Interdomain Interaction in the FimH Adhesin of Escherichia coli Regulates the Affinity to Mannose*

Received for publication, March 8, 2007, and in revised form, June 13, 2007. Published, JBC Papers in Press, June 13, 2007, DOI 10.1074/jbc.M702037200

Pavel Aprikian1‡1, Veronika Tchesnokova1‡1, Brian Kidd5, Olga Yakovenko5§, Vladimir Yarov-Yarovoy5, Elena Trinchina5, Viola Vogel5, Wendy Thomas5***‡, and Evgeni Sokurenko1***‡

From the Departments of 1Microbiology, 5Bioengineering, 5Pharmacology, and **3Nanotechnology Center, University of Washington, Seattle, Washington 98105 and the 1Department of Materials, Laboratory of Biologically Oriented Materials, Swiss Federal Institute of Technology, ETH Zurich, 8093 Zurich, Switzerland

FimH is a mannose-specific adhesin located on the tip of type 1 fimbriae of Escherichia coli that is capable of mediating shear-enhanced bacterial adhesion. FimH consists of a fimbria-associated pilin domain and a mannose-binding lectin domain, with the binding pocket positioned opposite the interdomain interface. By using the yeast two-hybrid system, purified lectin and pilin domains, and docking simulations, we show here that the FimH domains interact with one another. The affinity to mannose is greatly enhanced (up to 300-fold) in FimH variants in which the interdomain interaction is disrupted by structural mutations in either the pilin or lectin domains. Also, affinity to mannose is dramatically enhanced in isolated lectin domains or in FimH complexed with the chaperone molecule that is wedged between the domains. Furthermore, FimH with native structure mediates weak binding at low shear stress but shifts to strong binding at high shear, whereas FimH with disrupted interdomain contacts (or the isolated lectin domain) mediates strong binding to mannose-coated surfaces even under low shear. We propose that interactions between lectin and pilin domains decrease the affinity of the mannose-binding pocket via an allosteric mechanism. We further suggest that mechanical force at high shear stress separates the two domains, allowing the lectin domain to switch from a low affinity to a high affinity state. This shift provides a mechanism for FimH-mediated shear-enhanced adhesion by enabling the adhesin to form catch bond-like interactions that are longer lived at high tensile force.

Receptor-ligand specific adhesion is among the most fundamental of biological phenomena in nature. This phenomenon underlies eukaryotic cell-cell or cell-surface attachment, initiates recognition and signaling events, binds bacteria or viruses to target cells, and mediates biofilm formation on medical implants. Usually, the adhesion is mediated by protein-protein, protein-lipid, or often, protein-saccharide interactions (1). Most of these adhesive interactions occur under a certain level of tensile force that arises from shear stress. Whereas a number of receptor-ligand binding interactions are weakened by tensile force, recent studies have shown that certain physiologically important biomolecular interactions can be enhanced by mechanical force (2–4). Although these studies have exposed a novel, so-called catch bond, mode of receptor-ligand adhesion, the precise structural mechanism for it remains elusive.

One type of receptor-ligand interaction that is enhanced by mechanical force is that of the most common family of bacterial adhesins, mannose-specific type 1 fimbriae. In Escherichia coli, mannose-specific adhesion is mediated by the FimH adhesin at the tip of type 1 fimbriae. FimH has two domains, the mannose-binding lectin domain (Ld)4 (1–156 aa) and the fimbria-incorporating pilin domain (Pd) (160–273 aa), which are connected via a 3-aa interdomain linker peptide chain (5). The Ld is a β-barrel fold with the monomannose (1M)-binding site located in FimH complexed with the chaperone molecule that is wedged between the domains. The shear-enhanced adhesion of FimH is reminiscent of shear-dependent rolling of leukocytes on the endothelium surface mediated by the interaction between P- and L-selectin protein and Lewis X antigen oligosaccharides (9). The FimH and P- and L-selectin-mediated binding have been suggested to occur via a catch bond mechanism (2, 3, 6, 10–14), where the receptor-ligand interaction is enhanced by tensile force. This mechanism differs from a slip bond of traditional interactions that...
are inhibited by force. Despite data that show longer interaction lifetimes with increasing force (10, 12−14), the existence of catch bonds remains controversial due to limited experimental evidence that would provide a structural basis for how mechanical force regulates protein−ligand interaction.

Here we show that the fimbriae-anchoring Pd could play a direct role in modulating the activity of the mannose-binding Ld. This modulation occurs through interdomain interactions that keep the Ld in the 1M weak-binding state. This state can switch to a 1M strong-binding state when the Ld is separated from Pd. Thus, the interdomain regulation we report here provides a mechanism for switching from weak to strong binding under high shear stress.

**Experimental Procedures**

**Reagents**—Monomannosylated-BSA (Man1-BSA) was a generous gift of Dr. Y. C. Lee, Johns Hopkins University, Baltimore, MD. All other reagents were obtained from Sigma.

**Strains**—Recombinant strains utilized here were constructed using a fim null K-12 derivative, AAEC191A (provided by Dr. Ian Blomfield, University of Kent, UK), and were described previously (15). fimH allele encoding the FimH_{k12} variant was derived from *E. coli* MG1655 and is identical to the one encoding FimH in *E. coli* J96 that was used for the x-ray crystallography studies of Ref. 5.

**Site-directed Mutagenesis**—Structural replacements and mutations were introduced as described previously (6).

**Bacterial Binding**—Static assays of bacterial adhesion to immobilized 1M/ligands (1M/BSA or yeast mannan) were carried out in 96-well plates as described previously (15). Binding under shear stress were performed using parallel plate flow chambers as described in Ref. 7. Rolling adhesion was defined as movement of greater than one bacterial radius in 1 s.

**Yeast Two-hybrid Analysis**—Yeast two-hybrid analysis was performed in general as described previously (16). In brief, we used *Saccharomyces cerevisiae* strain L40 with the yeast his3 gene as a reporter that, when properly expressed, enables yeast growth on medium lacking histidine. Expression of his3 in L40 is driven by the minimal his3 promoter fused to multimerized binding sites for the DNA-binding protein LexA. Gene regions encoding for Ld of different variants of FimH were cloned to create in-frame fusion with the LexA DNA-binding domain, whereas different variants of Pd (with self-complementing β-strand) were cloned to create in-frame fusion with the activation domain of transcriptional activator VP16. Both expression vectors, carrying LexA-Ld and VP16-Pd fusions, were transformed into strain L40 and productive interaction between Ld and Pd were detected by the ability of transformants to grow on plates of minimal YNB media lacking histidine with and without 3-aminotriazole inhibitor of the his3 protein.

