The FimH protein is the adhesive subunit of *Escherichia coli* type 1 fimbriae. It mediates shear-dependent bacterial binding to monomannose (1M)-coated surfaces manifested by the existence of a shear threshold for binding, below which bacteria do not adhere. The 1M-specific shear-dependent binding of FimH is consistent with so-called catch bond interactions, whose lifetime is increased by tensile force. We show here that the oligosaccharide-specific interaction of FimH with another of its ligands, trimannose (3M), lacks a shear threshold for binding, since the number of bacteria binding under static conditions is higher than under any flow. However, similar to 1M, the binding strength of surface-interacting bacteria is enhanced by shear. Bacteria transition from rolling into firm stationary surface adhesion as the shear increases. The shear-enhanced bacterial binding on 3M is mediated by catch bond properties of the 1M-binding subsite within the extended oligosaccharide-binding pocket of FimH, since structural mutations in the putative force-responsive region and in the binding site affect 1M- and 3M-specific binding in an identical manner. A shear-dependent conversion of the adhesion mode is also exhibited by P-fimbriated *E. coli* adhering to digalactose surfaces.

Bacterial adhesion, a critical initial step in colonization and biofilm formation, is commonly mediated by carbohydrate-binding lectin-like proteins expressed on the bacterial surface as part of hair-like fimbrial appendages or as nonfimbrial components (1–4). Lectins are a structurally diverse class of receptor proteins that bind monosaccharide or, more commonly, oligosaccharide ligands in a noncovalent and nonenzymatic fashion (5). In general, receptor-ligand interactions (including lectin-saccharide binding) are thought to occur via slip bonds, where the strength of the binding is highest under no tensile force (6, 7). Thus, the surface adhesion of eukaryotic or bacterial cells is expected to be strongest and have the highest level of surface accumulation at the lowest shear stress. However, several studies have demonstrated shear-enhanced bacterial and cell adhesion where surface binding under static or low shear conditions is too weak to mediate adhesion of whole cells but becomes significantly stronger at increased shears, resulting in dramatically higher surface accumulation of cells at elevated shear stresses (i.e. a shear threshold for surface adhesion is seen). This has been shown for von Willebrand-mediated adhesion of platelets (8, 9), for saccharide-specific leukocyte surface rolling mediated by P- and L-selectin (10–12), and for adhesion of *Escherichia coli* mediated by binding of the fimbrial lectin FimH to monomannose surfaces (13). It has been suggested that shear thresholds for binding could result by forming not slip bonds but catch bonds whose lifetime is increased by flow-induced tensile forces (14–17).

Type 1 fimbriae are the most common type of adhesive organelles in *E. coli* and other enterobacteria and mediate mannose-specific adhesion via the fimbrial tip-associated lectin-like subunit FimH (30 kDa). FimH is a two-domain protein consisting of the mannose-binding lectin domain and the fimbria-incorporating pilin domain. On the top of the lectin domain, distal from the pilin domain, is the ligand-binding pocket that accommodates a 1M residue (18). The ability of FimH to bind to 1M under static, no-flow conditions is a highly variable property. *E. coli* binding to 1M via most common naturally occurring FimH variants (especially ones originating in intestinal *E. coli* isolates) is very low. In contrast, adhesion of uropathogenic *E. coli* (primarily those causing pyelonephritis) is commonly mediated by the FimH variants that carry point structural mutations enabling them to bind relatively well to 1M in static assays (19, 20).

When mediated by FimH, *E. coli* adhesion to 1M-BSA surfaces is dramatically enhanced under flow conditions (after shear stress reaches a certain level) compared with static conditions (16). Thus, binding to 1M is enhanced by shear and requires shear threshold for adhesion to occur. Using steered molecular dynamics simulations and site-directed mutagenesis of FimH, we have shown that the affinity to 1M is probably enhanced by the tensile force-induced extension of a 3-amino acid linker chain between the two domains of FimH, distal from the mannose-binding site (13).

Whereas most variants of FimH bind only weakly to 1M substrates in static conditions, all natural FimH variants exhibit strong oligosaccharide-specific binding to 3M already in the absence of flow that is up to 20 times higher than the binding to 1M (19, 21). This is thought to be due to the presence of an extended FimH binding pocket with multiple subsites corresponding to the size of a trimannose (3M) and, possibly, pentasaccharide (22–24). Indeed, although some lectins are capable of binding simple monosaccharides, it is rather typical for lectins to bind specifically to complex oligosaccharide ligands that ensure high affinity and specificity for cell-cell or cell-surface interactions (25).

