Integrin-like Allosteric Properties of the Catch Bond-forming FimH Adhesin of Escherichia coli

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FimH is the adhesive subunit of type 1 fimbriae of the Escherichia coli that is composed of a mannose-binding lectin domain and a fimbria-incorporating pilin domain. FimH is able to interact with mannosylated surface via a shear-enhanced catch bond mechanism. We show that the FimH lectin domain possesses a ligand-induced binding site (LIBS), a type of allosterically regulated epitopes characterized in integrins. Analogous to integrins, in FimH the LIBS epitope becomes exposed in the presence of the ligand (or “activating” mutations) and is located far from the ligand-binding site, close to the interdomain interface. Also, the antibody binding to the LIBS shifts adhesin from the low to high affinity state. Binding of streptavidin to the biotinylated residue within the LIBS also locks FimH in the high affinity state, suggesting that the allosteric perturbations in FimH are sustained by the interdomain wedging. In the presence of antibodies, the strength of bacterial adhesion to mannos is increased similar to the increase observed under shear force, suggesting the same allosteric mechanism, a shift in the interdomain conformation. Thus, an integrin-like allosteric link between the binding pocket and the interdomain conformation can serve as the basis for the catch bond property of FimH and, possibly, other adhesive proteins.

A wide variety of proteins has shown an allosteric shift in their affinity for ligands or enzymatic substrates. In the cell adhesion receptor protein family of integrins, such a shift has been linked to the quaternary configuration between binding and nonbinding domains, where an increased affinity toward fibrinogen and other ligands (the integrin activation) involves a switchblade-like shift from the bend to the straightened conformation of the integrin domains (1–4). One of hallmarks of the integrin activation is exposure of ligand-induced binding site (LIBS) epitopes in both binding and nonbinding domains.

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The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.

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4 The abbreviations used are: LIBS, ligand-induced binding site; PAb, polyclonal antibody; MAb, monoclonal antibody; Ld, lectin domain; αMMP, α-methyl-D-mannopyranoside; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; BSA, bovine serum albumin; PBS, phosphate-buffered saline; SPR, surface plasmon resonance.
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nature of the FimH-mannose interaction has also been shown recently by using atomic force microscopy.5

Based on steered molecular dynamics simulations and site-directed mutagenesis studies, we have proposed that enhancement of FimH binding to a mannosylated surface might be associated with extension of the interdomain linker chain by shear-generated tensile force (18). We recently showed that interaction between Ld and pilin domain regulates the affinity to mannose, with amino acid mutations that disrupt the interaction resulting in a shift of Ld from a low affinity conformational state toward mannose to high affinity state (28, 32). We also proposed that the shift toward increased binding strength between FimH and its mannosyl ligand under tensile force is allosteric in nature (17, 28), and a mathematical model for such a mechanism has been advanced (16). However, it has not been demonstrated directly that the FimH protein is capable of shifting in an allosteric fashion between low and high affinity conformations.

Here we show that the interaction of soluble or surface-immobilized mannose with FimH under equilibrium (no force) conditions results in exposure of an LIBS epitope region in Ld that is located away from the mannosyl-binding pocket, close to or in the interface between the Ld and pilin domain. Binding of monoclonal antibodies to this LIBS pocket leads to a dramatic increase in the FimH affinity for mannose, similar to the increased adhesion observed under shear stress. Furthermore, the LIBS is constitutively exposed in FimH mutant variants with a disrupted interdomain interaction or in FimH with molecular chaperone wedged between the domains. Thus, there is an allosteric link between the high affinity state of ligand-binding pocket and the open conformation of interdomain interface that is analogous to one demonstrated in integrins. Because tensile force would favor the open interdomain configuration, this type of allostery provides a mechanism for the shear force-enhanced behavior of the FimH adhesin and, likely, other receptor proteins shown or proposed to form catch bonds.

EXPERIMENTAL PROCEDURES

Reagents—Monomannosylated BSA (Man1-BSA) was obtained from Dextra Laboratories, Ltd. (Reading, UK). All other reagents were obtained from Sigma unless stated otherwise.

Antibodies—Mouse monoclonal antibodies (MAb) and rabbit polyclonal antibodies (PAb) were raised against E. coli K12 FimH-lectin domain (1–160 amino acids) by PickCell Inc. (Netherlands).

Strains—Recombinant strains utilized here were constructed using a fim null E. coli K12 derivative, AAEC191A (provided by Dr. Ian Blomfield, University of Kent, UK), and were described previously (50). Briefly, AAEC191A was transformed with the recombinant plasmid pPKL114 (provided by Dr. Per Klemm, Danish Technical University, Copenhagen, Denmark) to create strain KB18. Plasmid pPKL114 is a pBR322 derivative containing the entire fim gene cluster from the E. coli strain K12, but with a translational stop-linker inserted into the unique KpnI site of the fimH gene. Strain KB18 cells express only a few long, nonadhesive fimbriae. For the studies reported here, strain KB18 was cotransformed with a series of isogenic pGB2–24-based plasmids, containing fimH alleles from various clinical isolates, as well as fimH alleles created by site-directed mutagenesis. The fimH allele encoding the wild-type FimH variant was derived from E. coli strain K12 and is identical to that encoding FimH in E. coli strain J96, which was used to determine the X-crystallographic structure (30). Site-directed mutagenesis of the fimH gene was performed using a Quick-Change kit from Stratagene. The presence of the mutation was verified by sequencing.

