Comparative Structure-Function Analysis of Mannose-Specific FimH Adhesins from *Klebsiella pneumoniae* and *Escherichia coli*

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FimH, the adhesive subunit of type 1 fimbriae expressed by many enterobacteria, mediates mannose-sensitive binding to target host cells. At the same time, fine receptor-structural specificities of the FimH from different species can be substantially different, affecting bacterial tissue tropism and, as a result, the role of the particular fimbriae in pathogenesis. In this study, we compared functional properties of the FimH proteins from *Escherichia coli* and *Klebsiella pneumoniae*, which are both 279 amino acids in length but differ by some ~15% of residues. We show that *K. pneumoniae* FimH is unable to mediate adhesion in a monomannose-specific manner via terminally exposed Manα(1-2) residues in N-linked oligosaccharides, which are the structural basis of the tropism of *E. coli* FimH for uroepithelial cells. However, *K. pneumoniae* FimH can bind to the terminally exposed Manα(1-3)Manβ(1-4)GlcNAcβ1 trisaccharide, though only in a shear-dependent manner, wherein the binding is marginal at low shear force but enhanced sevenfold under increased shear. A single mutation in the *K. pneumoniae* FimH, S62A, converts the mode of binding from shear dependent to shear independent. This mutation has occurred naturally in the course of endemic circulation of a nosocomial uropathogenic clone and is identical to a pathogenicity-adaptive mutation found in highly virulent uropathogenic strains of *E. coli*, in which it also eliminates the dependence of *E. coli* binding on shear. The shear-dependent binding properties of the *K. pneumoniae* and *E. coli* FimH proteins are mediated via an allosteric catch bond mechanism. Thus, despite differences in FimH structure and fine receptor specificity, the shear-dependent nature of FimH-mediated adhesion is highly conserved between bacterial species, supporting its remarkable physiological significance.

The most common type of adhesive organelle in the Enterobacteriaceae is the type 1 fimbria, which has been most extensively studied in *Escherichia coli*. The corresponding structures of *Klebsiella pneumoniae* are similar to those of *E. coli* with regard to genetic composition and regulation (15). Type 1 fimbriae are composed primarily of the structural subunit FimA, with minor amounts of three ancillary subunits, FimF, FimG, and the mannose-specific adhesin FimH. The FimH adhesin is an allosteric protein that mediates the catch bond mechanism of adhesion where the binding is increased under increased shear stress (48).

It has been demonstrated in *E. coli* that FimH has two domains, the mannose-binding lectin domain (from amino acid [aa] 1 through 156) and the fimbria-incorporating pilin domain (from aa 160 through 279), connected via a 3-aa-long linker chain (6). A mannose-binding site is located at the top of the lectin domain, at aa 160 through 279), connected via a 3-aa-long linker chain (6). A mannose-binding site is located at the top of the lectin domain, at the opposite end from the interdomain linker (17).

Several studies have demonstrated that type 1 fimbriae play an important role in *E. coli* urinary tract infection (UTI) (7, 21, 23, 35). In addition, in urinary *E. coli* isolates, the FimH adhesin accumulates amino acid replacements which increase tropism for the uroepithelium and various components of basement membranes (21, 30, 35, 47). Most of the replacements increase the monomannose binding capability of FimH under low shear, by altering allosteric catch bond properties of the protein (48). The mutated FimH variants were shown to provide an advantage in colonization of the urinary tract in the mouse model (35) and correlate with the overall extraintestinal virulence of *E. coli* (16). Thus, FimH mutations are pathoadaptive in nature.

*Klebsiella pneumoniae* is recognized as an important opportunistic pathogen frequently causing UTIs, septicemia, or pneumonia in immunocompromised individuals (29). It is responsible for up to 10% of all nosocomial bacterial infections (18, 41). *K. pneumoniae* is ubiquitous in nature, and it has been shown that environmental isolates are phenotypically indistinguishable from clinical isolates (22, 26, 27, 29, 33). Furthermore, it has been demonstrated that environmental isolates of *K. pneumoniae* are as virulent as clinical isolates (28, 45).