The abbreviations used are: 1M, monomannose; 3M, trimannose; PBS, phosphate-buffered saline; BSA, bovine serum albumin; RB, RNase B; pN, piconents. aMM, methyl-α-D-mannopyranoside.
We show here that the number of *E. coli* binding to 3M surfaces lacks a shear threshold (i.e. is both high and maximal at the lowest shear). Despite this, the surface adhesion strength of individual bacteria on 3M was still enhanced by shear stress; at low shear, bacteria rolled along the surface, but as the shear was increased, the bacteria stopped and adhered firmly. Thus, we show here that, in analogy to FimH-1M, the lifetime of the FimH-3M complex can also probably be switched by force from short to long lived. Finally, shear-enhanced adhesion independent of FimH, but without a binding shear threshold, is demonstrated by *E. coli* expressing the P fimbrial adhesin PapG, a bacterial adhesin that binds specifically to digalactose.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains**—Recombinant bacterial strains expressing naturally occurred and mutant FimH variants were constructed as described previously (13). *E. coli* expressing FimH from model fecal isolate F18 (with a low 1M binding ability under static conditions) were used for all FimH experiments except those testing the effect of point mutations (Figs. 5 and 6), where FimH from a model uropathogenic isolate J96 (with a moderate 1M binding under static conditions) was used. A recombinant P-fimbriated strain of *E. coli* G1122 was provided by Dr. Steve Moseley (University of Washington, Seattle, WA). Bacteria were grown overnight in SB broth (type 1 fimbriated and nonfimbriated E. coli G1122) and used to inoculate 5 ml at a density of 109 colony-forming units/ml of TSB broth. Cells were refreshed with 10 ml of TSB broth and grown for 2 h at 37 °C with 100 μg/ml bovine RNase B for the P-fimbriated strain, 2% methyl-α-D-mannopyranoside was also added in order to inhibit any potential mannose-specific interactions.

**Coated with a 3M Ligand**—Glycoprotein-coated dishes were prepared by incubating 35-mm tissue culture dishes (Corning) for 75 min at 37 °C with 100 μl of 0.02% bicarbonate buffer containing one of the following: 200 μg/ml mannosamine-BSA (1M-BSA; EY Laboratories); 0.2, 2, 20, 100, or 200 μg/ml RNase B (3M-RB; Sigma); or a dilution of purified pigeon ovalbumin (provided by Dr. J. Johnson, University of Minnesota, Minneapolis, MN). Incubation was followed by quenching with PBS-BSA. Unless otherwise noted, 2 μg/ml RNase B was used for 3M experiments, since this resulted in a similar peak number of bacteria adhered as on 200 μg/ml 1M-BSA. In order to increase adhesion, 20 μg/ml RNase B was used for both static and flow trials of the pocket mutant strains.

**Parallel Plate Flow Chamber Setup**—All experiments were performed at room temperature in PBS-BSA buffer. The protein-coated dishes formed the bottom of a 2.5 cm (length) x 2.5 cm (width) x 250 μm (height) parallel plate flow chamber (GlycoTech). One or more Harvard Apparatus or Warner Instruments syringe pumps set at various flow rates were used to produce various wall shear stresses at the flow chamber wall. The bound bacteria were recorded using a Nikon TE200 or a Nikon Diaphot inverted microscope with a 10× phase-contrast objective, a Roper Scientific high resolution CCD Cascade camera, and MetaMorph® or MetaView® (Universal Imaging Corp.™) video acquisition software or with a Nikon TE2000-E microscope, Hamamatsu Photonics EM CCD-C9100 camera, and SimplePCI video acquisition software (C-Imaging Systems). The field of view was 500 x 370 μm with a resolution of 0.77 μm/pixel for the cascade camera, whereas for the Hamamatsu camera, 50–60% larger fields of views where used, and results were subsequently normalized for the smaller cascade field of view, which was predominantly used. The bound bacteria were recorded in time lapse digital videos. The camera shutter was held open for a time that depended upon the shear rate so that free-floating bacteria were blurred out, whereas surface-interacting bacteria could be clearly visualized, as previously described (16). In any given experiment, less than 2% of the surface area was covered by bacteria, so that any interactions between bacteria were minimal. Images were acquired every 0.5–2 s, depending on the experiment.

**Analyzing the Bacterial Adhesion in Time Lapse Videos**—The digital videos were analyzed using MetaMorph® and MetaView™ Imaging System (some additional manual counting was done with SimplePCI). To count bound bacteria, we manually determined an intensity threshold that correctly distinguished the bacteria from the background and used the automated cell counting package in MetaMorph®. To measure the level of bacterial adhesion (accumulation, i.e. the overall number of surface-bound bacteria), we counted the number of bacteria adhered to the surfaces after allowing bacteria to flow over the noted surface for 5 min at the given shear.