**Interdomain Docking**—Docking predictions were performed using the docking mode of the Rosetta program (17). The sequences of the Ld and Pd from the FimH variant of the starting crystal structure were identical to the FimH_k12 variant used in these studies. To isolate the two domains for the docking predictions, the linker chain residues (Pro^{157}−Gly^{159}) and the FimC chaperone molecule were computationally removed from the x-ray structure of FimH (Protein Data Bank code 1klf (18)). The domains were allowed to move freely relatively to each other in the predictions, although there was a distance constraint of 13.0 Å between the C-terminal residue of the Ld and N-terminal residue of Pd to ensure the linker chain connection in the docked structure. A total of 5,000 models were generated followed by clustering of the top 200 scoring models (17) and five models were selected based on their relative score rank among all models. However, two of the top-scoring models presented severe conformational constraints that would have prevented repositioning the linker chain between the domains, whereas another model created overlaps between the Ld and groove into which the β-strand of the FimC chaperone and, in native fimbriae, the FimG subunit is being incorporated. The remaining two models were identical within 0.9 Å and included the highest scoring model. The highest scoring model was selected from the five models and the interdomain linker was successfully rebuilt in the selected model using the loop mode of the Rosetta program (19).

**Steered Molecular Dynamics**—Domain-domain interactions were examined using molecular dynamics (MD) and steered molecular dynamics simulations. All simulations were performed using NAMD 2.5, which was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign (20). To set up the simulations, a-d-mannose and crystal waters were aligned to the docked structure based on the x-ray crystal structure (18). The Pd from the docked structure was aligned to the Pd from the crystal structure (PDB code 1klf (18)) and we selected the coordinates of residues Asn^{101}−Ile^{111} from the chaperone FimC. These coordinates match an alignment that simulates the donor β-strand of the FimHC complex (5, 18). The final structure was solvated in a periodic box of explicit water molecules that ranged in size from 129 to 140 × 78−91 × 84−91 Å^3. Disulfide bonds were assumed between residues Cys^3−Cys^44 and Cys^161−Cys^187. Residue His^45 was assumed to have both nitrogen atoms protonated due to the position of the negatively charged Asp^47 and Asp^100 residues (18). Ions (sodium and chlorine) were randomly added to the water box to: 1) give the solvent an ionic strength of 150 mM and 2) ensure the system had a zero net charge as required for the Particle Mesh Ewald algorithm. The computational mutation (A188D) was performed on the docked structure using the side chain prediction algorithm SCRWL3.0 (21).

All simulations were performed using a 1-fs time step. Minimization was carried out in 80 steps and four stages to allow the entire system to reach a stable energy minimum. Thermalization was achieved in 40 ps by increasing the temperature in steps of 10 K every 1 ps until a final temperature of 310 K. Equilibration was conducted for 2 ns. During equilibration, the system was held at a temperature of 310 K and anisotropically coupled to a Berendsen pressure piston. The pressure was set to 1.013 bar with a relaxation time of 1 ps and a compressibility factor of 4.57 × 10^{-5} bar^{-1}.

To simulate separation of the two domains by mechanical force, the Ca atom on the N terminus of the donor β-strand (FimC Asn^{101}) was pulled with a constant force in one direction.
Interaction between Binding and Anchoring Domains of FimH

and the O-1 atom of \(\alpha\)-d-mannose was pulled with an equal force in the opposite direction. Individual simulations were performed for 500 to 1000 ps at constant forces that ranged from 50 to 250 pN.

**Fimbriae Purification**—Fimbriae were purified from recombinant *E. coli* strains expressing type 1 fimbriae with different FimH essentially as described previously (8).

**Ld-His\(_6\), Expression and Purification**—Different variants of FimH Ld were expressed and purified as described in Refs. 22 and 23 with some modifications. Cells were grown at 37 °C in LB media to \(A\_{600}\) = 0.6, induced with 1 mM isopropyl 1-thio-\(\beta\)-d-galactopyranoside, with induction continuing overnight at 16 °C. Cells were harvested by centrifugation and periplasmic fractions were prepared using osmotic shock. Periplasmic proteins were precipitated with 55% ammonium sulfate and dialyzed overnight in 50 mM NaH\(_2\)PO\(_4\), 300 mM NaCl, 10 mM imidazole. His-tagged proteins were purified on Ni-NTA-agarose according to standard procedures (Qiagen Inc.), dialyzed in 50 mM sodium acetate buffer, pH 6.5, 150 mM NaCl, and further purified on gel filtration column Superdex-75.

**Pd-His\(_6\), Expression and Purification**—Pilin domain (residues 160–279) with dsc-extension was created as described for dsc-FimH (24) with addition of His\(_6\), at the C-terminal end, amplified by PCR, and cloned into Ncol-BamHI digested pET22b expression plasmid. Expression, periplasmic preparation, and purification on Ni-NTA-agarose was done as described for Ld (see above). Ni-NTA-purified fraction was dialyzed in 50 mM NaAc, pH 5.25, and loaded on a HiTrap CM column. Pd containing fraction was eluted in 50 mM NaAc, pH 5.25, buffer containing 0.5 M NaCl and further fractionated on Superdex 75 gel filtration column.

**Purification of Fimbrial Tips**—Genes encoding for FimC (with His\(_6\), tag at the C-terminal end) and FimF-FimG-FimH were cloned into the pRSET-B expression plasmid. Expression, preparation of periplasmic fraction, and purification on Ni-NTA-agarose was done as described above. A FimC-His\(_6\),—FimFGH containing fraction after elution from Ni-NTA-agarose was dialyzed in 50 mM HEPES, pH 7.0, 0.5 M NaCl and loaded on \(\alpha\)-d-mannose-agarose column. After extensive washing tips were eluted with 10% \(\alpha\)-d-mannose and fractionated on Superdex 75 gel filtration column. Binding of the tips to the Co\(^{2+}\) beads was done as described for Ld-His\(_6\),.

