. ducreyi* FN binding was marginal and inconsistent. It was possible that the animal FNs in the FetalPlex present in the CA medium inhibited subsequent human FN binding. Therefore, we examined FN binding for *H. ducreyi* grown on CA with or without the addition of FetalPlex, as well as that for cells grown on heme plates. *H. ducreyi* grown on heme agar bound FN at levels much greater than that of bacteria grown on CA, regardless of the addition of FetalPlex (Fig. 1A, panel 1, first three lanes). In the ^{125}I-labeled FN binding assay, strain 35000HP grown on heme agar bound 28 and 19 times more FN (Fig. 1C, first three bars) than that grown on CA, with and without FetalPlex, respectively ($P < 0.01$ for CA with or without FetalPlex compared to heme agar [Fig. 1C). We concluded that the medium used for growth profoundly affected FN binding by *H. ducreyi*. Therefore, all subsequent FN binding assays used bacteria grown on heme agar.

Since DsrA also mediates *H. ducreyi* binding to VN (16), the effect of growth medium on VN binding was also investigated. As opposed to FN binding, VN binding by *H. ducreyi* was not affected by the growth medium. Bacteria grown on CA, with or without the addition of FetalPlex, bound VN as well as those grown on heme plates (Fig. 1A, panel 2).

DsrA is the major OMP mediating FN binding by *H. ducreyi*. To determine the role of DsrA in FN binding by *H. ducreyi*, we compared FN binding by the wild-type (wt) parent strain 35000HP and the *dsrA* isogenic null mutant FX517 (Fig. 1). The parent strain bound approximately seven times more FN than did the *dsrA* mutant strain ($P < 0.01$) (Fig. 1C) in the ^{125}I-labeled FN binding assay. Similar results were observed in the unlabeled FN binding assay, where the *dsrA* mutant bound very little purified FN compared to strain 35000HP (Fig. 1A, panel 1, lanes 3 and 6).

We previously reported that the *H. ducreyi* lectin DltA strongly binds to a number of N-linked glycoproteins, including FN, in a ligand blot format (31). However, in that study, DltA did not have a major role in FN binding by whole cells of *H. ducreyi*. This conclusion is supported by the data presented in Fig. 1A, where it is shown that even though the levels of expression of DltA are identical between the parent and the *dsrA* mutant strains (Fig. 1A, panel 4), the FN binding phenotypes are different. Thus, binding of FN by *H. ducreyi* did not correlate with expression of DltA.

The data presented above strongly suggest that DsrA is involved in FN binding by *H. ducreyi*. However, the data also show that there might be a second component involved in FN binding, since there was residual FN binding by *H. ducreyi dsrA* mutant strain FX517 in the absence of DsrA in both the un-

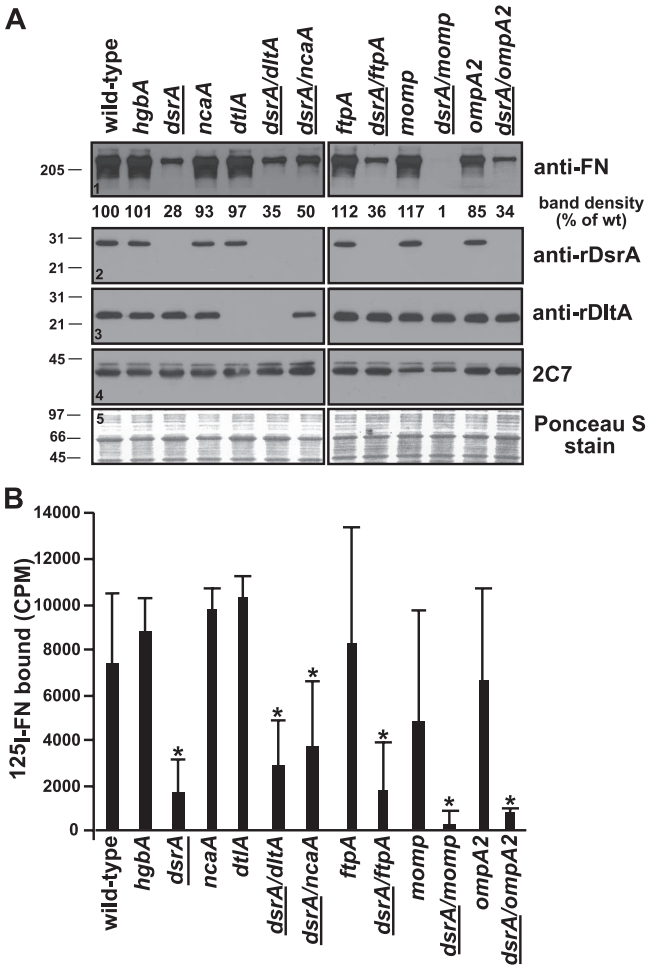

FIG. 2. Expression of MOMP affects FN binding by *H. ducreyi*. Both unlabeled (A) and ^{125}I-labeled (B) FN binding assays were repeated, using a panel of mutants constructed in the 35000HP background (Table 1). Asterisks indicate strains with levels of FN binding statistically different ($P < 0.05$) from the level of FN binding by the wt parent strain 35000HP. Strains not expressing *dsrA* are indicated with underlining. The results presented in panel A are representative of at least three other similar experiments. Ponceau S staining (panel 5) of the nitrocellulose membrane indicated approximately equal loading of total proteins for all the strains.

labeled and ^{125}I-labeled FN formats of the FN binding assay (Fig. 1A, panel 1, and C). To determine if another component(s) of the outer membrane of *H. ducreyi* was involved in FN binding, double mutants that did not express *dsrA* and other outer membrane protein (OMP) genes, such as *dltA*, *ncaA*, *ftpA*, *momp*, and *ompA2*, were constructed. Single and double mutants (Table 1) were then tested for FN binding.