Type 1 fimbriated bacteria were defined as rolling if they moved at least one-half bacterial diameter (roughly 2 pixels) over 20 s, since rolling bacteria should move at least 2 μm on 3M surfaces in this time (supplemental Fig. S1). For P-fimbriated bacteria, rolling is shown (Fig. 8) with a 1-s rather than 20-s definition or rolling, to reflect the faster bacterial movement on pigeon ovalbumin compared with 3M. The number of rolling (and, to a lesser extent, detaching) bacteria at a given time was identified by subtracting the intensity values in each pixel in the second image from the first; the stationary bacteria then blended in with the gray background, whereas the rolling bacteria appeared as a pair of dark and light spots, which were counted using a threshold and either the automated or manual object counting package. The rest of the bound bacteria were then assumed to be firm. In Figs. 3 and 5, the fraction firm was determined by examining the last 20 s of the 5-min accumulation videos, described above.

When examining the movement of bacteria expressing FimH pocket 1M mutants, the fraction firm bacteria was determined over a 15-min time interval (rather than 20 s as above) to show the nearly complete lack of movement of the parent strain and the Y137A mutant and to ensure that slow bacterial movement was captured. For both 0.15- and 1.5-pN/μm² runs, bacteria were first loaded at 0.15 pN/μm² before the flow was switched to a buffer stream set at the appropriate shear and the 15 min experiment was started. Since the mutant strains Y137A and D141A adhered at much lower numbers than the parent strain and the D140A strain, they were loaded at a concentration of 10³ colony-forming units/ml rather than the normal 10⁶ colony-forming units/ml.

**Static Adhesion Assay**—The bacterial binding level under static conditions was determined as described previously (13).

**Statistical Analysis**—When the number of attachments or accumulated bacteria was measured, the exact 95% confidence intervals for a Poisson variable were used. When instead a fraction was measured, the exact 95% confidence intervals for a binomial variable were used.

**RESULTS**

**Shear Stress Does Not Enhance Accumulation of *E. coli* on Surfaces Coated with a 3M Ligand**—We tested the ability of bacteria that expressed a shear-enhanced 1M-binding FimH phenotype (from *E. coli* strain F18) to adhere under flow conditions to surfaces coated with bovine RNase B, a model ligand containing N-linked high mannose type with terminally exposed 3M structures (3M-RB) (26). In contrast to adhesion to 1M-BSA (Fig. 1A), shear stress did not cause any significant increase in accumulation of *E. coli* on surfaces coated with 3M-RB (Fig. 1B). The bacteria adhered readily on the 3M surfaces at the lowest shears tested, and accumulation decreased with increased shear (occasional small increases in accumulation were not statistically significant). This pattern of adhesion did not change when the coating concentra-
Shear-enhanced Adhesion of E. coli to Trimannose

FIGURE 1. The effect of shear stress on bacterial accumulation on 1M and 3M. Shown is E. coli accumulation on 1M (A) and 3M (B) surfaces under variable shear conditions. Concentration of 3M-RB used for plates coating was as follows: 100 μg/ml (RB100; ▲), 2 μg/ml (RB2; △), and 0.2 μg/ml (RB0.2; ○). In control runs with 100 μg/ml (RB100; ●) and 2 μg/ml (RB2; ○)-coated plates, 8% αMM was added to the bacterial solution in order to inhibit mannose-specific adhesion. The nonfimbriated parent strain AAEC191A did not adhere to test surfaces (RB2, ×). The error bars show 95% confidence intervals.

Adhesion to both 1M (16) and 3M (Fig. 1B) is inhibited by the addition of soluble methyl-a-d-mannopyranoside (αMM) to the bacterial solution. Similarly, E. coli lacking the type 1 fimbrial plasmids were unable to adhere to the RB substrate (control strain AAEC191A; Fig. 1B). E. coli adhesion to the RB surface was thus specific to the FimH-mannose interaction.

Thus, oligosaccharide-specific binding of FimH to 3M does not require a shear threshold (i.e. it is high and maximal under the lowest shear), which is in contrast to the shear-enhanced 1M-specific binding of FimH.

Differences in 1M- and 3M-specific Accumulation under Shear Are Due to Differences in Both Attachment and Detachment Rates—We next determined whether 3M- and 1M-specific binding differ in the initial attachment rate or in the adhesion lifetime at different shears. The rate of initial E. coli interactions with the 1M and 3M surfaces was determined from accumulation videos, where images were collected twice/s. Shear stress inhibited initial attachments on both 3M-RB2 (Fig. 2A, ▲) and 1M-RB (■) but with a sharper decline in initial attachments on 3M compared with 1M. This indicates a slower on-rate of 3M-specific compared with 1M-specific adhesion under the test conditions.