**FimC-FimH Complex Purification**—Genes encoding for FimC and FimH were cloned in tandem in pRSET-C plasmid under control of the T7 promoter. Plasmid was transformed into BL21Star cells. Expression and purification of the complex was done as described previously (57).

**Bead-anchored FimH Construct Binding**—Dynabeads® Talon™ Co\(^{2+}\) beads (Dynal Biotech) were coated with His-tagged FimH constructs as advised by the manufacturer using 0.22 \(\mu\)M protein per 10 \(\mu\)L of beads. Briefly, beads were washed in sodium phosphate buffer with 0.1% Tween 20, mixed with protein, and rotated for 20 min at room temperature, then washed 5 times with 0.75 ml of same buffer. Prepared beads were used in parallel plate flow chamber experiments at a final dilution of 1:1000 in the same way as whole bacteria.

**Solution Affinity Measurement**—Solution affinity of purified fimbriae, fimbrial tips, and Ld was measured by surface plasmon resonance on Biacore2000™ (Biacore, Uppsala, Sweden) in solution competition experiments according to protocols described in the BIAsensitive Handbook (Biacore). In brief, 1M-BSA was immobilized on a CM5 sensor chip via amino coupling at 2000 resonance units (2000 pg of ligand mm\(^{-2}\)). A series of concentrations of fimbriae, tips, and Ld were injected over the 1M-BSA ligand at a flow rate of 20 ml min\(^{-1}\) in HBS-EP buffer (Biacore, Inc.) at 25 °C. To obtain the calibration curve, the average response value at a certain time point (in a 5-s time window) was plotted against concentration of the analyte. The fixed concentration of fimbriae, tips, or Ld (used for the calibration curve) was pre-equilibrated with several concentrations of \(\alpha\)-methyl-\(\alpha\)-mannopranoside (1M) and injected over the same surface. The average response value at the same time point as above was determined, and the concentration of free analyte (not bound to mannose in solution and therefore able to bind mannose immobilized on the chip) was calculated using the calibration curve. Free analyte was plotted against the concentration of 1 M, and solution affinity analysis was performed to determine solution affinity (\(K_D\), M) using a general fit solution affinity in BIAevaluation software 3.0 (Biacore AB, Inc.). All experiments were performed at least twice with every curve in triplicates, different chips, sugar batches, and protein preparations used.

**RESULTS**

**Structural Alteration of the Pilin Domain Increases Mannose Binding of FimH**—We previously compared the 1M-specific binding of bacteria expressing a hybrid adhesin containing residues 1–185 (including the entire Ld and N-terminal part of Pd) of the FimH variant from *E. coli* K12 (FimH\(_{K12}\)) and residues 186 to 273 (the rest of Pd) of the adhesin FocH, which has 80% homology to FimH (15). The hybrid exhibited very strong 1M binding activity under static conditions relative to FimH\(_{K12}\), suggesting an importance of the pilin domain to binding. At the same time, replacement of 202–273 residues with the corresponding FocH sequence did not affect binding (see supplemental Table 1S). Considering the results of the previous study, here we replaced residues 186–201 (YCAKSDKNLGYLSGTH) within the Pd of FimH\(_{K12}\) with the corresponding sequence from FocH (RCDTQTQSVSYTLSGSV) to look in more detail at the influence of Pd structure on 1M binding. This FimH\(_{K12}\) (186–201)FocH mutant exhibited very strong 1M binding (Table 1). To probe the exact mutations that are responsible for the change in phenotype, we screened a number of point substitutions within this sequence of 16 aa. Whereas most point replacements did not affect the FimH\(_{K12}\) phenotype (for some examples, see supplemental Table 1S), two substitutions, Y186R and A188D, increased 1M binding significantly (Table 1). Of these two mutations, A188D resulted in the largest change in 1M binding.

The increased 1M binding of bacteria was not due to an increased number of FimH subunits incorporated into the fimbriae (i.e., increased avidity). We have shown previously that neither the amount of FimH presented on the bacterial surface nor the fimbriae morphology change when a high 1M-binding mutant FimH or FimH/FocH hybrid is expressed (15, 25, 26). Here also, the fimbriae purified from the surface of bacteria...
expression either FimH_{K12} or mutant FimH had the same amount of FimH (see supplemental Fig. 1S).

To determine whether the increased 1M binding of bacteria was due an exposure of an additional 1M binding site within the mutant FimH, we introduced the mutation Q133A into FimH. This mutation alters a critical mannose-interacting residue within the 1M high-affinity state toward 1M in FimH_{K12} and a high-affinity state toward the 1M high-binding FimH variants.

**Mutations That Increase Affinity to Mannose Weaken the Interaction between the Ld and Pd**—Because the primary structures of FimH_{K12} and the 1M high-binding FimH mutants differ only in their Pd, we proposed that the Pd is capable of interacting with the Ld and affecting its 1M binding. Although the crystal structure of FimH shows virtually no interaction between the Ld and Pd (5), this fact could be due to the presence of a molecular chaperone protein, FimC, which was co-crystallized with FimH to ensure stability of the Pd. (FimC is complexed with FimH by a C-terminal fused chaperone protein, FimC, which was co-crystallized with FimH to ensure stability of the Pd. (FimC is complexed with FimH by a C-terminal fused chaperone protein, FimC, which was co-crystallized with FimH to ensure stability of the Pd.) To determine whether the Ld and Pd can interact in the absence of the chaperone, we employed a yeast two-hybrid system. Gene regions encoding the Ld and Pd of FimH_{K12} were fused to the LexA DNA binding domain and to the transcription activation domain of VP16, respectively, and expressed in yeast strain L40. To ensure the stability of the Pd in the absence of FimC, we used a self-complementing Pd construct with a C-terminal fused 13-aa peptide that supplies the missing $\beta$-strand to the Pd as done previously (24). The two domains from FimH_{K12} interact with each other in the yeast two-hybrid system, resulting in activation of transcription of a lacZ reporter gene and the -his3 reporter gene and the affinity of purified fimbriae, fimbrial tips, and lectin domains to soluble mannose in SPR experiments. All experiments were done in triplicate.