Bacterial Binding—Static assays of bacterial adhesion to immobilized Man1 ligands (Man1-BSA or yeast mannan) and Man3 ligand (bovine RNase B) were carried out in 96-well plates as described previously (50). Briefly, plates were coated with mannosylated substrates in 0.02 M NaHCO3 buffer, blocked by 0.1% BSA in PBS. Then 100 μl of E. coli suspension at A590 2.0 (radioabeled by growing overnight in the presence of [3H]thymidine) were incubated in the wells for 45 min at 37 °C in the absence or presence of 50 mM α-methyl-d-mannopyranoside (αMMP). After washing away unbound bacteria, the level of bacterial binding was measured by counting the radioactivity in each well. The actual number of bound bacteria was determined from calibration curves. Each data point was done in quadruplicate and then averaged. Binding under shear stress was performed using parallel plate flow chambers as described previously (18). Briefly, 35-mm polystyrene cell culture dishes (Corning, Inc.) were coated with Man1-BSA, and a parallel plate flow chamber (2.5 (long) × 0.25 cm (wide) × 250 μm (high), GlycoTech) was assembled on top of it. The entire assembly was then mounted on a Nikon TE2000-E microscope with a 10× phase-contrast objective, connected to high resolution CCD Cascade camera (Roper Scientific, Inc.). Bacteria in 0.2% BSA/PBS flowed into the chamber at different flow rates using a Warner Instruments syringe pump. Bacterial binding to the surface was recorded and analyzed using MetaMorph® or MetaView® (Universal Imaging Corp., PA) video acquisition software as described in the text.

Fimbriae Purification—Fimbriae were purified from recombinant E. coli strains expressing type 1 fimbriae with different FimH. Bacteria were grown overnight at 37 °C in LB growth media with 100 μg/ml ampicillin and 30 μg/ml chloramphenicol at 125 rpm, then harvested at 8000 rpm, resuspended in 50 mM Tris-HCl, pH 7.0, 150 mM NaCl buffer, osterized four times for 1 min with a 1-min pause on ice using PRO 200 homogenizer (PRO Scientific Inc., Oxford, CT). Cell debris was spun down, and fimbriae were precipitated from the supernatant with 0.2 M MgCl2 at least twice. Finally fimbriae were dissolved in HBS-EP buffer (10 mM Heps, 150 mM NaCl, 3 mM EDTA, 0.01% Tween 20, pH 7.4). Protein concentration was measured using BCA™ protein assay kit (Pierce) after the fimbriae were heated for 5 min at 99 °C in the presence of 0.1 M HCl.

ELISA—Monoclonal and polyclonal antibody binding to immobilized fimbriae or lectin domain was carried out in 96-well flat-bottom plates. Both fimbriae and Ld were immobilized in 0.02 M NaHCO3 buffer for 1 h at 37 °C and then washed 4–6 times with PBS. Fimbriae were immobilized at 200 μg/ml, Ld at 0.2 μM, unless otherwise stated. Plates were quenched for

5 O. Yakovenko, S. Sharma, M. Forero, V. Tchesnokova, P. Aprikian, B. Kidd, A. Mach, V. Vogel, E. Sokurenko, and W. E. Thomas, submitted for publication.
at least 15 min with 0.1% BSA/PBS. Different antibodies were added to the wells and incubated at 37 °C for 1 h with or without 50 mM αMMP. Bound antibodies were detected in direct ELISA using HRP-conjugated goat anti-rabbit (for PAb) or anti-mouse (for MAb) antibodies. The reaction was developed using 3,3',5,5'-tetramethylbenzidine peroxidase enzyme immunoassay substrate kit (Bio-Rad). Plates were read on Molecular Devices Emax microtiter plate reader and then analyzed. In comparative experiments concentrations of immobilized FimH constructs and fimbriae were adjusted to give similar response at binding of polyclonal Ab in ELISA.

**Horseradish Peroxidase Binding to Fimbriae**—Fimbriae (0.4 mg/ml) were immobilized in 96-well microtiter plates as described above. Where indicated, the immobilized fimbriae were pretreated with 50 mM αMMP and 1:200 diluted MAb separately or together for 1 h at 37 °C, as described under “Results.” After the pretreatment, followed by six washes with PBS, 40 µg/ml HRP in BSA/PBS was added to the wells with or without 50 mM αMMP, or a 1:200 dilution of monoclonal antibodies, and incubated at 37 °C for 1 h. Bound HRP was detected using the 3,3',5,5'-tetramethylbenzidine kit as described above.

**SPR Experiments**—SPR experiments were generally performed as described previously on a Biacore2000™ instrument (Biacore Inc.) (28). In brief, Man1-BSA was immobilized on a CM5 research grade chip via amino coupling at 4000 - 8000 response units using an amino-coupling kit (Biacore). A blank surface (activated and blocked with ethanolamine) was used as the reference surface. Purified fimbriae in HBS-EP buffer (Biacore Inc., Woburn, MA) with or without mannose for 1 h at 37 °C and detection with 3,3',5,5'-tetramethylbenzidine kit.