We next examined the proportion of surface-interacting bacteria that remained bound only transiently (for less than 1 s). On the 1M-RB-coated surface, 83% of E. coli surface interactions were transient at the low shears, and longer term interactions dominated only above 0.05 pN/μm² (Fig. 2B, ▲). The shear-induced transition from transient to longer term surface interaction (with a relatively slow decline of the on-rate with increased shear) was the main cause of the shear-increased bacterial accumulation on 1M-RB. In contrast to 1M-RB, over the same shear range, transient adhesion on the 3M-RB-coated surface remained relatively constant and at only 3–13% of total adhesion events, demonstrating a lower bacterial surface off-rate for 3M- than for 1M-specific FimH adhesion (Fig. 2B, ■).

Thus, the low bacterial detachment rate over a broad shear range combined with a sharp drop in on-rate under shear increase was responsible for the decreased accumulation of bacteria under shear observed on the 3M-RB surfaces.

Increased Shear Stress Results in a Transition from Rolling to Stationary E. coli Adhesion—The shear-enhanced adhesion mediated by FimH-3M bonds can be seen when examining the adhesion mode of already adherent bacteria. When FimH-expressing E. coli do adhere to 1M-RB, they exhibit two adhesion modes: firm stationary binding and weak rolling adhesion, as shown previously (16). In the latter mode, bacteria move along the surface at speeds significantly slower than the near surface fluid velocity. The rolling mode dominates at lower shears, but bacteria convert into stationary adhesion if the shear is increased (i.e. E. coli exhibits shear-dependent “stick-and-roll” adhesion on 1M (16)) (Fig. 3A).

To see if a shear-induced stick-and-roll transition from rolling to stationary adhesion could be seen on 3M, we determined the fraction of bacteria that were stationary (as opposed to rolling or, occasionally, detaching) across the shear spectrum. Bacteria were defined as stationary if they moved less than one-half cell diameter over the last 20 s of accumulation experiments. Within a lower shear stress range (0.01–0.1 pN/μm²), about 60 and 30% of surface-attached bacteria adhered firmly (Fig. 3B) on surfaces coated with 200 μg/ml (3M-RB200) and 2 μg/ml (3M-RB2), respectively. Within an intermediate shear range (0.1–1.0 pN/μm²), bacteria increasingly adhered in a firm mode on both 3M surfaces, and almost all bacteria were stationary at high shears (1.0–4.3 pN/μm²).

Whereas bacteria rolled along the surface at intermediate shears and transitioned into firm adhesion as the flow was increased on both
1M-BSA and 3M-RB, they rolled significantly slower on 3M than on 1M. On 1M-BSA, rolling velocities averaged 5–20 μm/s, compared with averages of 0.1–0.4 μm/s on 3M-RA at 0.37 pN/μm², despite a 100-fold lower 3M-RB than 1M-BSA concentration being used to prepare the surfaces (supplemental Fig. S1). Similarly, the stationary mode of bacterial adhesion was weaker on 1M than 3M; at 1.1 pN/μm², where bacteria adhere firmly (Fig. 3), 20% of initially adherent bacteria moved within 1 min on 1M, whereas it took 30 min for the same amount of movement to occur on 3M (supplemental Fig. S2).

Thus, whereas increased shear did not increase the overall number of bacteria adhering on the 3M surface, it still increased the strength of adhesion of individual adherent bacteria, causing a transition from rolling to stationary adhesion. However, on 3M, bacteria both rolled slower and had a longer stationary lifetime compared with on 1M.

Mutations Decreasing Shear Dependence of 1M Binding Similarly Affect 3M-specific Binding—We have proposed previously that the FimH-mediated adhesion of E. coli to 1M can be tuned by point mutations that affect extension (by shear-derived tensile force) of the linker chain between the pilin and the lectin domain of FimH (16). We tested whether the putative linker extension-inhibiting mutations Q32A/S124A or the extension-facilitating mutation V156P (Fig. 4, A and C), which increase or decrease, respectively, the shear dependence of binding to 1M, also affect 3M binding under shear stress. Neither V156P nor Q32A/S124A had a significant effect on E. coli accumulation on 3M-coated surfaces under shear (Fig. 5A), whereas the effect on the 1M-coated surfaces was obvious (Fig. 5B). However, the difference between the FimH variants in the shear-dependent stick-and-roll transition on 3M substrate was rather dramatic (Fig. 5C) and corresponded well to the effects on 1M-coated surfaces (Fig. 5D). On both surfaces, the linker extension-inhibiting mutations Q32A/S124A resulted in a 2-fold increase, on average, in the proportion of bacteria rolling rather than adhering firmly at low shears (i.e. made the adhesion more dependent on shear). In contrast, the extension-facilitating mutation V156P resulted in the vast majority of bacteria adhering in stationary mode at all shears on both 3M and 1M surfaces (i.e. made the adhesion significantly less dependent on shear).