All strains expressing *dsrA* bound FN comparably to parent strain 35000HP in the unlabeled FN binding assay (Fig. 2). Among strains not expressing *dsrA* (underlined *dsrA* in Fig. 2), all *dsrA* double mutants, save strain 35000HP *dsrA momp*, bound FN comparably to the single *dsrA* mutant in both assays. Most importantly, FN binding by strain 35000HP *dsrA momp* was not detected in the unlabeled FN binding assay (Fig. 2A, panel 1) and was barely detectable in the ^{125}I-labeled FN binding assay (Fig. 2B), which correlated with the absence of expression of *momp*. The values obtained in the ^{125}I-labeled FN binding assay for strains that had significantly different FN binding from the wt were not statistically different from the values obtained for the single *dsrA* mutant FX517 ($P = 0.402$ for the *dsrA dltA* mutant, $P = 0.109$ for the *dsrA ncaA* mutant, $P = 0.948$ for the *dsrA ftpA* mutant, $P = 0.115$ for the *dsrA momp* mutant, and $P = 0.238$ for the *dsrA ompA2* mutant).

H. ducreyi expresses two OmpA analogues, MOMP and OmpA2 (30). MOMP is estimated to be five times more abundant than OmpA2 in the outer membrane (44), and both proteins migrate as a 37- to 39-kDa doublet and a heat-modifiable 43-kDa species (30, 40). MAb 2C7 binds to both MOMP and OmpA2 (30, 40). The single *momp* mutant primarily lacked expression of the 37- to 39-kDa doublet (Fig. 2A, panel 4), as described previously (30, 44). However, the *ompA2* mutant did not exhibit altered binding of 2C7 (Fig. 2A, panel 4) due to the fact that it expresses MOMP.

To further define the role of MOMP in FN binding by *H. ducreyi*, we cloned the *momp* gene under the control of the *lac* promoter in the shuttle vector pLSKS and expressed it in *E. coli* DH5α, in *H. influenzae* strain KW20 Rd, and in the *H. ducreyi* double *dsrA momp* mutant. We were unable to observe expression of the *momp* gene in *E. coli* and *H. influenzae*, even in the presence of IPTG (isopropyl-β-D-thiogalactopyranoside), and although the double *dsrA momp* mutant expressed this construct, it did not do so to wt levels (data not shown). Furthermore, the *dsrA momp* mutant complemented with the *momp* construct did not bind more FN than the mutant complemented with an empty vector (data not shown). In hopes of increasing the expression level of *momp*, and therefore confirming a role for *momp* in FN binding by *H. ducreyi*, we attempted to clone the *momp* gene into pLSKS with its native promoter. However, we were unable to isolate a clone for any of the three strains described above.

To confirm that the mutation in the *dsrA* gene was responsible for the observed FN binding phenotype of the *dsrA* mutant FX517, it was complemented in *trans* with plasmid pUNCH1260 (20), containing intact *dsrA* from strain 35000HP, and was assayed for FN binding as described above. In the unlabeled FN binding assay, expression of *dsrA* class I from pUNCH1260 ($dsrA_I$) restored FN binding by the *dsrA* mutant to levels comparable to that seen for the wt parent strain 35000HP (Fig. 3A, panel 1). Consistent with these results, the ^{125}I-labeled FN binding assay showed that FN binding by the *dsrA* mutant FX517 expressing $dsrA_I$ in *trans* was significantly increased compared to that of the *dsrA* mutant complemented with an empty vector ($P = 0.003$). Taken together, the data presented above suggest that DsrA is the major OMP involved in FN binding by *H. ducreyi* and that MOMP may have a minor role in this phenotype.