**Cysteine Labeling with [14C]Iodoacetamide**—Purified fimbriae expressing FimH:N29C variant were immobilized in microtiter strip wells as described above, and 10 mM [14C]Iodoacetamide (Amersham Biosciences) was added to fimbriae with or without 50 mM αMMP and incubated for 1 h at 37 °C and then overnight at 4 °C. Strips were broken, and at least triplicates for each data point were checked for cysteine labeling using the scintillation method.

**Cyssteine Labeling with Biotin-maleimide**—Purified fimbriae expressing FimH:N29C variant were immobilized as described above. Wells were quenched with 0.1% gelatin in PBS to reduce nonspecific labeling. Biotin-maleimide was added at 2 µg/ml with or without mannose and were allowed to bind for 1 h at 37 °C. To detect fimbriae-bound biotin, wells were incubated with HRP-conjugated streptavidin (1:10,000 dilution; Endogen Inc., Woburn, MA) with or without mannose for 1 h at 37 °C. To test for activation of biotin-labeled fimbriae with streptavidin, 100 µl of unconjugated streptavidin (1 µg/ml in 0.1% of gelatin in PBS) were incubated for 1 h at 37 °C with the immobilized fimbriae (biotinylated in the presence of αMMP as described above), followed by extensive washes, then incubation with HRP (40 µg/ml) for 1 h at 37 °C, and detection with the 3,3',5,5'-tetramethylbenzidine kit.

**Statistical Analysis of ELISA, HRP Binding, and Cysteine Labeling Experiments**—Each experiment was repeated at least twice, with triplicates or quadruplicates for every data point. Results are represented on graphs as the average of replicates in each experiment, with standard deviation used for error bars.

**RESULTS**

Monoclonal Antibodies against Lectin Domain Bind to Fimbria-incorporated FimH Only in the Presence of Mannose—We have raised polyclonal and monoclonal antibodies by immunizing rabbit and mice with the purified Ld. Polyclonal antibodies recognized both Ld and purified fimbriae immobilized on the
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To test this, we first used soluble horseradish peroxidase (HRP) that contains N-linked mannosylated oligosaccharide chains and binds to FimH in a mannose-specific manner while retaining its oxidative activity. After combined pretreatment of surface-immobilized fimbriae with mannose and MAb21, followed by extensive washes, mannose-specific binding of HRP to the fimbriae increased severalfold (Fig. 2A). The increase was not because of multimerization of the fimbrial FimH by antibodies as the same results were obtained when purified Fab fragments of MAb21 (Fab-MAb21) were used instead of full-size antibodies (not shown). Sequential pretreatment with mannose and then MAb or pretreatment only with mannose or MAb did not result in the increased HRP binding (Fig. 2A).

We directly determined whether the increased binding could be a result of increased affinity of the fimbria-incorporated FimH for mannose. The affinity for mannose was measured in SPR using soluble fimbriae with and without pretreatment with Fab-MAb21 and mannose. Upon pretreatment of fimbriae with Fab-MAb21, the affinity for soluble mannose increased from \( \frac{9}{28} \pm 53 \) to \( 6.08 \pm 0.64 \) \( \mu M \) as determined by the ability of soluble mannose to inhibit binding of the fimbriae to immobilized Man1-BSA. The binding sensograms show complex multistate kinetics, with both binding and unbinding occurring at multiple rates (Fig. 2B). This might be expected for an allosteric protein that has a low and a high affinity state, but it makes it difficult to interpret rate constants. Nevertheless, it is clear that Fab-MAb21 decreases unbinding of FimH from Man1-BSA, because the SPR response has a lower fractional drop during the wash step for the fimbriae pretreated with Fab-MAb21. Therefore, a decrease in unbinding kinetics is at least responsible in part for the increased affinity caused by Fab-MAb21 pretreatment.

Thus, the mannose interactions with the FimH-binding pocket and MAb21 binding to the LIBS epitope are reciprocal, i.e. the mannose binding increases the MAb binding and vice versa. This demonstrates that the exposure of LIBS epitope in FimH by binding of mannose is allosteric in nature. In a manner analogous to activation of integrins with LIBS-specific antibodies, binding of MAb21 to FimH leads to the activation of the adhesin.

In the Absence of Mannose, MAb21 Recognizes FimH Variants That Have Increased Mannose Binding Capability under Static Conditions—We screened the antibody binding capability of different fimbria-incorporated FimH variants with amino acid replacements in various regions of the Ld and pilin domain (Fig. 3A). These replacements include naturally occurring and recombinant mutations that either do not affect the static Man1 binding of type 1 fimbriated bacteria or increase it to various degrees (supplemental Table 1S).

All plastic-bound fimbrial samples reacted with polyclonal anti-Ld antibodies at a similar level, whether in the presence or absence of mannose (not shown). When the fimbrial samples were probed with the monoclonal antibodies in the presence of mannose, MAb21 reacted well with all fimbrial samples (Fig. 4, closed circles), except for those incorporating six FimH variants (Fig. 4, closed triangles) that are discussed further below. However, in the absence of mannose, the FimH variants exhibited a strong correlation \( R^2 = 0.833 \) between the static Man1 bind-
ing capability of the fimbriae and recognition of the corresponding fimbriae by MAb21 (Fig. 4, open circles). Thus, in the absence of mannose, the accessibility of LIBS is directly associated with the level of mannose binding ability of FimH variants under static equilibrium conditions, in other words with the (pre)-activation effect of the FimH structural mutations.