Thus, mutations that increase or decrease shear dependence of 1M binding also do so to the 3M-specific interaction, although in the latter case affecting mainly the stick-and-roll behavior under shear. These results strongly indicate that a similar mechanism involving the inter-domain linker region of FimH is responsible for the shear-dependent binding of bacteria via FimH to 1M and 3M surfaces.

Mutations in the 1M-binding Pocket Similarly Affect 1M- and 3M-specific Binding under Shear—To test further to what extent the 1M-binding subsite is involved in the 3M-specific interaction under shear, we modified some of the residues that constitute the 1M-binding site according to the crystal structure (18) and determined their effect on the 3M-specific binding under various shear stresses. Most of the 1M-binding residues are primarily clustered on two larger loops that form a hydrophobic ring around the FimH 1M-binding site, with the N terminus also contributing to the ligand interaction. Most of the mutations tested here have been tested previously in static conditions only and in the shear-dependent FimH variant (from E. coli strain J96) with relatively weak 1M-binding under static conditions (18). Instead, we introduced these and other mutations into the background of the same FimH variant but with the V156P mutation that mediates a high level and strength of adhesion to both 1M and 3M ligands already in static conditions (see above). This provides better means to examine quantitatively the functional effects of binding site substitutions.

Residues Asn46, Ile53, Asp54, Asp140, and Gln133, which all hydrogen-bond to 1M in the crystal structure (18), and residues Tyr137 and Asp141, which also define the 1M subsite, were replaced one by one with alanine (Fig. 4). In order to assess the mutations that probably block access to the 1M-binding site, residues Asn46 and Asp54 were also mutated to glutamic acid and glutamine, respectively. Finally, a glycine was introduced between the 1M-binding residue Phe1 and residue Ala2, which are both located at the base of the pocket (mutation 1G2). None of the
mutations affected incorporation of FimH into the surface-expressed fimbriae as determined with anti-FimH antibodies (not shown).

In static assays, individual pocket mutations caused a similar reduction in binding on 3M and on 1M or, in the case of the IG2 and D54A mutations, conversion into non-mannose-specific binding mode (Fig. 6A). This suggests that the 3M ligand of 3M-RB is likely to bind to the extended FimH binding pocket in such a way that its terminal mannose residue is incorporated into the 1M pocket in a similar way as the 1M ligand of 1M-BSA. Thus, the 1M-binding site of FimH identified in the crystal structure is a critical subsite within the 3M-binding extended pocket of FimH.

Under flow, replacements N46A and I52A resulted in nearly complete inhibition of bacterial accumulation over both 3M-RB and 1M-BSA surfaces over a wide range of shears (Fig. 6, B and C). The mannose-resistant adhesion seen for IG2 and D54A was very limited already at low shear, without any stick-and-roll adhesion pattern (Fig. 6, B and C).

The D140A mutation caused E. coli to adhere more poorly to 1M surface under shear than the parent strain, while not significantly affecting bacterial accumulation on 3M surfaces (Fig. 6, B and C), consistent with previously published results for static adhesion of a D140A FimH mutant (18). However, this mutation caused a shear-dependent transition from mobile to stationary adhesion on 3M (Fig. 6D), which is not seen with the strongly adherent bacteria expressing the background FimH V156P variant.

The mutation D141A, which partially reduced binding in static assays to both 1M and 3M, completely eliminated adhesion to 1M under flow and reduced adhesion to 3M. The D141A bacteria remained in a rolling mode of adhesion on 3M surfaces over the range of shears tested but without transition to stationary adhesion with shear increase.

Thus, individual 1M-binding site mutations had very similar effects on the binding to both 1M and 3M under shear stress, indicating the critical importance of 1M-specific binding in the shear-associated phenotype of FimH over the 3M surface.

Shear Stress Enhances P-fimbrial E. coli Adhesion to Gal-1,4-Gal-containing Glycoprotein Surfaces—We next tested whether we could use the stick-and-roll test of the effect of shear stress on bacterial adhesive mode to detect shear enhanced bacterial attachment that does not involve the FimH adhesin and that lacks a shear threshold for bacterial accumulation. We tested the adhesion of E. coli expressing the digalactose-specific P-fimbriae, which express the tip adhesin PapG and are one of the most common adhesive structures in E. coli next to type 1 fimbriae.