DsrA is sufficient to confer FN binding to the non-FN-binding *H. influenzae* strain KW20 Rd. *Escherichia coli* is typically used as the host strain for expression of foreign genes and analysis of resulting phenotypes. However, *dsrA* is unclonable in *E. coli* due to its toxicity to this species (20). Thus, to study the role of DsrA in the absence of other *H. ducreyi* factors, FN binding was studied in *H. influenzae* strain KW20 Rd expressing $dsrA_I$ from plasmid pUNCH1260. *H. influenzae* strain KW20 Rd does not appear to express a homolog of *dsrA*, since only a homologous pseudogene of YadA with three frameshifts was found in this strain (25, 26). In the unlabeled FN binding

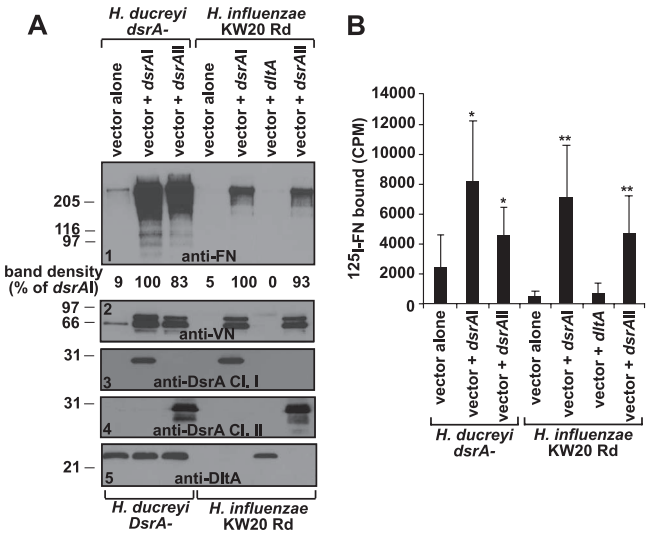

FIG. 3. Either class of DsrA is sufficient to confer FN binding to the non-FN-binding strain *H. influenzae* KW20 Rd. The same FN binding assays described in the legend to Fig. 1 (unlabeled [A] and ^{125}I-labeled [B] FN binding assays) were used to test FN binding by the *H. ducreyi dsrA* isogenic mutant FX517 (DsrA negative) and *H. influenzae* KW20 Rd expressing either an empty vector (pLSKS), pLSKS containing *dsrA* class I ($dsrA_I$), pLSKS containing *dltA*, or pLSKS containing *dsrA* class II ($dsrA_{II}$). All strains were grown on heme plates. Single asterisks represent statistically significant results ($P \leq 0.01$) compared to the *H. ducreyi dsrA* mutant expressing the empty vector pLSKS, while double asterisks use *H. influenzae* KW20 Rd expressing the empty vector pLSKS as the comparison strain ($P \leq 0.01$). The results presented in panel A are representative of at least three other similar experiments. Ponceau S staining (not shown) of the nitrocellulose membrane indicated approximately equal loading of total proteins for all the strains.

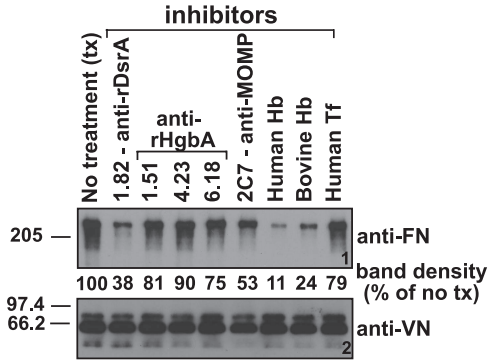

FIG. 4. FN binding by *H. ducreyi* is inhibited by an anti-DsrA MAb and by hemoglobin. The effect of preincubation of MAbs or purified protein on the ability of *H. ducreyi* to bind FN was measured using the unlabeled FN binding assay (see Materials and Methods). A panel of MAbs (purified IgGs), as well as hemoglobin (Hb) and transferrin (Tf), each at 50 μg/ml, were tested as potential inhibitors of FN and VN binding by *H. ducreyi*. Ponceau S staining (not shown) of the nitrocellulose membrane indicated approximately equal loading of total proteins for all the strains.

assay, expression of $dsrA_I$ in *H. influenzae* KW20 Rd conferred FN binding to this non-FN-binding strain (Fig. 3A, panel 1), although the binding was not at the level seen for the *H. ducreyi dsrA* mutant complemented with $dsrA_I$. In the ^{125}I-labeled FN binding assay, FN binding was 10 times higher in strain *H. influenzae* KW20 Rd expressing $dsrA_I$ ($P < 0.001$) than in the *H. influenzae* strain expressing an empty vector (Fig. 3B, double asterisks). Expression of $dsrA_I$ in *H. influenzae* KW20 Rd also conferred binding to VN to levels seen in the *dsrA* mutant complemented with $dsrA_I$ (Fig. 3A, panel 2), while expression of the *dltA* gene in *H. influenzae* did not confer FN or VN binding (Fig. 3A, panels 1 and 2, and B), as expected. These data confirm that DsrA is sufficient to confer FN binding to *H. influenzae* in the absence of other *H. ducreyi* components.