LIBS Epitope Is Readily Recognized in a FimH Variant with Disrupted Interdomain Interaction—We have recently demonstrated that Ld and pilin domains of FimH interact with each other and that disruption of the interaction results in a shift of FimH from low to high affinity state toward mannose, i.e. in FimH activation (28). In particular, FimH with an A188D mutation in the pilin domain was shown to strongly interfere with the interdomain interaction and result in very strong Man1 binding under static conditions. Here we found that binding of MAb21 to FimH:A188D fimbriae was especially strong even in the absence of mannose (Fig. 4), suggesting an inverse correlation between the strength of Ld-pilin domain interaction and the strength of MAb21 binding. To ensure that the strong binding of MAb21 to FimH:A188D fimbriae is not because of a higher level of the mutant FimH in the fimbrial rod, instead of fimbriae we also tested purified fimbrial tips that possess FimH adhesin in complex with two minor subunits of type 1 fimbriae, FimG and FimF (the latter complexed with FimC chaperone), but no FimA subunits that compose the fimbrial rod. The MAb21 binding pattern to the tips (in the absence or presence of mannose) was the same as to the corresponding purified fimbriae, with MAb21 binding to FimH:A188D tips in the absence of mannose being almost as high as MAb21 binding to wild-type FimH tips in the presence of mannose (Fig. 5A). Only a minor additional increase of MAb21 binding to FimH:A188D tips was observed in the presence of mannose. Thus, the LIBS epitope appears to be well exposed in the FimH variant with a disrupted interdomain interaction, suggesting that LIBS is masked by the interaction of Ld and pilin domain.

Mutations in the Bottom Region of Ld Abolish Recognition by the MAb—Six FimH variants, FimH:N29G, FimH:N152A, FimH:D153A, FimH:V154A, FimH:V155P, and FimH:V156P, were not recognized by MAb21 with or without mannose (Fig. 4B, open and closed triangles, respectively). These variants lost their ability to bind MAb21 independently of whether their static Man1 binding capability was high or low. The lack of recognition by MAb21 of these variants was not because of a lower amount of FimH in the fimbriae, because they bound polyclonal antibodies similar to other mutants (Fig. 4A, open and closed triangles). The mutations are located in positions 152–156 that comprise the C-terminal end of Ld connecting it to the pilin domain via the linker chain (Fig. 3A). It also includes residue Asn-29, which is positioned close to the residue Val-156 in the crystal structure (Fig. 3B). Mutation of these residues also drastically decreases MAb21 binding to corresponding purified Ld variants (not shown). All six residues are positioned far away from the mannose-binding pocket of FimH and are instead in the region of the Ld near the interface with the pilin domain.

We propose that positions 152–156 and 29 comprise a three-dimensional LIBS epitope recognized by MAb21. The three-dimensional nature of the epitope is supported by the fact that screening of synthetic peptides in this region failed to identify a linear binding epitope for MAb21 (not shown). The location of residues in the interdomain interface suggests that interaction between Ld and pilin domain could interfere with accessibility of the LIBS epitope to MAb21 and that mannose binding could lead to a more open configuration of the domains, unmasking the epitope and possibly changing its conformation. Interestingly, this position of the FimH LIBS is also similar to the position of LIBS epitopes in integrins, i.e. away from the binding pocket(s) and within the interface between the integrin subunits.

Mannose Binding Is Required for the Residue 29 to Be Exposed to Labeling—We investigated the effect of mannose in unmasking of the putative LIBS region by assessing accessibility of the residue 29 to labeling. Asparagine in position 29 was replaced with cysteine that also abolished the MAb21 binding but did not affect the static Man1-specific binding of FimH (not shown). The unpaired Cys-29 in FimH:N29C was probed in purified fimbriae with free SH group-reacting iodoacetamide labeled with 14C. The 14C labeling could be accomplished only in the presence of mannose (Fig. 6A) showing directly that, in native mannose-free form of FimH, residue 29 of Ld is not accessible to even relatively small soluble compounds but becomes exposed in the presence of ligand. Labeling of Cys-29 with another SH-reacting compound, biotin-maleimide, was also accomplished exclusively in the presence of mannose. Moreover, after the biotinylation, the biotin-Cys-29 complex in FimH:N29C could be accessed by streptavidin only in the presence of mannose (Fig. 6B).

Thus, residue 29 that is identified as being a part of the FimH LIBS epitope is indeed hidden in the mannose-free low affinity state of FimH but becomes exposed upon mannose binding. The cryptic position of residue 29 is likely to be due to a location of its side chain within or close to the interface between the domains rather than to its interior position inside Ld. The latter scenario, for example, would not readily explain the inaccessibility of a relatively bulky and hydrophilic Cys-29-linked biotin to streptavidin in the absence of mannose.