When P-fimbriated E. coli were allowed to accumulate on pigeon ovalbumin, a Gal-1,4-Gal-presenting surface (27, 28), for 5 min at shears ranging from 0.01 to 4.3 pN/μm² (Fig. 7A), no shear threshold was seen, and accumulation decreased with increased shear. Soluble methyl-α-D-mannopyranoside (2%) was added to the test solution to inhibit any mannose-specific interactions of the bacterial strain with the pigeon ovalbumin surface. Except for the lowest shear, where bacteria adhered in a stationary manner, roughly 20–50% of adherent bacteria
rolled along the surface until the highest shear tested (4.3 pN/μm²), where the few bacteria that adhered did so in a stationary manner (not shown).

We therefore examined the behavior of surface-bound P-fimbriated E. coli using a variable shear stick-and-roll test to see how bacteria adhered at 4.3 pN/μm² after being allowed to initially adhere at a shear where significant accumulation is possible. Bacteria were loaded at the pigeon ovalbumin surface at a low shear (0.01 pN/μm²) until about 100–150 bacteria had accumulated, followed by a switch to a pure buffer stream with the shear set to 1.1 pN/μm² and, after 100 s, to 4.3 pN/μm², the shear where stationary adhesion was seen in the accumulation experiment. At 1.1 pN/μm², ~50% of bacteria were adhering in a rolling mode, with the rest being attached firmly (Fig. 7B). As the shear was increased to 4.3 pN/μm², bacteria transitioned into stationary adhesion, although not as abruptly as on 3M-RB- or 1M-BSA-coated surfaces and at a significantly higher shear. When the shear was decreased from 4.3 back to 1.1 pN/μm², stationary bound bacteria rapidly reverted back to rolling adhesion. Less than 4% of bacteria detached at the highest shear (not shown).

Thus, we can demonstrate shear-enhanced adhesion manifested as a shear-induced transition from loose, rolling bacterial adhesion to firm, stationary adhesion for a bacterial adhesion system not involving FimH or a mannosylated ligand.

**DISCUSSION**

Here we show that E. coli adhesion to surfaces coated with 3M or digalactose ligands is enhanced by shear, although the level of surface adhesion is inhibited by increased shear stress. Binding is high and maximal under static conditions (i.e. does not demonstrate shear threshold of binding), although the adhesive strength of individual bacteria is significantly enhanced by increased shear stress. This is a novel, and possibly widespread, type of shear-enhanced adhesion that is in contrast to previously reported shear-enhanced binding mediated by E. coli FimH. For example, whereas bacteria expressing the FimH D140A mutant reverts the parent phenotype to shear-activated adhesion that only binds under static conditions, the shear-enhanced binding of rigid 1M-coated beads to surfaces coated with the remote adhesion interface is regulated by a shear-dependent switch from rolling to stationary adhesion on the 3M-coated surface occurs via the same or a similar mechanism at high shear. Thus, compared with 1M, bacterial binding to 3M results in a stronger, but still shear-enhanced, surface interaction.

**Additional FimH Binding Sites for 3M outside the 1M-binding Pocket Synergistically Enhance Binding**—Although there is no crystal structure of FimH bound to the oligosaccharide of 3M-RB or to any other 3M-like moiety, binding studies suggest two possible binding regions of 3M to FimH: one involving the Manα1-3[Manα1-6]Man moiety of the high mannos type on oligosaccharides (16, 19, 21) and another involving the trisaccharide Manα1-3Manβ1-4GlcNAc structure (22). In any case, the 3M binding is oligosaccharide-specific (in contrast to monosaccharide-specific 1M binding) and requires a binding pocket with FimH of an extended structure.

The critical importance of the 1M-binding pocket subsite within the 3M-binding region of FimH is directly supported by the fact that each 1M-binding residue mutation had an analogous effect on binding to the 1M and 3M ligands. Thus, the 1M-binding site is not only a critical part of the 3M-binding pocket but also appears to accommodate the terminal mannos of its ligand in a similar conformation for 1M and 3M. Indeed, the crystal structures of FimH bound to both monomannose and butyl-mannose show that the mannos residues of this two ligands also bind FimH in a very similar orientation to each other (18, 29). It is possible that many, if not most, mannos structures that bind to FimH incorporate into the 1M subsite in a similar manner. This allows for a general structural mechanism for how shear can enhance FimH bond lifetimes to a range of mannosylated compounds. Also, whereas two mutations, N46A and I52A, abolished all measurable 1M-binding of E. coli but diminished the 3M binding only partially under static conditions, no mutants that abolished 1M binding under static conditions could mediate adhesion to either 1M or 3M under flow, indicating a critical role of the 3M site in FimH-mediated bacterial adhesion under shear. It is possible that, in contrast to the bulky N46Q and D54E substitutions that completely abolish both 1M and 3M binding, the N46A and I52A mutations keep the 1M site accessible but with very low affinity so that additional synergistic interactions with residues outside the 1M pocket are needed for stable bacterial adhesion to occur. In the absence of 1M pocket binding, however, the external 3M contacts are apparently insufficient to mediate adhesion under shear. But when the 1M pocket is functional (even in a reduced capacity), the external 3M subsites synergistically enhance this binding, allowing for bacterial adhesion at least in static conditions.