*K. pneumoniae* possesses a number of known virulence factors, including a pronounced capsule, type 3 fimbriae, and type 1 fimbriae (29, 44). Type 1 fimbriae produced by *K. pneumoniae* are described as functionally and structurally similar to type 1 fimbriae from *E. coli* (25) and have been shown to play a significant role in *K. pneumoniae* UTI (32, 43).

We have previously shown that mature FimH from 54 isolates of *K. pneumoniae* (isolated from urine, blood, liver, and...
TABLE 1. Strains and plasmids used in this study

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<tr>
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**MATERIALS AND METHODS**

**Bacterial isolates and plasmids.** Bacterial isolates and plasmids are listed in Table 1.

**Construction of *K. pneumoniae* fimH mutant.** The *K. pneumoniae* fimH mutant was constructed as described elsewhere (43). Briefly, the *fimH* gene in C3091ΔmrkA-F was deleted by allelic exchange with a tetracycline resistance-encoding cassette flanked by regions homologous to the regions up- and downstream of the *fimH* gene. All primers used are listed in Table 2. The cassette was generated by using a modification of a three-step PCR procedure described previously (8). In the first step, the tetracycline resistance-encoding gene (tet) was amplified from pAR82 by use of primers Ucas and Dcas (31). Second, from C3091ΔmrkA-F chromosomal DNA regions flanking the *fimH* gene were PCR amplified by use of primers FimH1 and FimH3 and primers FimH2 and FimH4, respectively. At their 5' ends, FimH3 and FimH4 contained 20-bp regions homologous to the extremities of the tet gene. In the third step, the flanking regions were added on each side of the tet gene by mixing 100 ng of each fragment, followed by PCR amplification using primers FimH3 and FimH4. The PCR product was purified and electroporated into C3091ΔmrkA-F harboring the therosensitive plasmid pKOBEGApra, which encodes the λ Red recombinase. The C3091Δ*fimH* mutant was selected by growth on LB medium plates containing tetracycline at 37°C, and loss of the pKOBEGApra plasmid was verified by the inability of the mutant to grow on LB agar plates containing apramycin. Correct allelic exchange was verified by PCR analysis using combinations of primers inside the tet gene (primers TcD and TcU) (43) and primers flanking the deleted *fimH* gene (primers Up_fimH and Dw_fimK) (data not shown). To verify that the mutant was not able to express FimH, the *fimH* mutant and the wild-type strain were analyzed for the ability to agglutinate guinea pig erythrocytes. As expected, the wild-type strain exhibited mannose-sensitive agglutination, whereas the *fimH* mutant did not agglutinate guinea pig erythrocytes.

**Construction of isogenic recombinant isolates.** *E. coli* and *K. pneumoniae* recombinant isolates were constructed by *fimH* subcloning as described elsewhere (38). Briefly, *fimH* genes were cloned from *K. pneumoniae* cas663 and cas665 with *fimH* TOP2 and *fimH* BOT4 (Table 2) by PCR and subcloned into the pACYC184-based plasmid pGB17 under the *bla* promoter. The *fimH*-expressing plasmids were introduced either into *fimH*-null *K. pneumoniae* strain C3091ΔmrkA-F Δ*fimH* (see above) or into *fim*-null *E. coli* strain AAEC191A with the pPKL114 plasmid encompassing the entire *E. coli* *fim* operon, excluding *fimH* (38).

**PPFC experiments.** Bacterial binding under different flow conditions was tested as described previously with minor modifications (47). Isogenic strains expressing FimH adhesins from cas663 and cas665, respectively, were grown overnight at 37°C in LB broth with shaking at 125 rpm and then washed with phosphate-buffered saline (PBS) and adjusted to 3 x 10^7 CFU/ml in 0.2% bovine serum albumin (BSA) in PBS. Cell culture dishes (35 x 10 mm, treated, polystyrene; Corning Inc., NY) were coated with 1 monomannosylated BSA (M-BSA) (200 g/ml) or RNase B (100 g/ml) in 0.02 M bicarbonate buffer for 1 h at 37°C and washed with 0.2% BSA in PBS. A parallel plate flow chamber (PPFC) measuring 2.5 cm (length) by 0.25 cm (width) by 250 μm (height) (GlycoTech) was assembled on top of the dish according to the manufacturer's instructions. The entire assembly was then mounted on a Nikon TE2000-E microscope with a 10x phase-contrast objective, connected to a high-resolution charge-coupled device Cascade camera (Roper Scientific, Inc., GA). Bacteria were spun into the chamber at various flow rates using a syringe pump (SP210C, Warner Instruments).