DsrA class II confers FN and VN binding in both *H. ducreyi dsrA* mutant FX517 and *H. influenzae* KW20 Rd. We and another group (36, 47) were unable to mutagenize the *dsrA* and LOS genes from *H. ducreyi* class II strains. Thus, to circumvent this limitation, we studied the role of the *dsrA* class II gene ($dsrA_{II}$) in FN binding by expressing $dsrA_{II}$ from pUNCH1296 in both the null *H. ducreyi dsrA* mutant FX517 and the non-FN-binding strain *H. influenzae* KW20 Rd. Expression of $dsrA_{II}$ restored FN binding to wt levels in FX517 ($P = 0.008$ versus vector alone) and conferred FN binding to the non-FN-binding strain *H. influenzae* KW20 Rd (Fig. 3A, panel 1, and B) ($P = 0.0002$ versus *H. influenzae* KW20 Rd vector alone). $dsrA_{II}$ also restored VN binding in the *dsrA* class I mutant, as well as conferred VN binding to the non-VN-binding *H. influenzae* strain KW20 Rd (Fig. 3A, panel 5). These data suggest that DsrA proteins from both classes of *H. ducreyi* strains mediate FN and VN binding.

Inhibition of *H. ducreyi* FN binding by antibodies. To determine the effect of antibodies on the binding between FN and DsrA, MAbs to *H. ducreyi* OMPs were preincubated with *H. ducreyi* cells prior to the addition of FN. FN was then added to the bacterial suspension, and the unlabeled FN binding assay was carried out as previously described (see Materials and Methods). The anti-DsrA MAb 1.82 (this study) and the anti-MOMP MAb 2C7 (11) were selected because they have been shown to bind to the surface of *H. ducreyi*. Three other MAbs (35), chosen as irrelevant control MAbs for this experiment, were also used. These were MAbs 1.51, 4.23, and 6.18, which are directed against HgbA. As shown in Fig. 4 (panel 1), MAb 1.82 partially blocked FN binding by *H. ducreyi*, while binding was reduced to intermediate levels by MAb 2C7. None of the other MAbs affected FN binding. VN binding by *H. ducreyi* strain 35000HP was not affected by any MAb tested (Fig. 4, panel 2).

Inhibition of *H. ducreyi* FN binding by selected proteins. Earlier, we showed that FN binding by *H. ducreyi* grown on CA was barely detectable and that FetalPlex could not account for this inhibition (Fig. 1). Based on these data, we hypothesized that hemoglobin (Hgb) present in the CA may inhibit FN binding by *H. ducreyi*. To test this hypothesis, *H. ducreyi* strain 35000HP was preincubated with human or bovine Hgb or human transferrin, a protein not present in CA medium, prior to the addition of FN or human serum (a source of VN). While Hgb from both sources greatly reduced FN binding by *H. ducreyi* (Fig. 4, panel 1), neither affected the binding of VN by *H. ducreyi* (Fig. 4, panel 2), consistent with the results shown in Fig. 1. As expected, human transferrin did not affect FN or VN binding by *H. ducreyi*.

Incubation of *H. ducreyi* with Hgb profoundly affected FN binding by this microorganism (Fig. 4, panel 1). To determine

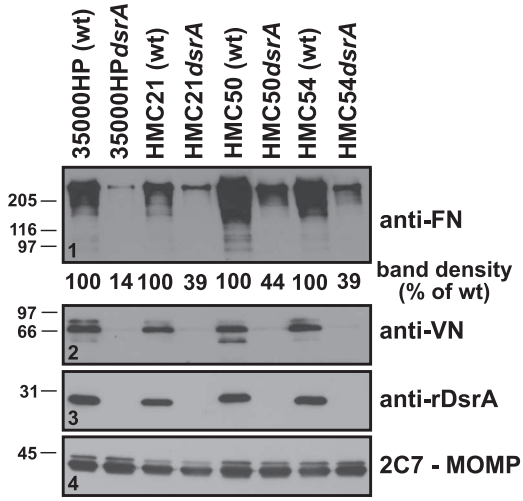

FIG. 5. DsrA is the major OMP involved in FN binding by other wt *H. ducreyi* class I strains. FN binding by three other wt *H. ducreyi* class I strains and their respective *dsrA* isogenic mutants was determined using the unlabeled FN binding assay described in the legend to Fig. 1A. Results are representative of similar experiments which were done twice. Ponceau S staining (not shown) of the nitrocellulose membrane indicated approximately equal loading of total proteins for all the strains.

if this could be due to steric hindrance, we evaluated FN binding of the *hgbA* mutant (FX504) and the parent strain 35000HP upon growth on CA (with Hgb in the medium). We reasoned that the *hgbA* mutant, which is unable to bind Hgb (19), should be able to bind FN in the absence of Hgb bound to the surface of the cell if Hgb prevents binding of FN by steric hindrance. The *hgbA* mutant did not bind more FN than the parent strain (data not shown) in the presence of Hgb, suggesting that steric hindrance is not involved in preventing FN binding by *H. ducreyi* in the presence of Hgb.

DsrA is the major OMP involved in FN binding by other class I *H. ducreyi* strains. Although class II DsrA proteins are almost identical at the amino acid level (47), class I DsrA proteins are more variable (85% identity) (20). To determine if the differences in amino acid sequence among the DsrA class I proteins could affect FN and VN binding, previously constructed and characterized *dsrA* mutants FX529 and FX530 (1) and a newly constructed *dsrA* mutant, FX528, as well as their respective wt parent strains (HMC54, HMC50, and HMC21, respectively), were assayed for FN and VN binding. Each *dsrA* mutant bound less FN than its respective parent strain, although the level of FN binding was heterogeneous among the wt parent strains (Fig. 5, panel 1). VN binding phenotypes were also as expected, with all wt parent strains tested binding about equally to VN and their respective isogenic mutants unable to bind VN (Fig. 5, panel 2). Thus, in all *H. ducreyi* strains tested, DsrA was the major FN and VN binding protein.