This synergistic effect of the additional 3M contact points outside of the binding pocket is probably reflected in the significantly lower off-rate of bacteria binding to 3M compared with the 1M-ligand. In turn, this lower off-rate probably translates into the high surface accumulation of bacteria on 3M surfaces already under static conditions, the slower rolling on 3M compared with 1M ligand at intermediate shear, and the longer lived stationary state after the transition to firm adhesion at high shear. Thus, compared with 1M, bacterial binding to 3M results in a stronger, but still shear-enhanced, surface interaction.

**The Remote Interface between Lectin and Pilin Domains Regulates Shear-enhanced Binding of FimH to Both 3M and 1M**—Mutations predicted to affect the force sensitivity of the linker region between the ligand-binding lectin domain and the fimbriae-incorporating pilin domain of FimH had a similar effect on 1M and 3M adhesion, suggesting that the shear-dependent switch from rolling to stationary adhesion on the 3M-coated surface occurs via the same or a similar mechanism as on 1M and involves extension of the interdomain linker chain of FimH. Furthermore, the force-induced conformational changes in the interdomain linker region of FimH probably result in an affinity-increasing change in the FimH binding region, since the shear-dependent phenotype of FimH appears to be a finely regulated property of the FimH 1M-binding pocket. For example, whereas bacteria expressing the Y137A pocket mutant remain firmly adherent to 3M (like the strain expressing the parent FimH(V156P) variant), bacteria expressing the D141A mutant exhibit mobility at all shears, with no evidence of shear-enhanced binding. Meanwhile, bacteria expressing the FimH D140A mutant reverts the parent phenotype to shear-activated adhesion that switches from mobile to firm adhesion with increased shear stress.

**Catch Bond Model of FimH-mediated Adhesion**—Whereas it is debatable for eukaryotic cells whether shear-enhanced adhesion results from a shear-enhanced bond formation rate or from a force-induced increase of the lifetime of individual receptor-ligand interactions (catch bonds), we believe that our previous studies taken together support a shear-enhanced increase of the FimH-mannose bond lifetime (catch bond). Indeed, (a) the effect of point mutations within FimH on the shear-dependent binding suggests that shear-enhanced binding depends on the receptor-ligand bond properties rather than bond number (13, 16); (b) shear-weakened E. coli adhesion to surfaces coated with FimH antibodies shows that the shear-enhanced adhesion is specific to the FimH-mannose bond (16); (c) the enhancement of binding by increased viscosity demonstrates that the binding strength depends on shear stress (and therefore force) rather than shear rate (13, 16); and (d) the shear-enhanced binding of rigid 1M-coated beads to surfaces coated with purified type 1 fimbriae (30) argues against the possible role of...
bacterial flattening under shear. In addition, our recent studies use reduced receptor concentrations, the addition of soluble FimH inhibitors, and mathematical modeling of bond lifetimes at various shears to show that bond number is unlikely to be a major factor in the observed shear-enhanced adhesion (31, 32).

We propose that FimH forms a catch bond with 1M as well as with 3M. It is possible that ligand can be bound to two alternative states of FimH: a weak short-lived state (S\textsubscript{short-lived}), with a high ligand off-rate, and a long-lived state (S\textsubscript{long-lived}) with a slow off-rate (13, 16, 30, 31) For the switch from S\textsubscript{short-lived} to S\textsubscript{long-lived} to occur, an energy barrier (∆E\textsubscript{linker}) must be overcome. It is likely that the structural mechanism for overcoming ∆E\textsubscript{linker} involves the force-induced extension of the interdomain linker chain between the lectin and pilin domain, which in turn allosterically regulates (by a yet unknown mechanism) the affinity of the distally located mannose-binding site.

If ∆E\textsubscript{linker} is more force-sensitive than the energy barrier for unbinding from S\textsubscript{short-lived} then higher forces will accelerate the switch to S\textsubscript{long-lived}. Whereas bacteria roll along the surface as weak S\textsubscript{short-lived} bonds continually break and reform, the long lifetime of S\textsubscript{long-lived} results in firm bacterial adhesion. Compared with 1M, the overall stronger adhesion to 3M is probably a result of significantly higher energy barriers for unbinding for both states due to the additional receptor-ligand interaction subsites outside the 1M pocket.