Bacterial binding to the surface was recorded in time-lapse videos with the shutter time set to blur out all free-floating bacteria and analyzed using Meta...
domain of FimH (aa 1 to 156) and 21 in the pilin domain (aa 160 to 279) (green in Fig. 1A, B, C, and D), i.e., without any domain preference.

None of the between-species variations overlapped with any of nine naturally occurring mutations found within E. coli FimH known to be pathoadaptive in nature and to increase 1M binding (monomannose binding) under static/low-shear conditions (residues 27, 56, 62, 66, 78, 119, 139, 163, and 166, shown in red in Fig. 1) (39). In contrast to the between-species variations, most of the within-species E. coli mutations (seven versus two) were located in the lectin domain (P < 0.05). Similarly to E. coli, K. pneumoniae FimH also exhibits within-species variability, with a total of seven naturally occurring variations from the consensus FimH found by analysis of 54 K. pneumoniae strains. As in E. coli FimH, most of the natural within-species K. pneumoniae mutations (five versus two) were mapped to the lectin domain. Of the within-species K. pneumoniae mutations found in the lectin domain, two occurred within E. coli as well (purple in Fig. 1A and B): at residue 27, alanine (Ala) had been replaced by threonine (Thr), and at residue 62, serine (Ser) had been replaced by alanine. Residue 27 is a known hot-spot position for pathoadaptive mutations in E. coli FimH and has been shown to be associated with E. coli urovirulence by increasing 1M binding, through rather moderately (16, 37, 39). The S62A mutation produces a drastic increase in the 1M binding in E. coli and is found in a highly pathogenic clonal group of extraintestinal strains (19). Interestingly, the S62A mutation was found as the only point variation in FimH among a large clone of nosocomial uropathogenic K. pneumoniae strains in Denmark, with the mutation obviously occurring during the endemic circulation of the clone (42). Because the S62A mutation confers increased virulence in E. coli, we wanted to investigate if the same effect occurs in K. pneumoniae, by comparing the wild type with the mutant. Thus, the functional effect of the S62A mutation on the mannose-binding capabilities of K. pneumoniae FimH was investigated in detail.

Functional effect of the S62A mutation on shear-dependent binding. E. coli FimH mediates shear-dependent binding, wherein adhesion is weak under static or low-shear conditions but significantly increased with flow. As mentioned above, the S62A mutation belongs to the naturally occurring FimH mutations that increase the mannose-binding capability of FimH under static or low-shear conditions (1, 19). Thus, we examined whether K. pneumoniae FimH can mediate shear-dependent binding as well and whether mutation S62A has the same effect of increasing binding as in the E. coli FimH.

It is difficult to make quantitative comparisons of FimH-mediated adhesive properties in different clinical isolates because of the phase variability of type 1 fimbiae (20), as well as interference with fimbrial adhesion and expression by other adhesive organelles, such as type 3 fimbriae (15, 29). There-
FIG. 1. (A and B) FimH lectin (top) and pilin (bottom) domains shown from each side. The location of the carbohydrate binding pocket is indicated in light yellow (4). Polymorphisms distinguishing E. coli F18 and K. pneumoniae cas663 are shown in green. Within-species K. pneumoniae naturally occurring mutations are shown in blue; residues 94 and 105 (the latter is not visible in Fig. 1) have mutations both within K. pneumoniae and between E. coli and K. pneumoniae. Within-species E. coli naturally occurring mutations which increase binding to mannose are shown in red. Point mutations with pathoadaptive functional changes occurring both in E. coli and in K. pneumoniae (V27T and S62A) are shown in purple. Mutations in residues 105 (blue) and 106 (green) are hidden in the figures. (C) Binding pocket of FimH lectin domain. Polymorphisms distinguishing E. coli F18 and K. pneumoniae cas663 are shown in green. Mutations which functionally change the properties of FimH binding to 1M are shown in yellow. Mutations which functionally change the properties of FimH binding to 3M are shown in orange. (D) LIBS epitope (residues 29 and 152 to 157) shown in gray at bottom of the lectin domain of FimH. Polymorphisms distinguishing E. coli F18 and K. pneumoniae cas663 are shown in green.
in a mannose-specific manner (not shown), demonstrating that the recombinant *K. pneumoniae* FimH is functional.