Wedgeing of Interdomain Interface Leads to FimH Activation—To test whether FimH activation by MAb21 binding to the LIBS epitope is because of an antibody-specific interaction or a mere wedging effect, we tested whether streptavidin binding to exposed biotinylated residue Cys-29 leads to activation of the FimH:N29C variant. Indeed, binding of streptavidin to the biotinylated FimH:N29C fimbriae (in the presence of mannose) results in a significant increase in the ability of fimbriae to bind soluble HRP in a mannose-specific way (Fig. 7A). Thus, both MAb21 and streptavidin binding to the LIBS region have a similar activation effect on FimH, suggesting a nonspecific, interdomain wedging effect for both proteins. Although interdomain location of FimH-bound MAb21 or streptavidin is still hypothetical, the molecular chaperone FimC is known to be wedged between the Ld and pilin domains according to the crystal structure of FimH-FimC complex. In fact, FimH/FimC affinity toward soluble HRP is high with or without antibodies bound (Fig. 7A), indicating that the FimC-complexed FimH is likely to be in an activated state. This corresponds to our previous finding that FimH/FimC has a high affinity toward mannose (i.e. is in an activated state) (28). Furthermore, MAb21
readily recognizes the FimH in complex with the FimC chaperone in either the absence or presence of mannose, i.e. the LIBS is well exposed in FimH/FimC (Fig. 7B). Taken together, these results indicate that LIBS-bound MAb21 that leads to FimH activation is likely to function as a molecular wedge between the Ld and pilin domains, preventing their interaction.

Strength of FimH-mediated Bacterial Adhesion Is Enhanced in the Presence of MAb —To determine how the presence of antibodies would affect FimH interaction with a mannose-coated surface, we first tested binding of soluble fimbrial tips to Man1-BSA-coated surface. In the absence of MAb21, wild-type FimH tips bound significantly weakly to the Man1-surface than FimH:A188D tips, analogous to the Man1-binding properties of the corresponding fimbriated bacteria (see Fig. 4). In the presence of MAb21, binding of the wild-type FimH tips increased to the level of binding of FimH:A188D tips (Fig. 5B). The increased binding was not because of FimH multimerization by antibodies as the same effect was seen with purified Fab fragments of MAb21 (not shown). Also, based on the reactivity with anti-mouse secondary IgG, the tips bound to Man1-BSA surface were in complex MAb21. Thus, binding of FimH to surface-immobilized Man1-BSA under equilibrium conditions results in exposure of the LIBS epitope as in case of the FimH binding to soluble mannose. Apparently, MAb21 binding to the exposed LIBS results in locking the surface-bound FimH in the activated state, leading to an increase in the level of tips binding to the surface.

We then determined how strength of bacterial adhesion is affected by the presence of either MAb or shear stress. E. coli cells expressing wild-type FimH were loaded into a parallel plate flow chamber coated with Man1-BSA, at a concentration that allows about 300 bacterial cells to be settled onto the ligand-coated surface (within a
chosen field of view under ×20 magnification). After 15 min of the static post-loading incubation, the fluid flow was started at either low shear stress (0.035 Pa) or high shear stress (1 Pa), and the bacterial behavior was observed with video microscopy. In comparison with the low shear wash, under the high shear the number of bound bacteria was increased severalfold (Fig. 8A). Also, although about half of the bacteria were bound at low shear in a loose, rolling manner, under the high shear all bacteria were bound in the stationary mode of adhesion (Fig. 8B), demonstrating the phenomenon of FimH-mediated shear-enhanced adhesion of type 1 fimbriated bacteria to the mannosylated surface.

When the same number of bacteria were loaded and incubated onto the flow chamber surface in the presence of MAb21 in the medium and then washed with low shear, the number of bacteria that remained attached on the surface increased almost 4-fold (Fig. 8A). Furthermore, in the presence of MAb, the vast majority of the surface-attached bacteria was bound in a firm fashion (Fig. 8B). Thus, the MAb21 treatment seems to increase the strength of the bacterial attachment to the Man1 surface in a fashion similar to the high shear stress. Under the high shear wash, the increase in the number of bacteria attached in the presence of MAb21 was much less significant relative to the wash of bacteria without MAb21 (Fig. 8A).

In contrast to the binding increase by high shear only, the increase of the binding strength by antibodies was irreversible. When the shear stress was switched from the high shear down to the low level, more than 40% of initially attached bacteria without antibodies detached from the surface after 5 min, but only a few of the MAb21-treated bacteria did so in the same time frame (Fig. 8C). Thus, the adhesion strengthened by shear is reduced with the reduction in shear, whereas the increase caused by the bound MAb is irreversible (in the time frame of our assays).

These results indicate that the FimH shift from the low to high affinity state is stabilized by bound MAb21 during FimH-mediated bacterial adhesion to mannosylated surface. Furthermore, the similar effect of either the shear stress or the MAb treatment on the increased strength of FimH-mediated adhesion indicates that the structural mechanism for FimH activation could be the same in both cases, i.e. allosteric in nature.

**DISCUSSION**

The fact that mannos binding to FimH causes a conformational change (epitope exposure) at the site distant from the ligand-binding site (in the bottom of the binding domain) suggests the allosteric nature of the change. This is further supported by the reciprocity of the structural changes in FimH, the ligand-induced conformational change in the domain interface enhances the binding of a MAb and, vice versa, the MAb21 binding increases the ability of the binding pocket to bind the ligand. Reciprocity is an expected attribute of allosteric regulation (33, 34), and in fact, there is no other well accepted theory to explain reciprocal activation. Therefore, these experiments directly demonstrate that FimH is an allosterically regulated protein, where interdomain configuration is linked to the conformation of the binding site via cross-domain structural perturbations (as yet unknown nature).