DISCUSSION

DsrA is the major component involved in FN binding by *H. ducreyi*. In this paper, we show four lines of evidence that DsrA is the major factor required for binding by *H. ducreyi* to the ECM protein FN. First, the single *dsrA* isogenic mutant strain bound significantly less FN than the wt parent strain 35000HP did. Moreover, the same phenotype was observed for three other class I *H. ducreyi* strains and their isogenic *dsrA* mutants. Second, expression of *dsrA* in *trans* in the single *dsrA* mutant restored FN binding to levels comparable to that of the parent strain, confirming that the reduced binding of FN by the *dsrA* mutant strain FX517 was due to a mutation in *dsrA* and not another locus. Third, an anti-DsrA MAb partially blocked FN binding by *H. ducreyi*. Fourth, mutants containing mutations in several outer membrane components bound levels of FN comparable to that of the parent strain, while most double mutants with a mutation in *dsrA* bound FN at levels similar to that of the single *dsrA* mutant.

MOMP may have a minor role in FN binding by *H. ducreyi*. The data presented in this report demonstrate that DsrA is the major FN binding factor in *H. ducreyi*. However, residual FN binding by the *dsrA* mutant strain was also observed. This residual FN binding might be attributed to MOMP, since a double *dsrA momp* mutant bound less FN than the single *dsrA* mutant FX517 in both FN binding assays, although the difference in levels of FN binding between the single *dsrA* and double *dsrA momp* mutants did not reach statistical significance in the ^{125}I-labeled FN binding assay ($P = 0.115$).

We attempted to demonstrate a role for MOMP in FN binding by expressing the *momp* gene in three different hosts, namely, *E. coli* DH5α, *H. influenzae* strain KW20 Rd, and the double *dsrA momp H. ducreyi* mutant (FX544). Although we were able to express *momp* under the control of the *lac* promoter in the *dsrA momp* mutant, it did not reach the levels expressed by the wt strain, and FN binding by the *dsrA momp* mutant complemented with this construct did not differ from that of the *dsrA momp* mutant complemented with an empty vector. Furthermore, we were unsuccessful in cloning the *momp* gene under the control of its native promoter, suggesting that this protein may be toxic when expressed at high levels, even in its natural host.

The DsrA proteins from class I and class II *H. ducreyi* strains are responsible for FN and VN binding. Similar to class I DsrA, the DsrA proteins from class II strains were also able to provide comparable FN and VN binding phenotypes to those obtained with class I DsrA. A cloned class II *dsrA* gene restored FN and VN binding in the isogenic *dsrA* mutant FX517 to the same levels as those with class I DsrA and conferred binding to the non-FN- and non-VN-binding *H. influenzae* strain KW20 Rd.

The DsrA proteins from the two classes of *H. ducreyi* strains share high identity in the C-terminal portion but very low identity in the N-terminal two-thirds of the proteins. The dissimilarity in these proteins raised the possibility that these proteins may have different functions. However, the data presented here and previously (47) suggest that both classes of DsrA have similar functions despite their divergent N termini, since both classes of DsrA mediate serum resistance as well as FN and VN binding. Either FN binding can be attributed to the C-terminal part of the protein, which is 88% identical among the DsrA proteins from the two classes, or features other than the primary sequence in the divergent N-terminal section of the proteins are responsible for this interaction. In order to dissect the multifunctional roles of DsrA in *H. ducreyi*, experiments are currently under way to construct DsrA pro-

teins that are involved only in adhesive properties and not serum resistance and vice versa. Furthermore, the virulence of these mutants will be examined in the experimental human infection model of chancroid.

Expression of the two classes of DsrA protein in a non-FN-binding *H. influenzae* strain conferred FN binding, although not to the levels seen for *H. ducreyi* strain FX517 complemented with $dsrA_{I}$ or $dsrA_{II}$. The absence of another component(s) may account for the lower levels of FN binding by *H. influenzae*. This assumption is supported by the results presented in Fig. 3. Expression of *dsrA* genes from both classes of *H. ducreyi* strains conferred VN binding by *H. influenzae* KW20 Rd to levels comparable to those seen in the *dsrA* mutant complemented with either *dsrA* gene (Fig. 3, panel 2). However, this was not the case for FN binding, where expression of either *dsrA* gene in *H. influenzae* KW20 Rd conferred <50% of the FN binding level of the *dsrA* mutant complemented with either class of *dsrA* gene. These data support the conclusion that MOMP may have a role in FN binding by *H. ducreyi*.