First we tested the *K. pneumoniae* recombinant strains for binding under low-shear (0.1 dyne/cm$^2$) and high-shear (1.0 dyne/cm$^2$) conditions in a PPFC with 1M-BSA-coated surfaces. The binding under any shear tested was low at baseline for both variants, and the difference between them could not be reliably assessed (Fig. 2A). Then the flow chamber surface was coated with another FimH substrate, bovine RNase, with a terminally exposed (nonreducing) Man$_5$(1-3)Man$_5$(1-4)GlcNAc$_5$ trisaccharide (3M), with a significantly higher affinity than that of 1M for the *E. coli* FimH (24). On the 3M, FimHkp-S62 mediated a much more pronounced binding that was clearly shear enhanced in nature (Fig. 2A), with 3.5-fold-lower binding under low shear than under high shear ($P < 0.0001$). In contrast, the 3M binding mediated by mutant FimHkp-A62 was high even under low shear, displaying a sevenfold increase in binding relative to that of FimHkp-S62 at 0.1 dyne/cm$^2$ ($P < 0.0001$).

In order to directly compare the S62A FimH mutant of *K. pneumoniae* FimH with that of *E. coli*, we tested both wild-type and mutant variants from both species after cloning them into the same recombinant background, the *fimH*-null *E. coli* strain AAEC191(pPKL114). All recombinant strains agglutinated yeast cells in the same manner (not shown), suggesting that there were no differences in fimbrial expression. To demonstrate further that indeed there was no significant quantitative difference in FimH expression by the recombinant strains, we tested them for the ability to bind polyclonal antibodies (Pab280), raised against FimH-Ld (lectin domain) (46). Both *E. coli* and *K. pneumoniae* FimH-expressing bacteria were able to bind anti-FimH-Ld polyclonal antibodies equally well...
when bacteria were immobilized on a plastic surface and evaluated by ELISA (Fig. 3A). Moreover, to confirm the equal binding even further, we immobilized Pab280 polyclonal antibodies on a plastic surface and added bacteria in different concentrations (Fig. 3B), in addition confirming that the equal binding of *E. coli* and *K. pneumoniae* FimH was not due to oversaturation of polyclonal antibodies.

In the *E. coli* background, FimHkp-S62 and FimHkp-A62 again bound at a very low rate to 1M under low and high shear alike (Fig. 2B). This was in contrast to the corresponding FimH variants of *E. coli*, where both wild-type FimH (FimHec-S62) and mutant FimH (FimHec-A62) mediated detectable levels of binding to 1M, though in distinct fashion. As expected, FimHec-S62 mediated shear-enhanced adhesion—weak binding under low shear but a 3-fold increase in surface accumulation under shear stress—while FimHec-A62 adhesion to 1M was very strong under even low shear (Fig. 2B). Thus, binding to 1M was significantly weaker for FimHkp variants than for FimHec variants.

When the *E. coli*-based recombinant strains were tested in parallel on a 3M-coated surface (Fig. 2C), FimHkp variants showed a binding pattern similar to that seen in the *K. pneumoniae* recombinant background. The 3M binding was in general more pronounced than the 1M binding, with FimHkp-S62 showing threefold-greater accumulation under high shear than under low shear and FimHkp-A62 displaying strong accumulation independently of shear strength. In contrast to FimHkp variants, both *E. coli* FimH-S62 and -A62 variants accumulated well on 3M under low shear. However, while under high shear the FimHec-S62 was slightly increased, the FimHec-A62 binding decreased.