**TABLE 1**

| FimH variant expressed | 1M-binding of bacteria | Purified fimbriae | Fimbrial tips | Lectin domain |
|------------------------|------------------------|------------------|--------------|--------------|
| FimH_{wt}              | 2.1 ± 0.5              | 298.0 ± 50.3     | 196.5 ± 29.0 | 1.2 ± 0.42   |
| FimH_{K12}(186–201)FocH| 15.1 ± 1.4             | 1.2 ± 0.42       | ND           | As above    |
| FimH_{K12}A188D        | 14.2 ± 0.9             | 18.2 ± 1.9       | 17.1 ± 2.34  | As above    |
| FimH_{K12}Y186R        | 8.3 ± 0.5              | ND               | ND           | As above    |
| FimH_{K12}             | 1.0 ± 0.3              | ND               | ND           | As above    |
| FimH_{K12}S52A         | 7.3 ± 0.4              | ND               | ND           | As above    |
| FimH_{K12}V118D        | 13.7 ± 1.2             | ND               | ND           | As above    |
| FimH_{K12}V27A:Δ(117–120) | 11.3 ± 0.7          | ND               | ND           | As above    |

* 10^9 cfu/well.
* K_{d}, × 10^{-6} (M).
* ND, not done.
* Lectin domain identical to FimH_{wt}.

![FIGURE 1. Detection of the interaction between Ld and self-complementing Pd in the yeast two-hybrid system](image)
zole (Fig. 1B). This indicates that structural alterations within Pd significantly weakened the interaction between the domains.

In addition, we tested the Ld/Pd interaction using Ld domains from two naturally occurring FimH variants, FimH\textsubscript{F18} and FimH\textsubscript{L18:S62A}, that mediate distinctly different 1M binding under static conditions (Table 1). FimH\textsubscript{F18} represents one of the most common FimH variants among intestinal \textit{E. coli} (27) and mediates even lower static 1M binding than FimH\textsubscript{K12} (Table 1). The high 1M binding FimH variant originated in the model uropathogenic \textit{E. coli} strain NU14 and differs from FimH\textsubscript{F18} in a single replacement, S62A, in the Ld.

Because the yeast two-hybrid assay only provides a qualitative indication of protein-protein interaction, we tested binding between the Ld and Pd regardless of whether the mutations are the low-affinity to the high-affinity state weaken the interaction.

To address this issue further, we also performed a structural comparison between our docked structure and a published crystal structure (5) using the Visual Molecular Dynamics software (28). This comparison showed that the Ld and FimC protein have an overlapping volume of 323 Å\(^3\) when the Pd of two structures are aligned (not shown). This amount of overlapping volume is sufficient to prevent the Ld and Pd from forming a docked structure in the presence of the chaperone protein, indicating that native interdomain interaction does not occur in the FimH-FimC complex and, as the result, the FimH molecule is locked in a stretched conformation.

Both 1M binding enhancing residues identified in the Pd, Ala\textsubscript{188} and Tyr\textsubscript{186}, were in the predicted interface (Fig. 3C). Because Ala\textsubscript{188} had the most significant functional effect among the point mutations tested, we looked into its role in the interface formation more closely. We tested the effect of the Ala\textsubscript{188}D mutation on the stability of the docked structure of FimH. Ala\textsubscript{188} was computationally mutated to Asp\textsubscript{188} and the stability of the interdomain interaction was assessed during 3 ns of MD equilibration. The MD simulations showed that the interdomain contacts remained relatively stable in the docked structure (supplementary materials Movie 2), which supports the important role of Ala\textsubscript{188} in the interdomain interaction.

As mentioned above, valine in position 118 of Ld forms the closest contact with Ala\textsubscript{188} (Fig. 3C). Considering the dramatic

\[ \text{OD} \text{ at } 600 \text{ nm} \]

\[ \text{Ld concentration, } \mu\text{g/ml} \]

\[ \begin{align*}
\text{FimH:wtPd} & \quad \text{FimH:A188D} \\
\text{FimH(186-201)FocH Pd} & \end{align*} \]

**FIGURE 2.** Binding of Ld to various Pd in an ELISA. Three Pd variants were immobilized on the surface and incubated with soluble Ld. After rinsing, bound Ld were identified with anti-Ld polyclonal antibodies. Bars indicate mean ± S.E. All experiments have been repeated at least three times.
effect of the A188D mutation, we introduced by site-directed mutagenesis the same type of mutation into the Val\(^{118}\) position in FimH\(_{K12}\) and examined the bacterial binding to 1M-BSA. As hypothesized, the A188D and V118D mutations had the same effect on 1M binding (Table 1). To further explore this interaction, we decided to see if an alternative structural change that minimizes or abrogates any interaction with Ala\(^{188}\) would have a similar effect. Eliminating the side chain with the mutation V118G significantly increased the 1M binding of bacteria as well (supplemental materials Table 1S).

Although the proposed docked structure is generally supported by experimental data, a thorough validation of the computationally predicted interdomain interface would be labor intensive and, in the absence of x-ray information, likely to remain inconclusive. Thus, the predicted docking between the domains should be treated with caution and only as an alternative possibility for the native structure in the absence of FimC chaperone.

**Shear Stress Induces the Shift from Low- to High-affinity States in FimH\(_{K12}\)**—Taken together, the yeast two-hybrid, ELISA, and computational structural predictions suggest that mutations that enhance 1M binding also weaken the interdomain interaction. A shift from the low- to high-affinity state of FimH appears to be associated with the separation between the Ld and Pd. To explore the functional role of this interdomain interaction, we decided to test whether the domains could be separated by tensile mechanical force applied via bound mannose ligand. Such force is likely to originate in the course of FimH-mediated adhesion to the 1M-coated surface under flow-induced shear stress. Following MD equilibration of the FimH docked structure, we performed steered molecular dynamics simulations to test the stability of the predicted Ld-Pd interaction under tensile mechanical force. Force was applied to the O-1 atom of the 1M ligand and the Ca atom on the N terminus of the donated \(\beta\)-strand that completes the \(\beta\)-barrel of the Pd. This force configuration ought to mimic how tensile force is applied to FimH when it is integrated via a donated \(\beta\)-strand into the fimbrial tip. In these simulations, the domains separated from each other before the ligand detached from the pocket (supplementary materials Movie 3). Interestingly, during the course of the steered molecular dynamics simulations, the side chains between Val\(^{118}\) and Ala\(^{188}\) were among the longest lived residue pair to remain in contact among all of the inter-domain interactions shown in the docked structure. Because the interdomain interface in A188D FimH was very unstable already at the equilibration stage, we did not evaluate its stability under tensile force. We also could not evaluate the effect of the docked pilin domain on mannose binding in the MD simulations because our simulations would not be expected to distinguish any effect. This is because the docking algorithm used did not allow for conformational changes in the binding pocket. Also, a propagation of changes from the interdomain region to the binding pocket would be unlikely to be observed even in state of the art MD simulations because they often occur on the microsecond time scale or longer.