**Physiological Significance of Shear-enhanced Bacterial Adhesion**—We show here that FimH variants common in both uropathogenic and intestinal E. coli mediate a transition from rolling to stationary bacterial adhesion to 3M surfaces with increased shear. The FimH variants used here are representative for E. coli FimH of different origin. FimH from intestinal E. coli strain F18 (used in most studies) is one of the evolutionary primary FimH variants that is common among fecal E. coli and is also found relatively frequently in uropathogenic strains. FimH from pyelonephritis E. coli strain J96 (used in the site-directed mutagenesis studies) has an A27V replacement that somewhat increases the 1M binding of FimH under static conditions relative to the FimH-F18 variant. The Val\textsuperscript{27} mutation is the most common type of variation from the evolutionary primary FimH variants and was shown to be associated with extraintestinal virulence of E. coli (33).

The demonstration of shear-enhanced adhesion on a broad variety of E. coli adhesion ligands supports the physiological importance of shear-enhanced adhesion. Shear-enhanced adhesion may enable bacteria to avoid clearance by adhesin-specific soluble inhibitors by allowing bacteria to adhere firmly to surfaces during periods of high flow when soluble inhibitors are most likely to be delivered, while still permitting reversible adhesion and bacterial spreading during periods of low flow (13, 32). Furthermore, it is likely that the activated state of FimH would predominate only when the bacterium is adhered to surface-bound ligands so that a large shear-induced tensile force can act on the adhesin, whereas interactions to soluble ligands, which are not similarly exposed to force, would remain short-lived. It is also possible that the differential level and strength of E. coli adhesion to different carbohydrate-ligand structures under shear allow the bacteria to tune their adhesion in a body niche-specific manner, depending on the ligand structures presented. The rolling mode of adhesion might provide bacteria with the means to explore a heterogeneous target surface without detaching from it when the shear stress increases due to flow fluctuations typical in vivo. Alternatively, bacteria attached in high shear regions might not be recognized well by host defense cells, since host immune cells are much larger and thus subjected to much higher drag forces than bacteria under the same conditions.

The role of shear-dependent adhesion in the pathogenesis of urinary tract infections caused by E. coli is not well understood. It has been shown that naturally occurring FimH variants with enhanced static 1M binding are more common among uropathogenic strains than fecal isolates and that such variants provide advantages in a murine model of bladder colonization (20). However, fecal-like, low 1M-binding FimH are still common among cystitis E. coli (although they are rather rare in pyelonephritis strains) (34). It is possible that in the course of the bladder infection, the shear-dependent FimH phenotype allows bacteria to resist clearance during the voiding by binding firmly to urothelium but permits bacteria to detach from it and spread around between urinations. In contrast, colonization of kidneys might require a relatively shear-independent phenotype, because shear stress in the renal tubule is continuously low (35). However, further studies are needed to determine the role of shear-dependent/-independent adhesion of E. coli in the urinary tract or other pathogenesis.

**Shear-dependent Adhesion of Non-type 1 Fimbriae**—Furthermore, our study indicates that increased shear may cause a significant increase in the strength of other types of bacterial adhesins even when the number of adherent bacteria is maximal in static conditions. Here we have shown that E. coli expressing P-fimbriae bind to surfaces coated with a galactose-1,4-galactose-presenting glycoprotein in the highest numbers at low shear. However, they exhibit rolling adhesion that transitions into firm stationary adhesion as the shear stress increases, although at a significantly higher shear level than is seen for FimH-mediated adhesion. The physiological relevance of this finding remains to be determined. The expression of P fimbriae and type 1 fimbriae is tightly co-regulated so that they are not co-expressed (36, 37), and it is unlikely that the adhesive patterns of the two would interplay during the same in vivo flow condition. Instead, the shear-enhanced adhesion demonstrated for the P-fimbriated strain shown here may be important in a different stage of bacterial colonization and infection.

P fimbriae are one of the most common adhesive structures on E. coli next to the type 1 fimbriae and mediate adhesion to the digalactose-presenting glycolipids and glycoproteins through the PapG subunit, which, similarly to FimH, is an adhesive subunit located on the tip of a fimbrial shaft (38). Also similar to FimH, PapG has a pilin domain connected to the carbohydrate binding lectin domain through an interdomain linker chain. However, whereas the stick-and-roll assay serves as a first indication for catch bond formation, additional tests are required to characterize the shear-enhanced binding seen here for the P-fimbriated E. coli. Proper testing of other bacterial adhesins under flow could also reveal their shear-enhanced binding to target surfaces.

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