According to the allosteric model of protein regulation, there should be an inactive and an active state of the receptor. We propose that in FimH the inactive state is characterized by the low affinity conformation of the mannose-binding pocket (located on the top of the lectin domain) and the LIBS-closed conformation of the lectin domain–pilin domain interface (Fig. 9A). Interaction of soluble mannos with the binding pocket can transiently shift the adhesion into the active state that is characterized by a high affinity conformation of the binding site and the LIBS-open configuration of the interdomain interface (Fig. 9B). Binding of MAb to the now accessible epitope sustains the active state conformation by preventing its reversion to the inactive state (Fig. 9C). We hypothesize that the accessibility of the LIBS epitope to MAb is modulated by the pilin domain interaction with the Ld. In the crystal structure of FimH, the pilin domain is complexed with the molecular chaperone, FimC, that is positioned in the domain interface, effectively preventing possible interaction between the domains. However, the existence of an interdomain interaction in the chaperone-free native form of FimH has been shown by using a yeast-two hybrid system, purified domains, and computational docking (28, 32). It also has been shown that mutations in or near the interdomain interface can significantly weaken this interaction as seen, for example, in the FimH:A188D mutant (28). The weakening of the interaction, in turn, eases the ability of FimH to shift from the low affinity to the high affinity state and at least some mutations can effectively sustain the active state conformation (Fig. 9D). Although some in silico predictions of the structure of interdomain interface were obtained and, at least, supported in part by site-directed mutagenesis, these predictions should be regarded as preliminary. Thus,
structural details of the domain-domain interaction and, consequently, LIBS epitope dynamics remain to be determined.

The ability of MAb21 to sustain FimH in the high affinity state is likely to be due to a structural interference of the LIBS-bound antibody with the domain-domain interaction (Fig. 9C). Consistent with this hypothesis, binding of streptavidin to a biotinylated residue 29 within the LIBS also results in a sustained increased affinity of FimH toward mannose. Also, in the FimH-FimC complex where FimC is wedged between the domains, the LIBS epitope is readily exposed even in the absence of mannose and affinity to mannose of the complex corresponds to the high affinity state of FimH (28).

Based on the evidence that the ligand-induced conformational change apparently involves opening of the interdomain interface, we propose that mechanical force also favors the allo-

FIGURE 3. Mapping of MAb21-binding epitope. A, β-sheet topology diagram of FimH, with lectin (upper) and pilin (lower) domains (from Ref. 30). Crosses correspond to relative position of residues comprising the binding pocket of FimH. Dots correspond to residues mutated in the FimH variants tested in this study. Boxed are positions of mutations that eliminate MAb21 binding. B, ribbon view of crystal structure of FimH Ld with epitope residues side chains shown as a sphere representation: positions 152–156 of linker chain in red and position 29 on adjacent loop in blue.

FIGURE 4. Screening of fimbrial FimH variants for MAb21 recognition. X axis, Man1/Man3 binding ratio of isogenic E. coli expressing different FimH variants. Unlike highly variable Man1-specific binding, Man3-specific binding is relatively strong and uniform among different recombinant FimH variants (50); it can thus be used to normalize the intrinsic Man1-binding versus the level of ManH expression by the bacteria. Y axis, binding of immobilized purified fimbriate to MAb21 versus PAb in the absence (open symbols) or presence (closed symbols) of mannose (α-MMP). Because all FimH variants are similarly recognized by PAb in the absence or presence of mannose, this binding serves to measure the amount of FimH in the microtiter-immobilized fimbriate. Thus, the MAb21/PAb ratio has been used to quantify FimH recognition by MAb21. All mutants are shown in circles except those having diminished recognition by MAb21 in both the absence and presence of α-MMP, which are shown in triangles. The positions of the data points corresponding to the wild-type FimH (wt), and those carrying A188D and putative epitope mutations are indicated. The straight lines in the figure denote linear regression plots for analyses of the relationship between the ability of E. coli FimH variants to bind Man1 under static conditions and to be recognized by MAb21 in the presence (solid line) or absence (dotted line) of α-MMP.

FIGURE 5. Differentiation between FimH and FimH:A188D variants in fimbrial tips constructs. A, MAb21 binding to surface immobilized tips with wild-type FimH (open bars) or FimH:A188D variant (closed bars), in the absence or presence of α-MMP. Error bars represent standard deviations. B, binding of soluble tips with wild-type FimH (open bars) or FimH:A188D variant (closed bars) to the Man1-BSA-coated surface. Tips were incubated on the Man1 surface either in the absence (experiment 1) or presence of MAb21 (experiments 2 and 3). In experiments 1 and 2, PAb and anti-rabbit secondary antibodies were used to detect tips bound to Man1-BSA. In experiment 3, anti-mouse secondary antibodies were used to detect binding of MAb21 to the Man1-bound tips. Tip binding to Man1 was inhibited >90% by soluble mannose in all tests (not shown). Error bars represent standard deviations.
FIGURE 6. Labeling of cysteine 29 in FimH:N29C. A, labeling of immobilized fimbriae with wild-type FimH (open bars) and FimH:N29C variant (closed bars) with [14C]iodoacetamide in the presence or absence of αMMP. Error bars represent standard deviations. B, labeling of FimH:N29C fimbriae with biotin-maleimide (BM) in the absence or presence of αMMP, with subsequent detection of Cys-29-coupled biotin by streptavidin conjugated with HRP (SAhrp), also in the absence or presence of αMMP. Error bars represent standard deviations.

