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Structural and Functional Characterization of the *FimH* Adhesin of Uropathogenic *Escherichia coli* and Its Novel Applications

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Abstract

Type 1 fimbriae are responsible for bacterial pathogenicity and biofilm production, which are important virulence factors in uropathogenic *Escherichia coli* strains. Many articles are published on *FimH*, but each examined a specific aspect of this protein. The current review study aimed at focusing on structure and conformational changes and describing efforts to use this protein in novel potential treatments for urinary tract infections, typing methods, and expression systems. The current study was the first review that briefly and effectively examined issues related to *FimH* adhesin.

Keywords: Uropathogenic *E. coli*; *FimH* Adhesion; *FimH* Typing; Conformation Switch; *FimH* Antagonists

Abbreviations

UTIs: Urinary Tract Infections; UPEC: Uropathogenic *E. Coli*; IBCs: Intracellular Bacterial Communities; QIR: Quiescent Intracellular Reservoir; LD: Mannose-Binding Lectin; PD: Fimbria-Incorporating Pilin; MBP: Mannose-Binding Pocket; LIBS: Ligand-Induced Binding Site; THP: Tamm-Horsfall Glycoprotein; MN: Mannan Yeast; MI: Mono-Mannose; MIL: Slow M1-binding; M1H: High M1-binding; SNPs: Single-Nucleotide Polymorphisms; PFGE: Pulsed-Field Gel Electrophoresis; MLST: Multilocus Sequence Typing; RFLP: Restriction Fragment Length Polymorphism; WGS: Whole-Genome Sequencing; ERIC: Enterobacterial Repetitive Intergenic Consensus-PCR; AIEC: Adherent and Invasive *E. Coli*

Introduction

Urinary Tract Infections (UTIs) are mostly caused by uropathogenic *Escherichia coli* (UPEC) strains. The UPEC binds the urothelium with type 1 fimbriae encoded by the *fim* operon [1]. Type 1 fimbriae are expressed in over 95% of all *E. coli* strains and this expression is partially regulated by phase variable inversion of the genomic structure comprising the *fimS* promoter, which eventually results in phase ON (expression) or OFF (non-expression) orientations [1,2]. The two *fimC* and *fimD* genes play a role in transcription and assembly of type 1 fimbriae. The *fimC*-encoded chaperone protein transports fimbrial proteins through the periplasmic space, and the *fimD*-encoded protein acts as an usher (Figure1) [3]. This fimbria has four different subunits, including FimA, *FimH*, FimF, and FimG. The bulk of the structure is FimA that is polymerized into a right-handed helical fibril and has a variable structure [4]. *FimH* is the fimbrial adhesin that is at the tip of the organelle and is highly conserved genetically and antigenically among *Enterobacteriaceae* genera. Perhaps FimF and FimG are required for the integration of *FimH* into fimbria [4,5]. Bacteria expressing type 1 fimbriae can agglutinate guinea pig red blood cells under mannose-sensitive conditions that require *FimH* to induce this phenotype [6]. *FimH* proteins play important roles in UPEC pathogenicity and the formation of bacterial biofilms [7]. *FimH* binds to mannosylated uroplakin proteins in the bladder lumen and invades into the superficial umbrella cells [8]. After the invasion, UPEC is expelled out of the cell in a TLR4 dependent process, or escape into the cytoplasm [9]. The rapid proliferation of bacteria under complex pathways inside the cell leads to the formation of intracellular bacterial communities (IBCs) that morphologically have distinct structures with biofilm characteristics that protect UPEC against the immune system [8]. In the sequel, UPEC forms another type of intracellular structure called the quiescent intracellular reservoir (QIR). QIRs are small collections of UPEC bacteria (up to 12 bacteria) with slow growth, known as recurrent UTIs agents [10]. Also, *FimH* is involved in the dissemination of infection to other tissues. For example, *FimH* is the cause of UPEC localization in the brain microvascular endothelium and invade to meninges [11]. The current review study aimed at overviewing *FimH* adhesin and emphasizing some general aspects of its biogenesis and role in bacterial colonization of host cell surfaces and virulence factors. The current study focused on the essential content of *FimH*, including the structure, mutations, and applications in biotechnology, subtyping, and treatment of UTIs.