While there were some quantitative differences observed for given FimHkp variants expressed within either *K. pneumoniae* or *E. coli* fimbriae, this could be attributed to the difference between the two isogenic strain backgrounds. For example, the *fimH*-expressing plasmid complements the *fimH*-null fimbral operon that is on the chromosome (i.e., in a single copy) in the *K. pneumoniae* strains but on a multicopy plasmid, pPKL114 (i.e., in multiple copies), in the *E. coli* strains. However, deciphering the exact nature of the different isogenic background effects might require further investigation.

Taken together, these results show that, similarly to *E. coli* FimH, *K. pneumoniae* FimH is capable of mediating shear-dependent binding, while naturally occurring mutation S62A significantly increases binding under low shear. At the same time, *K. pneumoniae* FimH appeared to differ in specificity from *E. coli* FimH, in particular by exhibiting significantly reduced ability to bind 1M substrate.

**Allosteric properties of *K. pneumoniae* FimH.** It has been shown that *E. coli* FimH possesses a three-dimensional LIBS epitope in the bottom of the lectin domain that, in shear-dependent FimH variants, is hidden in the interface between lectin and pilin domains and is inaccessible to LIBS-specific monoclonal antibodies (2, 46). The LIBS becomes exposed to
Inability of K. pneumoniae FimH to recognize terminal mannose residues. To analyze further the nature of binding specificity differences between K. pneumoniae and E. coli FimH, we tested the recombinant strains’ abilities to accumulate on SBA (Fig. 2D). SBA features uniform Man9GlcNAc2 oligosaccharides, where all terminally exposed (nonreducing) saccharides are of Man9(1-2) nature, and the Man9(1-3) residue in the high-affinity Man9(1-3)Manβ(1-4)GlcNAcβ1 trisaccharide is not terminally exposed (Fig. 5B). None of the K. pneumoniae FimH variants strongly recognized SBA whether under low shear or under high shear. At the same time, binding of E. coli FimH to SBA had the same pattern as that of binding to 1M-BSA- but not 3M-coated surfaces, with distinct shear-dependent binding mediated by FimHec-S62 wild-type variant and a significantly increased ability of the FimHec-A62 mutant to bind under low shear (Fig. 2D).

Thus, neither K. pneumoniae nor E. coli FimH recognizes Man9(1-3)Manβ(1-4)GlcNAcβ1 trisaccharide in the high-affinity, 3M-like manner when the Man9(1-3) residue is not in a terminally exposed (i.e., reduced) configuration (Fig. 5). It also appears that the inability of K. pneumoniae FimH to recognize 1M-BSA reflects its inability to recognize single terminal mannose residues per se, even when the latter are present in abundance. Interestingly, three amino acid variations between E. coli and K. pneumoniae, in residues 132, 141, and 145, are conserved in positions 152 to 157 and 29, are conserved in the K. pneumoniae FimH protein and specific monoclonal antibody (MAB21) recognizing the LIBS epitope of the lectin domain, bound to purified fimbriae of K. pneumoniae FimH-S62 (dark bars) and FimH-A62 (light bars). Data are shown as optical densities at 650 nm. (B) Polyclonal antibodies with affinity to the lectin domain of the E. coli FimH protein and specific monoclonal antibody (MAB21) recognizing the LIBS epitope of the lectin domain, bound to purified fimbriae of E. coli FimH-S62 (dark bars) and FimH-A62 (light bars). Data are shown as optical densities at 650 nm.
DISCUSSION

The major observations in this study are that *K. pneumoniae* FimH (i) exhibits the shear-dependent binding and allosteric properties observed with *E. coli* FimH; (ii) converts to a shear-independent phenotype by a single naturally occurring mutation, S62A; (iii) has an altered monomannose-binding pocket that recognizes only very weakly the single terminally exposed mannose residues in N-linked oligosaccharides, under any shear; and (iv) has a preserved extended binding groove around the pocket that enables strong, but shear-dependent, binding to the terminally exposed Manα(1-3)Manβ(1-4)GlcNAcβ(1→4) moiety found in Man5 oligosaccharides.