A difference in FN binding was detected between the two FN binding assays upon comparison of the *H. influenzae* strain KW20 Rd and the *H. ducreyi* single *dsrA* mutant expressing the *dsrA* gene (Fig. 3). FN binding measured by the ^{125}I-labeled FN binding assay was very similar between both *H. ducreyi* and *H. influenzae* strains, while there was a substantial difference between these strains in FN binding measured by the unlabeled FN binding assay. We do not understand the reason(s) for this observation. One possibility is that the affinity of FN for *dsrA* expressed in *H. influenzae* is less than that for *dsrA* expressed in *H. ducreyi*. In this case, the FN bound at the surface of the cell is more likely to be lost during the longer wash in the unlabeled FN assay than it is in the ^{125}I-labeled FN binding assay. This "reduced" affinity for FN binding by *H. influenzae* expressing *dsrA* may be due to additional *H. ducreyi* components which are absent in *H. influenzae* and that contribute to FN binding.

FN binding by *H. ducreyi* and virulence. Historically, limited studies of ECM binding by *H. ducreyi* have been reported. In the first in vitro ECM binding analysis, all 21 *H. ducreyi* strains tested bound FN, and a protein was speculated to be involved in this interaction (2). Later, Alfa and DeGagne showed that the addition of purified FN inhibited the binding of *H. ducreyi* to fibroblasts in a dose-dependent manner (4). A third in vitro study of ECM binding by *H. ducreyi* confirmed previous studies of FN binding by *H. ducreyi*, although no role for pili or full-length LOS in FN binding by *H. ducreyi* could be demonstrated (10). In the human experimental model of chancroid infection, *H. ducreyi* associates with the ECM proteins fibrin and collagen (9), and in natural chancroid, it is associated with fibrin only (12).

The discrepancy between FN binding by *H. ducreyi* in vitro and in vivo may be explained in several ways. *H. ducreyi* has not been visualized in the skin of experimentally infected volunteers in the first 48 h postinoculation (9) and may interact with FN early in the infection process, before organisms can be visualized. Also, the MAb used for the in vivo FN binding studies was developed against cellular FN (MAb 568; Novocastra Laboratories) (9), and it is not known whether this antibody reacts with plasma FN. In the present study, only plasma FN binding by *H. ducreyi* was examined, so we cannot make any conclusion(s) about the interaction of *H. ducreyi* with cellular FN.

We have shown that Hgb inhibits FN binding in vitro. Hgb is present at sites of *H. ducreyi* infection and is an important source of heme/iron for *H. ducreyi* in human experimental infection (6). Chancroidal ulcers are vascular (33), and blood at the site of infection is most likely a source of Hgb for *H. ducreyi*. Since *H. ducreyi* can be visualized only after 48 h of infection, it is possible that Hgb inhibits FN binding by *H. ducreyi* in vivo, consistent with our in vitro findings. Clearly, further studies are warranted to better understand how hemoglobin and fibronectin interact with the *H. ducreyi* cell surface.

FN binding by other Oca proteins. The amino acid sequence between amino acids 53 and 83 in YadA from *Yersinia pseudotuberculosis* is responsible for FN binding, and this region is absent in *Yersinia enterocolitica* YadA and *H. ducreyi* DsrA (24). Furthermore, FN binding by *Y. pseudotuberculosis* through the 53-83 region of YadA allows invasion through the $\alpha5\beta1$ integrin receptor. However, there was residual binding in Δ53-83 YadA of *Y. pseudotuberculosis*, suggesting that another section(s) of the protein may also bind FN. Experiments are under way to determine the residues of DsrA which are required for plasma FN binding by *H. ducreyi*.

In conclusion, we have shown that the OMP DsrA is the major mediator of human plasma FN binding by the gram-negative bacterium *H. ducreyi*. Our results also suggest that MOMP may play a minor role in the interaction of *H. ducreyi* with FN.

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REFERENCES

1. **Abdullah, M., I. Nepluev, G. Afonina, S. Ram, P. Rice, W. Cade, and C. Elkins.** 2005. Killing of *dsrA* mutants of *Haemophilus ducreyi* by normal human serum occurs via the classical complement pathway and is initiated by immunoglobulin M binding. Infect. Immun. **73:**3431–3439.
2. **Abeck, D., A. P. Johnson, and H. Mensing.** 1992. Binding of *Haemophilus ducreyi* to extracellular matrix proteins. Microb. Pathog. **13:**81–84.
3. **Albritton, W. L.** 1989. Biology of *Haemophilus ducreyi*. Microbiol. Rev. **53:**377–389.
4. **Alfa, M. J., and P. DeGagne.** 1997. Attachment of *Haemophilus ducreyi* to human foreskin fibroblasts involves LOS and fibronectin. Microb. Pathog. **22:**39–46.
5. **Al-Tawfiq, J. A., M. E. Bauer, K. R. Fortney, B. P. Katz, A. F. Hood, M. Ketterer, M. A. Apicella, and S. M. Spinola.** 2000. A pilus-deficient mutant of *Haemophilus ducreyi* is virulent in the human model of experimental infection. J. Infect. Dis. **181:**1176–1179.
6. **Al-Tawfiq, J. A., K. R. Fortney, B. P. Katz, A. F. Hood, C. Elkins, and S. M. Spinola.** 2000. An isogenic hemoglobin receptor-deficient mutant of *Haemophilus ducreyi* is attenuated in the human model of experimental infection. J. Infect. Dis. **181:**1049–1054.
7. **Al-Tawfiq, J. A., A. C. Thornton, B. P. Katz, K. R. Fortney, K. D. Todd, A. F. Hood, and S. M. Spinola.** 1998. Standardization of the experimental model of *Haemophilus ducreyi* infection in human subjects. J. Infect. Dis. **178:**1684–1687.
8. **Barnhart, M. M., and M. R. Chapman.** 2006. Curli biogenesis and function. Annu. Rev. Microbiol. **60:**131–147.
9. **Bauer, M. E., M. P. Goheen, C. A. Townsend, and S. M. Spinola.** 2001. *Haemophilus ducreyi* associates with phagocytes, collagen, and fibrin and remains extracellular throughout infection of human volunteers. Infect. Immun. **69:**2549–2557.
10. **Bauer, M. E., and S. M. Spinola.** 1999. Binding of *Haemophilus ducreyi* to extracellular matrix proteins. Infect. Immun. **67:**2649–2652.
11. **Bauer, M. E., and S. M. Spinola.** 2000. Localization of *Haemophilus ducreyi* at the pustular stage of disease in the human model of infection. Infect. Immun. **68:**2309–2314.