FIGURE 7. Effect of interdomain wedging with streptavidin or FimC chaperone. A, HRP binding to immobilized fimbriae with FimH:N29C-biotin without (open bar) or with (closed bar) pretreatment of fimbriae with streptavidin (SA) and αMMP simultaneously. Binding was inhibited >90% by soluble mannose (not shown). Error bars represent standard deviations. B, HRP binding to immobilized FimH-FimC complex in the absence (open bar) or presence (closed bar) of MAb21. Binding was inhibited >90% by soluble mannose (not shown). Error bars represent standard deviations. C, MAb21 binding to immobilized FimH-FimC complex in the absence (open bar), or presence (closed bar) of αMMP. Error bars represent standard deviations.

FIGURE 8. Effect of shear stress and MAb21 on the strength of FimH-mediated bacterial adhesion. A, FimH-expressing bacteria remaining attached to Man1-coated flow chamber surface after washing under low shear stress (0.035 Pa) or high shear stress (1.0 Pa). The bacteria were loaded into the chamber in the absence (open bars) or presence (closed bars) of MAb21. B, proportion of the Man1-attached bacteria in a weakly bound rolling mode during the low shear and high shear wash. Bars as in A. C, detachment of FimH-expressing bacteria from the Man1-coated surface upon the shift of the flow from high shear to low shear. Bacteria were loaded into the chambers at medium shear stress (0.27 Pa) in the absence (open circles) or presence (closed circles) of MAb21 and then subjected to high shear stress (1.0 Pa) wash for 5 min, followed by a switch to low shear (0.035 Pa) for 5 min of recording.

FIGURE 9. Schematic representation of conformational states of FimH. The pilin domain is represented by the lower rectangle, connected via a linker chain with the lectin domain above (the mannoside-binding site is a triangular indent on the top of the lectin domain). Black triangle, mannose ligand. Gray rectangle at the bottom of lectin domain, LIBS epitope. A, inactive state; B, active state (transient); C, active state sustained by the LIBS-specific antibody (body (dashed lines)). D, active state sustained by structural mutations (open circles). E, active state sustained by tensile forces (block arrows).

The finding of the existence of LIBS epitope(s) in FimH and of the activating effect of the LIBS-specific antibody brings up some provocative comparisons. The phenomenon of “activating” antibodies has been described for the transmembrane integrins involved in cell adhesion. In integrins with LIBS (or CLIBS for cation- and ligand-influenced binding site), the antibody epitopes become exposed when integrins become activated by either “outside-in” or “inside-out” signals (for review see Refs. 5, 11). Integrin LIBS do not overlap with ligand-binding sites and steric switch from closed to an open interdomain configuration and thus activates FimH. Obviously, sufficient level of tensile force applied to a FimH-mannose complex would facilitate and also sustain domain separation (Fig. 9E). This scenario explains why the bacteria rapidly transition from a weak rolling mode of adhesion to strong stationary adhesion when the force is increased. In contrast, reduction of tensile force would result in FimH de-activation by allowing re-docking of the domains. This, in turn, would lead to reversion of the active state to the weak binding state and increased probability of the mannose release from FimH, leading to the bacterial detachment that is commonly seen upon the reduction of shear. Thus, we propose that catch bonds are enhanced by tensile force via a force-regulated allostery, where the high affinity state can be induced by ligand binding even in the absence of tensile force, but tensile force both facilitates and maintains the conformational shift. The catch bond hypothesis and the importance of allostery in it is validated in our recent study in which the catch bond and force-induced shift in the FimH states are shown directly in single molecule studies using AFM experiments.

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are commonly found in nonbinding “legs” domains, like PSI and the hybrid domain in the β subunit or even in the most proximal β-tail (β subunit) or Cali-2 (α subunit) domains located next to the transmembrane regions. For example, anti-LIBS2 MAb binds to an epitope on β3 integrin that is 16 nm and several domains away from the fibrinogen-binding site of the αIIbβ3 integrin (8). Although the MAb21 LIBS epitope in FimH is located in the binding lectin domain, we expect that in the future LIBS might be found in the nonbinding pilin domain as well considering that interdomain shift during FimH activation is likely to unmask interface regions in both lectin and pilin domains. LIBS are found also in the ligand-binding domains of integrins, α-1/A (present in some integrins) and β-propeller domains in the α subunit and β-1/A domain in the β subunit that are distally positioned, forming the integrins “head.” In the binding domains of integrins, LIBS epitopes are mapped in various locations, either close to or far away from the ligand- or cation-binding sites or even, like in FimH, on the opposite side from these sites, close to interface regions with adjacent domains. The latter types include, for example, IAC-1 epitope in the α-1/A domain of 12G10 epitope in the β-1/A domain (7).

Similar to FimH, LIBS in integrins are exposed upon ligand (or cation) binding, and conversely binding of the antibodies to the epitopes sustains the high affinity, activated state of the receptor protein. As mentioned above, ligand/cation binding or inside-out activation leads to a switchblade- or flick-knife-like shift from a bent low affinity conformation of integrins to a straightened high affinity one (for review see Refs. 35–38). This involves significant quaternary re-arrangements across the domains (not limited to the “legs knees” region) and, ultimately, a shift in the conformation of the ligand-binding pocket via an allosteric mechanism (that is not yet well understood). Obviously, the interdomain re-arrangements lead to the exposure of multiple interface regions or changes in the tertiary structure of the various domains, resulting in exposure of LIBS epitopes. It has been proposed that binding of anti-LIBS antibodies stabilizes the activated conformation of integrins by displacing an equilibrium between different conformational states of the protein in favor of the activated state (reviewed in Ref. 39). It is possible that at least in some cases this stabilization is because of the interdomain wedging effect seen in FimH with MAb21 that sustains allosteric perturbations in tertiary structure of the binding domains. However, it is also possible that antibody directly recognizes and sustains the conformationally shifted tertiary (or even secondary) structure of the domain.