The phenomenon of shear-dependent adhesion by bacteria was originally described for *E. coli* FimH adhesin and is characterized by weak binding under low flow that strengthens manifold with shear increase. The structural basis of this shear-dependent binding is allosteric in nature, where conformation of the mannose-binding lectin domain of FimH is controlled by the interaction with the fimbria-anchoring pilin domain. When the domains interact, the mannose-binding pocket is in a low-affinity configuration; when the domains separate from each other (e.g., by tensile force under high shear), the pocket switches into high-affinity conformation. This force-enhanced, so-called catch bond mechanism of adhesion may be widespread in nature and indeed provides several possible advantages in comparison with traditional, force-inhibited (slip bond) interactions: for example, by reducing the inhibitory potential of soluble ligand-like molecules, providing the means for rapid surface spread by adherent bacterial cells, and increasing the on rate of adhesive interactions.

The *K. pneumoniae* FimH structure is 85% identical to that of the *E. coli* FimH. We showed here that the common structural (consensus) variant of *K. pneumoniae* FimH mediates distinct, shear-enhanced adhesion on 3M-coated surfaces: bacteria bind in far greater numbers under a 1.0-dyne/cm² level of shear than under 0.1 dyne/cm². As in *E. coli*, the shear-dependent properties of *K. pneumoniae* FimH are likely to be based on the allosteric properties of the lectin domain. The most direct test for the allosteric properties of FimH is unmasking of the LIBS epitope in the presence of mannose ligand (2, 46). The existence of LIBS was originally described in integrins, and LIBS-specific monoclonal antibodies were the major tool for studying the role of allostery in integrin function (3, 13). In *E. coli* FimH, the LIBS epitope has been mapped to the bottom of the lectin domain, in the interface between the latter and the fimbia-incorporating pilin domain of FimH, i.e., well away from the mannose-binding pocket located on the top of the lectin domain (2, 46). The ability of mannose binding to unmask the interdomain epitope reflects an allosteric conformational link between the binding pocket and interdomain interface regions. The LIBS recognition pattern in *K. pneumoniae* FimH is basically identical to the one observed in *E. coli*, demonstrating similar allosteric properties in the two adhesins and indicating that shear-enhanced binding of *K. pneumoniae* FimH to mannose is obviously occurring via the same allosteric catch bond mechanism.

Taken together, these results show that *E. coli* and *K. pneumoniae* FimH exhibit very similar structure/function properties. This is particularly notable in light of the substantial 15% divergence between the proteins (40 aa in the mature protein), given that even a single amino acid variation can result in elimination of the shear-dependent properties of the adhesin. Despite these differences in structure, the shear-enhanced allosteric property is fully preserved, indicating the significant physiological importance of the phenomenon of dependence of bacterial adhesion on shear stress.

We have previously described a urovirulent *K. pneumoniae* clone that acquired a point mutation in *fimH* in the course of its endemic circulation as a nosocomial pathogen, leading to replacement of residue S62 (isolate cas663) with A62 (isolate cas665) (42). The same mutation has been described in *E. coli* (19, 30), where it emerged under positive selection in uropathogenic isolates (39). In *E. coli*, the A62 FimH variants demonstrated increased mannose-binding capability under static or low-shear conditions, which translated, in turn, into increased bacterial tropism for uroepithelial cells (with the exception of the urethra during urination, low-flow conditions prevail in the urinary tract compartments) and thereby contributed to the general urovirulence of *E. coli* (36). Indeed, when the mannose-binding capabilities of *K. pneumoniae* FimH with and without the A62 mutation were compared, the mutant variant dramatically increased bacterial binding, suggesting that S62A has been acquired under positive selection in *K. pneumoniae* as well, likely in order to increase urovirulence.