We hypothesized that an increase in tensile force, caused by an increase in flow-induced shear stress, would separate the domains in FimH\(_{K12}\) and lead to a shift from a low-affinity to a high-affinity state. If this hypothesis were correct, bacteria expressing FimH\(_{K12}\) should shift from weak to strong adhesion under increased shear. In contrast, binding mediated by a 1M high-binding FimH mutant should be strong under any shear. To test this hypothesis, we compared the 1M binding of bacteria expressing FimH\(_{K12}\) and FimH\(_{K12}\):A188D under various flow conditions. These flow studies were conducted in a parallel plate flow chamber coated with a 1M-BSA. Under low flow conditions (shear stress of 0.01 pN/\(\mu\)m\(^2\), Fig. 4A), FimH\(_{K12}\) bacteria accumulated on the 1M surface in 10-fold fewer numbers than FimH\(_{K12}\):A188D bacteria. In addition, most of the FimH\(_{K12}\) bacteria that bound to the surface at low flow did so in

**FIGURE 3. Proposed docked structure of FimH domains.** A, crystal structure of FimH complexed with the FimC chaperone (from Ref. 18, PDB code 1klf), with lectin domain (silver), pilin domain (tan), linker chain (cyan), CPK spheres of mannose (green), and FimC (light blue) surface rendering. Small blue spheres depict the Ca atoms of FimH residues that interact with FimC. B, docked structure of FimH with the interface residues shown in small colored spheres. Blue spheres show the Ca atoms of residues that also interact with FimC in the crystal structure and orange spheres represent the Ca atoms of other interface residues in the docked structure. Images were created with PyMOL Molecular Graphics System (DeLano Scientific, Palo Alto, CA). C, close-up of the interdomain interface, with lectin domain (silver), pilin domain (tan), the linker chain (yellow), residue Ala\(^{188}\) (orange), residue Tyr\(^{186}\) (red), and residue Val\(^{118}\) (blue).
Interaction between Binding and Anchoring Domains of FimH

FIGURE 4. Effect of shear stress on FimH-mediated binding. In all panels, black symbols and lines show the number of bacteria or beads binding to the surface after 5 min. Gray symbols and lines show the fraction of these adherent bacteria or beads that are rolling at the end of 5 min (the remainder are firmly adherent). A, binding of isogenic bacteria to the 1M substrate at a range of shear stress. Bacteria express either FimHK12 (squares) or FimHK12:A188D (triangles). B, binding of 1-μm beads to which FimHK12 (squares) or FimHK12:A188D (triangles) fimbrial tips have been bound. C, binding of 1-μm beads with FimHK12:Ld D, binding of 1-μm beads with the FimHK12 FimH:FimC complex. Bars indicate mean ± S.E. All experiments has been repeated at least three times.

The binding pattern exhibited by the fimbriated bacteria was reproduced by 1.0-μm diameter cobalt beads coated with purified fimbrial tips that were anchored to the beads surface via the His6-tagged FimC (Fig. 4B). That is, the FimHK12 binding to 1M is weak under low shear, but strong under high shear, whereas the FimHK12:A188D binding is already strong under low shear. This demonstrates that a possible change in the conformation of the bacterial cells or the fimbriae under shear cannot explain the phenomenon of shear-enhanced binding mediated by FimHK12. Instead, we propose that shear stress apparently causes a shift in FimH from a low-affinity to a high-affinity state. As shear is expected to result in mechanical tension between the FimH domains that is likely to lead to separation of the domains, these results support the hypothesis that the increase in 1M affinity of Ld is associated with its separation from Pd.

In the Absence of Interaction with Pd, Ld Assumes the High-affinity State—In contrast to the native, tip-incorporated forms of FimHK12, cobalt beads coated with the isolated Ld from FimHK12 (anchored to the beads via the C-terminal-fused His6 tag) exhibited a pattern of binding like that mediated by FimHK12:A188D fimbrial tips rather than that mediated by FimHK12 fimbrial tips. The maximal surface accumulation of Ld beads was already under the lowest shear, with all beads binding in firm stationary mode under any shear (Fig. 4C). Similarly, beads coated with FimHK12 complexed with the molecular chaperone FimC (tagged with His6) exhibited the same binding pattern as isolated Ld (Fig. 4D). From these studies, the Ld appears to be in a high-affinity 1M-binding state either in the absence of Pd (in the isolated Ld) or when Ld is separated from Pd by the presence of chaperone (in the FimHK12:FimC complex).

The high-affinity state of isolated Ld was confirmed in SPR analysis, which showed that the K_D of the Ld and soluble 1M was the same as the K_D of the FimHK12:(186–201)FocH mutant. This result correlates with the notion that the inter-domain interaction in FimHK12:(186–201)FocH appears to be the weakest among FimH variants tested. In addition to the Ld from FimHK12, we also purified and tested 1M affinity of Ld from FimHK12, FimHF18, and FimHF18:V118D variants above as well as from an additional FimHK12:(186–201)FocH mutant. These FimH variants mediate up to 15-fold difference in static bacterial adhesion (Table 1) due to the differences in their Ld structure. However, in SPR assays with the isolated Ld, all Ld variants exhibited similar 1M affinity relative to each other. Thus, the 1M binding enhancing mutations in the Ld have a functional effect only in the native, two-domain structure of FimH. This effect supports the hypothesis that interaction with Pd plays a critical role in modulating the differential functional properties of FimH in the fimbriae-incorporated form.
Interaction between Binding and Anchoring Domains of FimH

DISCUSSION

The data presented here suggest that the interaction between the mannose-binding lectin domain, Ld, and fimbria-anchor pilin domain, Pd, keeps the native form of FimH in a low-affinity state. Domain separation is associated with a shift in the Ld from a low 1M-binding state to a high-affinity state. The interdomain interaction is reversible and provides a mechanism for responding to dynamic changes in force. When all this evidence is considered, this interdomain interaction where the Pd can regulate the Ld binding affinity provides a structural basis for the shear-enhanced adhesion of type 1 fimbriated E. coli.