12. **Bauer, M. E., C. A. Townsend, A. R. Ronald, and S. M. Spinola.** 2006. Localization of *Haemophilus ducreyi* in naturally acquired chancroidal ulcers. Microbes Infect. **8:**2465–2468.
13. **Bong, C. T., R. E. Throm, K. R. Fortney, B. P. Katz, A. F. Hood, C. Elkins, and S. M. Spinola.** 2001. DsrA-deficient mutant of *Haemophilus ducreyi* is impaired in its ability to infect human volunteers. Infect. Immun. **69:**1488–1491.
14. **Bozue, J. A., L. Tarantino, and R. S. Munson, Jr.** 1998. Facile construction of mutations in *Haemophilus ducreyi* using lacZ as a counter-selectable marker. FEMS Microbiol. Lett. **164:**269–273.
15. **Clarke, S. R., and S. J. Foster.** 2006. Surface adhesins of *Staphylococcus aureus*. Adv. Microb. Physiol. **51:**187–224.
16. **Cole, L. E., T. H. Kawula, K. L. Toffer, and C. Elkins.** 2002. The *Haemophilus ducreyi* serum resistance antigen DsrA confers attachment to human keratinocytes. Infect. Immun. **70:**6158–6165.
17. **Cotter, S. E., N. K. Surana, and J. W. St. Geme III.** 2005. Trimeric autotransporters: a distinct subfamily of autotransporter proteins. Trends Microbiol. **13:**199–205.
18. **Dutro, S. M., G. Wood, and P. Totten.** 1999. Prevalence of, antibody response to, and immunity induced by *Haemophilus ducreyi* hemolysin. Infect. Immun. **67:**3317–3328.
19. **Elkins, C., C. J. Chen, and C. E. Thomas.** 1995. Characterization of the *hgbA* locus of *Haemophilus ducreyi*. Infect. Immun. **63:**2194–2200.
20. **Elkins, C., K. J. Morrow, Jr., and B. Olsen.** 2000. Serum resistance in *Haemophilus ducreyi* requires outer membrane protein DsrA. Infect. Immun. **68:**1608–1619.
21. **El Tahir, Y., and M. Skurnik.** 2001. YadA, the multifaceted Yersinia adhesin. Int. J. Med. Microbiol. **291:**209–218.
22. **Fulcher, R. A., L. E. Cole, D. M. Janowicz, K. L. Toffer, K. R. Fortney, B. P. Katz, P. E. Orndorff, S. M. Spinola, and T. H. Kawula.** 2006. Expression of *Haemophilus ducreyi* collagen binding outer membrane protein NcaA is required for virulence in swine and human challenge models of chancroid. Infect. Immun. **74:**2651–2658.
23. **Hammond, G. W., C. J. Lian, J. C. Wilt, W. L. Albritton, and A. R. Ronald.** 1978. Determination of the hemin requirement of *Haemophilus ducreyi*: evaluation of the porphyrin test and media used in the satellite growth test. J. Clin. Microbiol. **7:**243–246.
24. **Heise, T., and P. Dersch.** 2006. Identification of a domain in *Yersinia* virulence factor YadA that is crucial for extracellular matrix-specific cell adhesion and uptake. Proc. Natl. Acad. Sci. USA **103:**3375–3380.
25. **Hoiczyk, E., A. Roggenkamp, M. Reichenbecher, A. Lupas, and J. Heesemann.** 2000. Structure and sequence analysis of *Yersinia* YadA and *Moraxella* UspAs reveal a novel class of adhesins. EMBO J. **19:**5989–5999.
26. **Hood, D. W., M. E. Deadman, T. Allen, H. Masoud, A. Martin, J. R. Brisson, R. Fleischmann, J. C. Venter, J. C. Richards, and E. R. Moxon.** 1996. Use of the complete genome sequence information of *Haemophilus influenzae* strain Rd to investigate lipopolysaccharide biosynthesis. Mol. Microbiol. **22:**951–965.
27. **Hynes, R. O.** 1990. Fibronectins. Springer-Verlag, New York, NY.
28. **Janowicz, D., I. Leduc, K. R. Fortney, B. P. Katz, C. Elkins, and S. M. Spinola.** 2006. A DltA mutant of *Haemophilus ducreyi* is partially attenuated in its ability to cause pustules in human volunteers. Infect. Immun. **74:**1394–1397.
29. **Joh, D., E. R. Wann, B. Kreikemeyer, P. Speziale, and M. Hook.** 1999. Role of fibronectin-binding MSCRAMMs in bacterial adherence and entry into mammalian cells. Matrix Biol. **18:**211–223.