That mutation S62A produces similar functional effects in *K. pneumoniae* and *E. coli* indicates that the structural-functional properties of FimH in the two species are very similar. Unlike that of *E. coli* FimH, however, the shear-dependent binding of *K. pneumoniae* FimH could not be demonstrated convincingly on a 1M-BSA-coated surface, to which binding was very low relative to that of *E. coli* FimH. *K. pneumoniae* FimH bound in a much more pronounced manner to 3M, where shear-dependent adhesion (and the effect of mutation A62) was apparent in the differing levels of surface accumulation under different shears. The 3M adhesion of *E. coli* FimH is quite strong under even low shear (i.e., there is no shear threshold for the binding), and the shear-dependent binding is manifested by conversion from the weak, rolling adhesion mode to the strong, stationary mode under increased shear (24). The fact that *K. pneumoniae* FimH binding is not clearly observed on 1M, but readily demonstrated on 3M, indicates that affinity of the *K. pneumoniae* FimH binding is stronger to 3M than to 1M (as in *E. coli*) but that both types of binding are significantly weaker than the corresponding *E. coli* behaviors. The differences in adhesion between *E. coli* and *K. pneumoniae* are unlikely to be due to differing quantities of FimH expressed, as the amounts of FimH in fimbiae of the isogenic strains were the same (the LIBS-related studies) and, under at least some conditions, the binding was equal between the *E. coli* and *K. pneumoniae* FimH-expressing bacteria (e.g., under high shear on 3M [Fig. 2C]).

The 1M-BSA and 3M binding involve different specificities. The former substrate has individual 1M residues coupled in various numbers to BSA, which are likely to interact with FimH via a single mannose interaction involving the monomannose-binding pocket on the top of the lectin domain. Mannose residues in the 3M substrate, bovine RNase B, are part of the high-mannose, type N-linked oligosaccharides, where about half are in the Man5GlcNAc2 configuration (14). In
Man\(_5\), there is a trisaccharide, Man\(_{\alpha}(1-3)\)Man\(_\beta(1-4)\)GlcNAc\(_\beta(1\rightarrow\alpha)\), with a terminally exposed Man\(_{\alpha}(1-3)\) residue (Fig. 5A) that has much higher affinity toward FimH than 1M (10, 11), due to Man\(_{\alpha}(1-3)\) interactions with the monomannose-binding pocket combined with additional interactions between the \((\rightarrow\alpha)\)Man\(_\beta(1-4)\)GlcNAc\(_\beta(1\rightarrow\alpha)\) portion of the trisaccharide and FimH residues around the monomannose-binding pocket (51). The configurations of the remaining 3M oligosaccharides in bovine RNase B range from Man\(_6\) to Man\(_9\), with Man\(_{\alpha}(1-3)\) in the trisaccharide being capped with one or two Man\(_{\alpha}(1-2)\) residues, i.e., the high-affinity trisaccharide is internal and does not have a terminally exposed mannose anymore. We tested here whether mannosylated N-linked oligosaccharides with only internal high-affinity trisaccharide mediate 1M-like or 3M-like binding to FimH by using SBA as a substrate, where all oligosaccharides are in Man\(_9\) configuration (Fig. 5B). The binding to SBA was clearly 1M-like for both \(E. coli\) and \(K. pneumoniae\) FimH, showing that unless Man\(_{\alpha}(1-3)\)Man\(_\beta(1-4)\)GlcNAc\(_\beta(1\rightarrow\alpha)\) trisaccharide has a terminally exposed mannose residue, mannosylated oligosaccharides are recognized by FimH via the 1M-specific mechanism. In general, the 1M mechanism of FimH binding might involve individual terminal residues that are either in Man\(_{\alpha}(1-2)\) (as in SBA), in Man\(_{\alpha}(1-3)\) (47), or, possibly, in Man\(_{\alpha}(1-6)\) configuration. In 1M-BSA, all mannoses are coupled via Man\(_{\alpha}(1\rightarrow\alpha)\) linkages, thus serving as an appropriate model substrate for the binding to terminal mannosides in N-linked oligosaccharides. In fact, the high-affinity Man\(_{\alpha}(1-3)\)Man\(_\beta(1-4)\)GlcNAc\(_\beta(1\rightarrow\alpha)\) trisaccharide is rarely exposed terminally (as in bovine RNase B) and Man\(_5\) glycoproteins are known to be unstable (12). Not surprisingly, the majority of glycoproteins tested so far bind FimH exclusively or primarily in a 1M-specific manner (35).