The proposed mechanism must be considered in light of the existing structural data. To date, the only crystal structure of full size FimH has been obtained in complex with the molecular chaperone, FimC, that stabilizes the tertiary structure of the Pd by donor strand complementation (5). This structure shows no interdomain interactions because the position of the chaperone prevents any interdomain contacts from forming. In principle, a crystal structure of a chaperone-free FimH could be obtained by fusing a self-complementing peptide to the C terminus of the Pd (29) or by allowing FimH to fold in the presence of a soluble complementing peptide (30). However, both of these strategies present large technical challenges for obtaining the chaperone-free FimH in the proper amount and/or conformational homogeneity that is sufficient for crystallization.

Despite the lack of x-ray data on the interdomain interaction, the existence of this interaction is supported here by detection of the Ld/Pd binding in two different experimental methods: (i) a yeast two-hybrid system and (ii) an ELISA with the purified domains. In addition, computational docking predicts specific interactions that were confirmed by mutational analysis. The fact that structural alterations in the Pd affect the functional properties of the Ld also suggest that the Ld conformation is either modulated by an interaction with the Pd in fimbriae-incorporated FimH or, alternatively, depends on the way the Ld is folded in the course of fimbrial biogenesis. The latter explanation remains a possibility considering the critical role the Pd plays in the interaction of FimH with the chaperone and its subsequent incorporation into the fimbrial tip. However, mutations in the Pd that alter function are positioned in the interface with the Ld in the crystal structure. Moreover, these mutations affect the interdomain interactions in the biochemical and computational tests used here. These results support the hypothesis that the interdomain interaction we have detected occurs in native FimH and that this interaction is important for FimH function.

The association between the FimH interdomain interaction and function is revealed by the fact that mutations in Pd that increase the 1M affinity also weaken the interdomain interaction. This association suggests that the Pd interacts with the Ld to keep the latter in a low-affinity state, whereas when the Pd is removed or interacts weakly with the Ld, the result is a high-affinity state of the binding site. Additional support for the inhibitory effect is provided by the observation that the purified Ld has an affinity to mannose that is more than 200-fold higher than the affinity of fimbriae- or tip-associated FimH_{K12} variant where the interdomain interaction is relatively strong. The affinities of the Lds toward mannose are similar to that of the FimH_{K12}:(186–201)FocH variant where the interdomain interaction has been abrogated by the 16-residue replacement in the Pd (Fig. 3). The Ld-mannose affinity levels obtained here are also similar to those obtained by others (31).

The notion that the quaternary structure plays a regulatory role is further supported when we consider the fact that the purified Ld variants show no functional difference but exhibit very distinct 1M binding phenotypes when they are part of native FimH. These data correspond to the results obtained by others who found that the Lds from functionally distinct FimH variants showed similar affinities toward different soluble mannosylated compounds (31). The similar binding pattern of different Ld variants contrasting with the variable binding pattern of different full-length FimH variants incorporated into fimbrial tips implies that native FimH has an additional factor that modulates the Ld affinity. This supports the hypothesis that the Ld/Pd interaction has a functional importance.

For several other receptor proteins, a connection has also been established between an interdomain interaction and the affinity toward ligand. In integrins, for example, low- and high-affinity toward ligands is associated with a bent (closed) and extended (open) conformation between the binding I-domains and the neighboring non-binding “thigh” domain of the β-integrin (32–34). Another adhesive protein, P-selectin, is a Lewis X-specific protein found on the surface of leukocytes, where a closed and open configuration between the binding lectin and anchoring epidermal growth factor-like domains is also observed (35) and is associated with low- and high-affinity binding (36). Also, binding of von Willebrand factor that mediates platelet adhesion to extracellular matrices is affected by interaction between binding A1 and the neighboring D’D3 domain (37). Thus, interdomain interaction as a mechanism for controlling binding properties of the adhesive proteins could be a common phenomenon. In some cases, like integrins, modulation of the affinity by the interdomain configuration is proposed to occur due to an allosteric connection between the interdomain interface and the binding site (32). In the case of von Willebrand factor, the neighboring domain is proposed to shield or mask the binding site (37). In the case of FimH, a shielding effect is unlikely due to the location of the mannos-binding site on the opposite side of the Ld from the Ld/Pd interface. Thus, it is most likely that the regulation of FimH affinity by the interdomain interaction occurs via an allosteric mechanism, the structural details of which remain to be determined.

We propose that the shift from the low- to high-affinity state of FimH upon separation of the Ld from the Pd provides a mechanism for how shear enhances FimH-mediated adhesion. The drag force that occurs upon binding of a bacterial cell or FimH-coated bead under shear stress is expected to result in a tensile force that is applied between the bound mannose and interdomain linker chain, the anchor point of the Ld to Pd. This would lead to disruption of the Ld/Pd interaction and thus a shift from low- to high-affinity interaction with mannose. We propose that such a shift is the basis of increased surface accumulation and binding strength of FimH_{K12} mediated adhesion.
of bacteria and tip-coated beads that occurs at increased flow. Consequently, strong adhesion under low shear would be expected and, in fact, is shown here for adhesion mediated by purified Ld, FimH-FimC complex, or the FimH variants, where the binding site might be either locked in or easily shifting toward the high-affinity state.

One needs to note here that FimC chaperone is not part of the fimbria, it is replaced with the FimG subunit in the course of fimbrial biogenesis via donor-strand swapping mechanism. Thus, FimH is complexed with FimG in the fimbrial tip. Although structural details of the FimH-FimG complex are unknown, FimG donates its N terminus as β-strand in the Pd groove, in the opposite direction from the complementing strand of FimC (5). Thus, FimG protein is expected to be on the opposite site of the pilin domain from the interdomain interface. In this position, it should not interfere with interdomain interaction and, thus, catch bond properties of FimH in the fimbriae as seen in the experiments with purified tips.

We have proposed previously that extension of the interdomain linker chain from the bottom of the Ld is associated with increased binding under shear stress (7). Our new observation that the Pd is an inhibitory modulator is consistent with this previous model of FimH activation under force because the linker chain extension would lead to separation of the Ld/Pd interaction. Also, the functional importance of the interdomain interaction provides a plausible explanation for why most of the naturally occurring mutations that enhance 1M binding of bacteria under static conditions are located within or in a close proximity to the interface between the two domains (38). However, the structural details of how the interdomain interaction is linked to a shift in affinity remains to be determined.