30. **Klesney-Tait, J., T. J. Hiltke, I. Maciver, S. M. Spinola, J. D. Radolf, and E. J. Hansen.** 1997. The major outer membrane protein of *Haemophilus ducreyi* consists of two OmpA homologs. J. Bacteriol. **179:**1764–1773.
31. **Leduc, I., P. Richards, C. Davis, B. Schilling, and C. Elkins.** 2004. A novel lectin, DltA, is required for expression of a full serum resistance phenotype in *Haemophilus ducreyi*. Infect. Immun. **72:**3418–3428.
32. **Lewis, D. A.** 2000. Chancroid: from clinical practice to basic science. AIDS Patient Care STDS **14:**19–36.
33. **Morse, S. A.** 1989. Chancroid and *Haemophilus ducreyi*. Clin. Microbiol. Rev. **2:**137–157.
34. **Mosher, D. R.** 1989. Fibronectin. Academic Press, San Diego, CA.
35. **Patterson, K., B. Olsen, C. Thomas, D. Norn, M. Tam, and C. Elkins.** 2002. Development of a rapid immunodiagnostic test for *Haemophilus ducreyi*. J. Clin. Microbiol. **40:**3694–3702.
36. **Post, D. M., R. S. Munson, Jr., B. Baker, H. Zhong, J. A. Bozue, and B. W. Gibson.** 2007. Identification of genes involved in the expression of atypical lipooligosaccharide structures from a second class of *Haemophilus ducreyi*. Infect. Immun. **75:**113–121.
37. **Riesbeck, K., T. T. Tan, and A. Forsgren.** 2006. MID and UspA1/A2 of the human respiratory pathogen *Moraxella catarrhalis*, and interactions with the human host as basis for vaccine development. Acta Biochim. Pol. **53:**445–456.
38. **Schwarz-Linek, U., M. Hook, and J. R. Potts.** 2004. The molecular basis of fibronectin-mediated bacterial adherence to host cells. Mol. Microbiol. **52:**631–641.
39. **Spinola, S. M., M. E. Bauer, and R. S. Munson.** 2002. Immunopathogenesis of *Haemophilus ducreyi* infection (chancroid). Infect. Immun. **70:**1667–1676.
40. **Spinola, S. M., G. E. Griffiths, K. L. Shanks, and M. S. Blake.** 1993. The major outer membrane protein of *Haemophilus ducreyi* is a member of the OmpA family of proteins. Infect. Immun. **61:**1346–1351.
41. **Sullivan, M.** 1940. Chancroid. Am. J. Syph. Gonorrhea Vener. Dis. **24:**482–521.
42. **Taylor, L. A., and R. E. Rose.** 1988. A correction in the nucleotide sequence of the Tn903 kanamycin resistance determinant in pUC4K. Nucleic Acids Res. **16:**358.
43. **Thomas, W. D., Jr., S. P. Wagner, and R. A. Welch.** 1992. A heterologous membrane protein domain fused to the C-terminal ATP-binding domain of HlyB can export *Escherichia coli* hemolysin. J. Bacteriol. **174:**6771–6779.
44. **Throm, R. E., J. A. Al-Tawfiq, K. R. Fortney, B. P. Katz, A. F. Hood, C. A. Slaughter, E. J. Hansen, and S. M. Spinola.** 2000. Evaluation of an isogenic major outer membrane protein-deficient mutant in the human model of *Haemophilus ducreyi* infection. Infect. Immun. **68:**2602–2607.
45. **Totten, P. A., and W. E. Stamm.** 1994. Clear broth and plate media for the culture of *Haemophilus ducreyi*. J. Clin. Microbiol. **32:**2019–2023.
46. **Trees, D. L., and S. A. Morse.** 1995. Chancroid and *Haemophilus ducreyi:* an update. Clin. Microbiol. Rev. **8:**357–375.
47. **White, C. D., I. Leduc, B. Olsen, C. Jeter, C. Harris, and C. Elkins.** 2005. *Haemophilus ducreyi* outer membrane determinants, including DsrA, define two clonal populations. Infect. Immun. **73:**2387–2399.
48. **Wood, G. E., S. M. Dutro, and P. A. Totten.** 1999. Target cell range of the *Haemophilus ducreyi* hemolysin and its involvement in invasion of human epithelial cells. Infect. Immun. **67:**3740–3749.

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