FimH is a recognized virulence factor in uropathogenic \(E. coli\). FimH-mediated binding to uroepithelial cells is clearly 1M specific (17, 37) and, therefore, is dependent on shear. Between voidings, urine flow and, thus, shear stress in the urinary tract are very low, creating suboptimal conditions for the FimH-mediated attachment of uropathogenic \(E. coli\). However, the naturally occurring mutations in FimH, such as S62A, increase FimH capability to bind under low shear and are positively selected in the uropathogenic \(E. coli\) strains, i.e., are pathoadaptive (19, 24, 36, 37).

The finding that, unlike \(E. coli\) FimH, \(K. pneumoniae\) FimH recognizes 1M very weakly, even under high shear, and depends on shear for strong 3M binding may have pronounced implications for \(K. pneumoniae\) pathogenesis in the urinary tract. While FimH is important to the ability of \(K. pneumoniae\) to cause UTI in mice (43), it appears to be involved in pathogenesis only at the later stages of infection, whereas in \(E. coli\), FimH is critical in the early stages (32). The S62A mutation in FimH was acquired by a uropathogenic \(K. pneumoniae\) strain that belongs to a large endemic clone in Denmark that causes nosocomial UTI (42). Identity of this mutation with one occurring in FimH of highly uropathogenic \(E. coli\) strains and its acquisition during endemic circulation of a uropathogenic \(K. pneumoniae\) clone together support the hypothesis that mutation S62A is pathoadaptive in nature and positively selected in human uropathogens (42).

The mutant FimH may confer a selective advantage on \(K. pneumoniae\) by enhancing (though only slightly) binding to 1M residues under high shear. Such high shear exists periodically during urination along the epithelial surfaces of the urethra and bladder, as well as in urinary catheters, where \(K. pneumoniae\) is a leading colonizer. (One needs to note that in \(E. coli\), while FimH mutations that enable shear-independent binding are adaptive, a large portion of uropathogenic \(E. coli\) strains express nonmutated, shear-dependent FimH variants that are nonetheless critical for colonization.) Another possible advantage provided by the S62A mutation in uropathogenic \(K. pneumoniae\) strains is the enhanced ability to mediate bacterial binding to 3M residues under low shear. While the 3M mechanism of adhesion is likely to play a secondary role, it may still contribute to urinary tract pathogenesis. For example, there are some 3M-like oligosaccharide moieties in Tamm-Horsfall protein (4, 35), which is secreted in urine but also expressed on the luminal surface in the loop of Henle (34). Thus, it might serve as a receptor for bacteria in the kidney compartment but, due to low-shear conditions there, requires a shear-independent mode of bacterial binding enabled by FimH mutations.

Taken together, this study reinforces two important concepts in bacterial adhesion and pathogenesis, namely, that (i) the shear-dependent catch bond mechanism of adhesive interactions is a conserved and, thus, physiologically important phenomenon, possibly widespread in nature, and (ii) naturally occurring point mutations in virulence-related adhesins can modify their receptor-binding properties in a pathoadaptive manner, providing an advantage to and being positively selected in pathogenic isolates. Regarding pathoadaptive mutations, however, one needs to remember the complex natural dynamics that govern any functional genetic change: the change could be adaptive under certain, even transient, conditions but maladaptive under other, possibly more stable, conditions (40). Such selective trade-offs are likely to prevent pathoadaptive changes from becoming fixed in the population and possibly explain why the S62A mutation was identified in only a single \(K. pneumoniae\) strain. At the same time, the broad occurrence of transient habitat conditions provides ample opportunities for mutations with short-term fitness advantage to occur frequently and to target a substantial subset of genes shared by pathogenic and nonpathogenic strains from the same bacterial species (5).

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