Also similar to FimH, various structural mutations have been identified in integrins that cause increased affinity of the ligand binding (as well as anti-LIBS antibody binding) but are located away from the ligand-binding site (reviewed in Refs. 11, 40). Some of these activating mutations are mapped as far away as in the transmembrane regions of integrins (40), although others are located in the binding domains. Interestingly, analogous to FimH, a great number of the activating mutations in the binding β-1/A domain are mapped oppositely from the binding site, in the interdomain region facing the Hybrid domain. Sometimes, the locations of activating mutations are close to or even overlap with a LIBS epitope (11, 12, 41). By analogy with FimH, some of the activating mutations in integrins might affect inter-domain interaction, shifting equilibrium toward the quaternary configuration that sustains the high affinity state. However, at least some of the activating antibodies, in both integrins and FimH, are likely to affect directly the allosteric pathway that leads to the high affinity conformation of the binding pocket.

Integrins are much more larger and clearly more complex receptor proteins than the FimH adhesin, with more than a dozen domains, multiligand binding and multiregulatory properties, including an inside-out activation that FimH lacks. However, the basic LIBS-associated allosteric characteristics appear to be the same in both receptors as follows: (i) ligand binding results in unmasking of an epitope located far away from the binding pocket; (ii) antibody binding to the LIBS sustains both receptor in a high affinity activated state, and (iii) LIBS exposure is connected to the quaternary configuration between binding and nonbinding domains located distally and, respectively, proximally toward the cell surface. Although integrins were not shown yet to form force-dependent catch bonds with their ligand, by analogy with FimH this might be expected to be the case. Indeed, it has already been suggested that mechanical regulation can activate integrins to make them catch bonds (4, 24). By the same token, same could be true for another cell adhesion protein where LIBS epitope has been demonstrated, galactose/N-acetylgalactosamine-specific calcium-type lectin (mMGL) on macrophages and lymphocytes, where MAbs recognize the LOM-11 epitope that becomes accessible when mMGL is activated by either calcium or ligand binding (42). When bound, LOM-11 MAb stabilizes the lectin in its “active” conformation so that the latter is able to bind ligands even in the low calcium conditions. Like FimH and integrins (and indeed vast majority of cell-adhesion receptors), mMGL has separate carbohydrate-binding domain and cell-anchoring domains (43).

On the other hand, receptor proteins that were shown to form catch bonds might also possess allosteric properties similar to FimH. For example, association of interdomain interaction with ligand binding has been proposed for P-selectin, for which alternative crystal structures have been obtained previously. Selectin binding to sialy-Lewis X saccharides mediates shear-dependent adhesion of leukocytes to the endothelial surface (19–22, 44). P-selectin is a multidomain protein, where the binding domain interacting with the neighboring domain (epidermal growth factor), with a bend in the hinge region between the domains in the absence of ligand and a straightened conformation of the domains in a ligand-bound form of the protein (45). It also has been shown that glycosylation of a residue positioned in the interdomain interface results in increased affinity of the receptor (26). The glycosylation is likely to have a wedging effect between the domains that is similar to the effect of the LIBS-specific antibody or streptavidin in FimH. Thus, it has been proposed that the straightened conformation is favored by tensile force and results in a stronger binding of the ligand (26). However, neither a LIBS epitope nor an allosteric shift between low and high affinity states in selectins has yet been demonstrated. We have also shown that E. coli adhesion mediated by di-galactose-specific P fimbriae occurs via shear-enhanced mechanism (46). The PapG adhesin of the P-fimbria has a ligand-binding and fimbria-anchoring domain as does FimH (and many other bacterial adhesins) and thus could also be an
allosteric protein, the active state of which is sustained by tensile force. A similar mechanism could be involved in the von Willebrand factor-mediated platelet adhesion that requires shear stress to occur (47). Catch bond mechanisms could also be involved in the interaction of T cells with antigen-presenting cells, where conformational stabilization of the receptor subdomains is associated with increased lifetime of the receptor-ligand interaction (48, 49). Another example where significant interdomain changes occur in the course of protein binding is the actin-myosin interaction, and these too have been shown recently to be catch bonds (23). It is quite possible that in the future integrin- and FimH-like LIBS epitopes will be discovered for these receptors proteins as well, with a similar activating effect by anti-LIBS antibodies.

Further studies need to be undertaken to understand the details of the conformational changes between the FimH-binding pocket and interdomain interface as well as whether or not the ligand-induced and force-facilitated allosteric mechanism represents a general principle of catch bond-like receptor-ligand interactions. However, the connection between the presence of LIBS epitope and the allosteric mechanism of catch bonds outlined here provides a conceptual framework and practical means for understanding the mechanism of biological interaction that occurs under mechanical tension and discovering new receptor proteins that form catch bonds. The relatively small size of FimH and ease of manipulation make it an excellent system to explore the basic principles of allosteric regulation in catch bonds.

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