The shear-enhanced binding phenomenon has been shown for P-, L-, and E-selectins that mediate flow-dependent adhesion of leukocytes to the endothelium (9, 39). For this receptor-ligand interaction, a connection between the binding affinity and interdomain interaction has also been proposed. Disruption of the native interdomain structure by structural mutation or glycosylation leads to stronger selectin-mediated adhesive interactions (36, 40). Considering the similarities between structure-functional properties of selectins and FimH, the basic mechanism of shear-enhanced binding could be the same in both systems.

One needs to note, however, that it has also been proposed that shear-enhanced adhesion of leukocytes does not involve a shift in binding affinity, but instead is driven by an increase in the number of selectin bonds forming at high shear (41–43). However, force enhancement of the individual P- and L-selectin bonds is supported by single molecule studies employing atomic force microscopy (12, 13). Similarly, the phenomenon of shear-enhanced FimH-mediated adhesion was also suggested to be due to an increased number of FimH-surface bonds that might form upon uncoiling of the fimbrial rod under tensile force (44). Our data presented here show that fimbrial rod is not needed for shear-enhanced FimH-mediated adhesion, because the phenomena can be reproduced by purified fimbrial tips anchored to metal beads, which lack the fimbrial rods. Instead, we have recently shown that uncoiling/recoiling of the fimbriae is important for sustaining the optimal level of tensile force on the FimH-1M bond to prolong the lifetime of the high-affinity state of FimH (45).

Tensile force-enhanced receptor-ligand interactions have been called “catch bonds” to distinguish them from the traditional slip bonds that break more rapidly if force is increased (11). In addition to FimH and P- and L-selectin-mediated binding, a force- or shear-enhanced mode of interaction was shown for digalactose-specific E. coli adhesion (46), actin-myosin interaction (47), and platelet adhesion mediated by von Willebrand factor (48, 49). The catch bond mechanism has also been proposed for integrins based on structural evidence that high-affinity (activated) states are stretched relative to low-affinity structures (32, 34, 50, 51). All of these interactions are mediated by multidomain receptor proteins and thus might have a common mechanism of mechanical regulation of binding strength.

A number of alternative mathematical or biophysical models have been suggested for the catch bond mechanism. These include a shift in the binding pocket conformation as proposed by the allosteric catch bond model (52), a dissociation of the ligand through a force-inhibited pathway as proposed by various two-pathway models (14, 53–55), and a change in the angle of detachment that would affect the ability to rebind as suggested by the sliding-rebinding model (40, 56). However, it remains unknown whether any of the receptor-ligand interactions that mediate shear-enhanced adhesion involve any of these mechanisms. The finding of interdomain regulation of adhesin binding in FimH provides an important step in dissecting the structural basis of FimH-mediated catch bonds and, possibly, other biological adhesive interactions shown or expected to be modulated by force or shear stress.

REFERENCES

1. Deller, M. C., and Yvonne Jones, E. (2000) Curr. Opin. Struct. Biol. 10, 213–219
2. Isberg, R. R., and Barnes, P. (2002) Cell 110, 1–4
3. Konstantopoulos, K., Hanley, W. D., and Wirtz, D. (2003) Curr. Biol. 13, R611–R613
4. Zhu, C., and McEver, R. P. (2005) Mol. Cell Biochem. 2, 91–104
5. Choudhury, D., Thompson, A., Stojanoff, V., Langermann, S., Pinkner, J., Hultgren, S. R., and Knight, S. D. (1999) Science 285, 1061–1066
6. Thomas, W. E., Nilsson, L., Forero, M., Sokurenko, E. V., and Vogel, V. (2004) Mol. Microbiol. 53, 1545
7. Thomas, W. E., Trintchina, E., Forero, M., Vogel, V., and Sokurenko, E. V. (2002) Cell 109, 913–923
8. Sokurenko, E. V., Chesnokova, V., Doyle, R. J., and Hasty, D. L. (1997) J. Biol. Chem. 272, 17880–17886
9. Finger, E. B., Puri, K. D., Alon, R., Lawrence, M. B., von Andrian, U. H., and Springer, T. A. (1996) Nature 379, 266–269
10. Thomas, W. E., Forero, M., Yakovenko, O., Nilsson, L., Vicini, P., Sokurenko, E. V., and Vogel, V. (2006) Biophys. J. 90, 753–764
11. Dembo, M., Torney, D. C., Saxman, K., and Hammer, D. (1988) Proc. R. Soc. Lond. B Biol. Sci. 234, 55–83
12. Marshall, B. T., Long, M., Piper, J. W., Yago, T., McEver, R. P., and Zhu, C. (2003) Nature 423, 190–193
13. Sarangapani, K. K., Yago, T., Klopotcki, A. G., Lawrence, M. B., Fieger, C. B., Rosen, S. D., McEver, R. P., and Zhu, C. (2004) J. Biol. Chem. 279, 2291–2298
14. Evans, E., Leung, A., Heinrich, V., and Zhu, C. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 11281–11286
15. Sokurenko, E. V., Schembri, M. A., Trintchina, E., Kjaergaard, K., Hasty, D. L., and Klemm, P. (2001) Mol. Microbiol. 41, 675–686
Interaction between Binding and Anchoring Domains of FimH

16. Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993) Cell 74, 205–214
17. Gray, J. J., Moughon, S., Wang, C., Schuler-Furman, O., Kuhlman, B., Rohl, C. A., and Baker, D. (2003) J. Mol. Biol. 331, 281–299
18. Hung, C. S., Bouckaert, J., Hung, D., Pinkner, J., Widberg, C., DeFusco, A., Auguste, C. G., Strouse, M., Langemer, S., Waksman, G., and Hultgren, S. J. (2002) Mol. Microbiol. 44, 903–915
19. Rohl, C. A., Strauss, C. E., Misura, K. M., and Baker, D. (2004) Methods Enzymol. 383, 66–93
20. Kale, L., Skeel, R., Bhandarkar, M., Brunner, R., Gursoy, A., Krawetz, N., Phillips, J., Shinozaki, A., Varadarajan, K., and Schulten, K. (1999) J. Comput. Phys. 151, 283–312
21. Canutescu, A. A., Shelenkov, A. A., and Dunbrack, R. L., Jr. (2003) Protein Sci. 12, 2001–2014
22. Bouckaert, J., Berglund, J., Schembri, M., De Genst, E., Cools, L., Wuhrer, M., Hung, C. S., Pinkner, J., Slattegard, R., Zavialov, A., Choudhury, D., Langerner, S., Hultgren, S. J., W. L., Kremer, S., Oscarson, S., Knight, S. D., and De Greve, H. (2005) Mol. Microbiol. 55, 441–455
23. Schembri, M. A., Hasman, H., and Klemm, P. (2000) FEMS Microbiol. Lett. 188, 147–151
24. Barnhart, M. M., Pinkner, J. S., Soto, G. E., Sauer, F. G., Waksman, G., Frieden, C., and Hultgren, S. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7709–7714
25. Sokurenko, E. V., Chesnokova, V., Dykhuizen, D. E., Ofek, I., Wu, X. R., Krogfelt, K. A., Struve, C., Schembri, M. A., and Hasty, D. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8922–8926
26. Sokurenko, E. V., Feldgarden, M., Trintchina, E., Weissman, S. J., Avagyan, S., Chattopadhyay, S., Johnson, J. R., and Dykhuizen, D. E. (2004) Mol. Biol. Evol. 21, 1373–1383
27. Weissman, S. J., Chattopadhyay, S., Aprikian, P., Obata-Yasuoka, M., Yarova-Yarova, Y., Stapleton, A., Ba-Thein, W., Dykhuizen, D., Johnson, J. R., and Sokurenko, E. V. (2006) Mol. Microbiol. 59, 975–988
28. Humphrey, W., Dalke, A., and Schulten, K. (1996) J. Mol. Graph. 14, 28–35
29. Barnhart, M. M., Sauer, F. G., Pinkner, J. S., and Hultgren, S. J. (2003) J. Bacteriol. 185, 2723–2730
30. Vetsch, M., Puorger, C., Spirig, T., Grauschopf, U., Weber-Ban, E. U., and Glockshuber, R. (2004) Nature 431, 329–333
31. Bouckaert, J., Mackenzie, J., De Paz, J. L., Chipwaza, B., Choudhury, D., Zavialov, A., Mannerstedt, K., Anderson, J., Pierard, D., W. L., Kremer, S., Oscarson, S., De Greve, H., and Knight, S. D. (2006) Mol. Microbiol. 61, 1556–1568
32. Xiao, T., Takagi, J., Coller, B. S., Wang, J. H., and Springer, T. A. (2004) Nature 432, 59–67
33. Vinogradova, O., Haas, T., Plow, E. F., and Qin, J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1450–1455
34. Takagi, J., Petre, B. M., Walz, T., and Springer, T. A. (2002) Cell 110, 599–611
35. Graves, B. I., Crowther, R. L., Chandran, C., Rumbaugh, J. M., L. S., Huang, K. S., Presky, D. H., Familietti, P. C., Wolitzky, B. A., and Burns, D. K. (1994) Nature 367, 532–538
36. Phan, U. T., Waldron, T. S., and Springer, T. A. (2006) Nat. Immunol. 7, 883–889
37. Ulrichs, H., Udvardy, M., Lenting, P. J., Pareyn, I., Vanhoozerbeke, K., and Deckmyn, H. (2006) J. Biol. Chem. 281, 4699–4707
38. Schembri, M. A., Sokurenko, E. V., and Klemm, P. (2000) Infect. Immun. 68, 2638–2646
39. Lawrence, M. B., Kansas, G. S., Kunkel, E. J., and Ley, K. (1997) J. Cell Biol. 136, 717–727
40. Lou, J., Yago, T., Klopocki, A. G., Mehta, P., Chen, W., Zarrinsyra, V. L., Bovin, N. Y., Zhu, C., and McEver, R. P. (2006) J. Cell Biol. 174, 1107–1117
41. Chang, K. C., and Hammer, D. A. (1999) Biophys. J. 76, 1280–1292
42. Dwir, O., Solomon, A., Mangan, S., Kansas, G. S., S. M., and Alon, R. (2003) J. Cell Biol. 163, 649–659
43. Chen, S., and Springer, T. A. (1999) J. Cell Biol. 144, 185–200
44. Miller, E., Garcia, T. L., Hultgren, S., and Oberhauser, A. (2006) Biophys. J. 91, 3848–3856
45. Forero, M., Yakovenko, O., Sokurenko, E. V., Thomas, W. E., and Vogel, V. (2006) PLoS Biol. 4, 1509–1516
46. Nilsson, L. M., Thomas, W. E., Trintchina, E., Vogel, V., and Sokurenko, E. V. (2006) J. Biol. Chem. 281, 16656–16663
47. Guo, B., and Guilford, W. H. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 9844–9849
48. Marchese, P., Saldivar, E., Ware, J., and Ruggeri, Z. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 7837–7842
49. Doggett, T. A., Girdhar, G., Lawshe, A., Schmidtko, D. W., Laurenzi, I. J., Diamond, S. L., and Diacovo, T. G. (2002) Biophys. J. 83, 194–205
50. Salas, A., Shimaoka, M., Phan, U., Kim, M., and Springer, T. A. (2006) J. Biol. Chem. 281, 10215–10221
51. Luo, B. H., Takagi, J., and Springer, T. A. (2004) J. Biol. Chem. 279, 1107–1117
52. Thomas, W. (2006) J. Cell Biol. 174, 911–913
53. Barsegov, V., and Thirumalai, D. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 1835–1839
54. Pereverzev, Y., Prezhdzo, O. V., Forero, M., Sokurenko, E., and Thomas, W. (2005) Biophys. J. 89, 1446–1454
55. Pereverzev, Y. V., Prezhdzo, O. V., Thomas, W. E., and Sokurenko, E. V. (2005) Phys. Rev. E Stat. Nonlin. Soft. Matter Phys. 72, 010903
56. Lou, J., and Zhu, C. (2007) Biophys. J. 95, 1471–1485
57. Jones, C. H., Pindner, J. S., Nicholes, A. V., Soman, L. N., Abraham, S. N., and Hultgren, S. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8397–8401