FimH structure

FimH protein is the precursor of 300 amino acids. It is then processed to 279 mature forms of amino acids (Figure 3) [12]. It is composed of two domains, the mannose-binding lectin (Ld, 1–156 amino acids) and the fimbria-incorporating pilin (Pd, 160–273 amino acids) connected via a short linker. At the tip of Ld, there is a β-barrel-shaped mannose-binding pocket (MBP) [13]. The MBP of *FimH* is stable amongst sequenced *E. coli* strains causing UTIs. Although some residues outside the MBP (positions 27, 62, 66, and 163) under positive pressure in UPEC isolates compared with intestinal strains [14]. The high affinity of *FimH* to mannose causes this deep pocket. It has three loops, the loops 2 and 3 are involved in polar interactions with mannose and a water molecule, and loop

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1, like a clamp, dissolves the mannose [15]. The interior of the MBP in *FimH* mostly consists of amino acids with hydrophilic side chains, and its exterior has amino acids with hydrophobic side chains [16]. The hydrophobic ridge directs the sugar into the binding pocket and facilitates polar interactions in the pocket. The hydrophobic nature of the pocket ridge distinguishes type 1 fimbriae in *E. coli* from type 1 adhesins in *Salmonella* sp. [17]. The tyrosine gate located at the entrance of the binding pocket, TYR48, and TYR137, plays a pivotal role in the strength and specificity of the adhesion-receptor interaction [16,18]. Although Ld has an affinity for all high-mannose glycans in the nanomolar range, the difference among glycans is based on their capacity for interaction with the tyrosine gate [19]. The Ligand-Induced Binding Site (LIBS) epitope is located at the bottom of the Ld, away from MBP [20]. The interaction of mannose with *FimH* under static conditions leads to the exposure of the LIBS epitope. The binding of monoclonal antibodies to the LIBS epitope, similar to shear stress, leads to a significant increase in *FimH* affinity to mannose. The existence of LIBS in the interdomain region shows that *FimH* is an allosterically regulated protein [13].

Conformation Switch of *FimH*

Interdomain interaction regulates the *FimH* affinity through the allosteric mechanism. The interaction of Pd with Ld keeps Ld in the low-affinity state, while if Pd is removed or has a weak interaction with Ld, a high-affinity state arises [21]. By increasing the flow of urine and inducing shear stress, *FimH* conformation changes from medium- to high-affinity, resulting in bacteria with stronger and long-lived binding to mannose ligands resist against micturition [22]. But, under static conditions, breakage and regeneration of short-lived bonds between *FimH* and mannose cause colonization of mannosylated surfaces by the bacteria [23]. The MBP is wider and looser in the low-affinity state than in the high-affinity state. As a result, the mannose affinity in the low-affinity conformation is much lower than that of the high-affinity one [24]. Also, inter-domain interactions contribute to UPEC motility in the urothelial surface, which can significantly affect UTIs pathogenesis [25]. Changes in the structure and function of Ld that affect tensile mechanical force created by the shear stress are similar to a molecular fingertrap. This type of *FimH* activation is the basis of the catch bond mechanism of type 1 fimbriae underflow [26]. Catch bonds are a new type of receptor-ligand interactions, which increases by tensile force, while traditional slip bonds collapse with growing force [13]. The effect of external force on *FimH* destabilizes the hydrogen bonds between the linker region and Ld. As a result, with the extension of the linker, the binding stability of the MBP increases, and its conformation changes to the high-affinity state. Under such circumstances, the MBP and linker regions are located on opposite sides of Ld [27,28]. The advantages of this adhesin include resistance to soluble inhibitors and rapid colonization of the surface. For example, THP -the most abundant protein in mammalian urine- protects the urothelial surface by binding to *FimH*, but the long-lived binding of *FimH* to mannose-coated surfaces under shear stress conditions neutralizes the inhibitory function of THP. The catch bond interactions are a conserved mechanism, and hence, a physiological phenomenon in and it is suggested that it can be used as a powerful and smart adhesion factor in technological applications [29].

FimH Receptors

The primary natural receptor of *FimH* is an uroplakin Ia glycoprotein with high levels in the urinary tract epithelial cells [30]. Other *FimH* receptors include Tamm-Horsfall glycoprotein (THP), β1 and α3 integrins, CD48, collagen, laminin, fibronectin, and abiotic surfaces [17,18]. *FimH* detects a wide range of high-mannose glycoproteins. Hence, proximal tubular cells of the kidney, buccal cells, epithelial cells of the bladder, intestine, lung, and different inflammatory cells are the target of the type 1 fimbriae. Also, *FimH* is attached to yeast mannans and interferes with the agglutination of yeast cells [30,31]. Based on the adherence of wild and recombinant strains to three substrate types, *FimH* is phenotypically classified as M, MF, and MFP. M phenotype strains can be only bound to the substrate rich in mannose residues such as mannan yeast (MN). MF phenotype strains attach to complex oligosaccharides such as human plasma fibronectin. MFP phenotype strains bind to MN, fibronectin, and synthetic peptides lacking saccharide compounds [32]. *FimH* lectin alleles are not identical in the ability to recognize terminal mannose structures. Typically, *FimH* subunits are capable of adhering through trimannose residues, but only *FimH* of UPEC isolates can attach to mono-mannose (M1) residues. As a result, phenotypic differences can lead to variations in host tissue tropism [32]. Some of the M1-binding *E. coli* strains are also efficient to identify complex oligosaccharides with no terminally exposed mannose residues [33].

FimH Mutations

Generally, bacteria modify the structure of the adhesins- e g, *FimH*, to adapt to the new niche and improve binding under different conditions, static or shear stress [11]. The main point in evolutionary adaptation is to optimize the adhesin binding properties [28]. Accumulation of amino acid replacements in *FimH* adhesin increases tropism for urothelium [34]. But, UPEC strains with mutant variants of *FimH* have a relatively well bind to M1 due to the presence of point mutations [35]. Two phenotypes of slow M1-binding (M1L) and high M1-binding (M1H) are observed in *FimH*. M1L variants are more common in the intestinal isolates, but M1H variants are

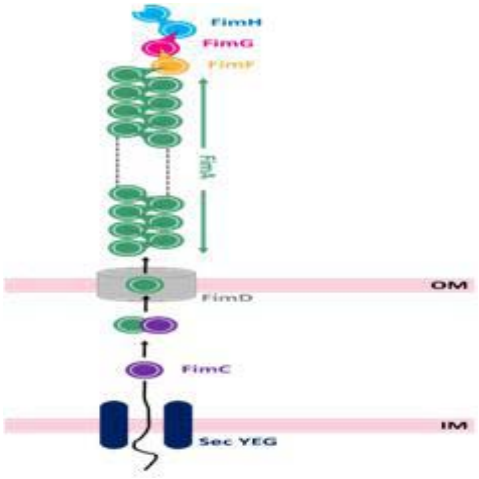

Figure 1: Schematic and simple illustration of the chaperone/usher pathway to assemble the Fimbriae; in the first stage, fimbrial subunits (A, F, G, H) enter the periplasmic space through the Sec system located in the inner membrane. Connection of the FimC chaperone to the subunits creates a soluble and stable form, and finally, the subunits are assembled through the FimD usher in the outer membrane and transferred to the cell surface. OM: Outer membrane, IM: Inner membrane.

Figure 2: Diagram of *FimH* Applications in Different Approaches.

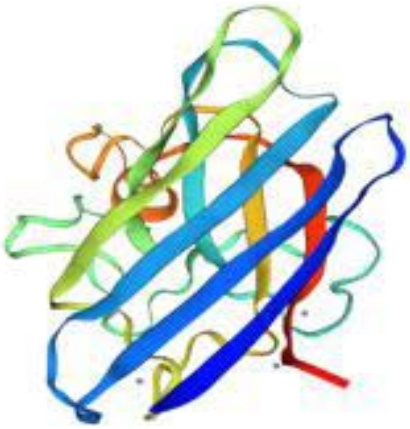

Figure 3: A schema of *FimH*-containing fimbrial tip crystal structure.

more commonly expressed in UPEC strains. They recognize cellular receptors and show less resistance to mannosylated compositions [2]. Probably, the conformational stability of the protein loop causes the strong binding of mutants to M1 that carries residues that interact with the receptor [33]. They change allosteric catch bond features of the protein observed in most *FimH* mutations, and increase binding to M1 residues. These mutations are called pathoadaptive and are responsible for increasing the virulence of the UPEC strains *in vivo* [20]. The pathoadaptive mutations are usually Single-Nucleotide Polymorphisms (SNPs) and modify the function of the protein by the replacement of amino acid. These changes are essential for the success of the pathogen [36]. The mutations occur in MBP and the interdomain region. Mutations in the interdomain area increase the Ld affinity to mannose up to 300 times [22,29,35]. Pocket zipper/clamp loop (in MBP), the β-bulge, the α-switch, and the interdomain swing, insertion, and linker loops, display an active role in the signal transition [37]. Mutant variants benefit from stable binding even under low flow conditions such as the upper urinary tract. Consequently, the allosteric catch-bond mechanism is necessary for the survival and transmission of pathogens [38]. On the other hand, strong adhesion to M1 can prevent the spread of UPEC biofilms inflow conditions. Also, these mutations increase the sensitivity to soluble mannosylated inhibitors [39]. Point mutations, along with mobile genetic elements, help to fast adaptation to *E. coli* strains and thus to spread infection by changing environmental conditions. Specific *FimH* variants could be indicative of unique *E. coli* adaptation in the inflammatory state and could be novel objects for the molecular characterization of such strains [34]. The mutations can also lead to the loss of multiple *FimH*-mannose interactions, and changes in the hydrogen bonding network and pocket geometry, which ultimately lead to adverse mannose-binding [17].

FimH Typing

Identification and typing of bacterial pathogens are particularly important in epidemiological studies, especially on *E. coli*, which is a common microorganism with high genetic diversity [40]. Multiple methods are applied to characterize *E. coli* strains, including Pulsed-Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Restriction Fragment Length Polymorphism (RFLP), and some types of PCR-based methods. PFGE is the gold standard in outbreak researches since its high discriminatory power or MLST is a useful and popular tool for global or long-term epidemiological studies [41,42]. Although these methods have high reproducibility and discriminatory power, they have disadvantages such as the need for high knowledge, time-consuming, high costs, and complexity of the work process; therefore, fast, simple, and cheap typing methods with reliable results can be very helpful [40].

Mutations that cause alterations in function occur as SNPs, which is a change in a nucleotide that causes amino acid replacements into essential pathogen proteins [36]. Most UPEC strains encode the *FimH* gene. It is located in an entirely recombinant region on the bacterial chromosome and is under positive pressure for functional mutations that alter the bacterial binding properties by the production of SNPs [43]. The high frequency of this gene among the UPEC strains, presence of sequence variants of this gene in GenBank, and the high heterogeneity in the gene sequences made it suitable for *E. coli* genotyping [44]. The *FimH* typing is a sequence-based and straightforward method for epidemiological studies [45]. Typically, *FimH* typing is performed by amplifying the *FimH* gene, followed by Sanger sequencing and alignment of the typing region with *FimH* variants contained in a database. However, this type can be performed quickly and easily on the Whole-Genome Sequencing (WGS) data [46]. The *fumC/FimH* typing is a rapid sequence-based typing method for UPEC isolates that have high discriminatory power. It could be cost-effective to evaluate large clinical isolate collections [43,47].

Furthermore, users can employ web tools designed to obtain *FimH* allelic information from simple Sanger sequences or WGS data [46]. The discriminative power of this method is suitable to investigate epidemiological events that occur over short periods or in a limited geographic area. This type can sometimes be used alongside other methods such as MLST and PFGE or even replace techniques such as Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR [44]. ERIC sequences are short, highly preserved 126-base pair noncoding regions found in *Enterobacteriaceae*. This method is a simple and cost-effective genotyping technology for discriminating different types of strains. The ERIC sequences are known in an enormous number of bacterial genomes, comprising *E. coli* [48].

Novel Therapeutic Target

The weakness of current therapeutic approaches to effectively combat resistant *E. coli* infections highlights the need for new

therapeutic strategies [49]. Today, attention is drawn to *FimH*-mediated adhesion as a potential target to treat UTIs [22]. Applying mannosides as a *FimH* inhibitor, preventing assembly and biogenesis of pilus by application of pilicides, using recombinant vaccines, and employing anti-*FimH* antibodies are the new approaches to treatment [34,50]. Over the last three decades, a large number of *FimH* inhibitors are introduced. The majority of studies are focused on the efficacy of tyrosine gate affinity [51]. Mannose-containing oligosaccharides inhibit *FimH*-mediated binding. This inhibition is based on the structural similarity between mannose and mannosylated receptors on urothelial surfaces. D-mannose binds to bacterial *FimH* to prevent attachment of bacteria to mannosylated receptors in the urinary tract, and ultimately flushing of urethral of unattached bacteria [52].

Some of them, such as α-D-mannosides with longer alkyl and aryl chains and heptyl mannose have a higher affinity for binding to *FimH* due to hydrophobic interactions with tyrosine gate [6,53]. *FimH* antagonists are a new therapeutic opportunity that does not lead to bacterial resistance in comparison with conventional antibiotics since they are not selective pressure on UPEC growth and do not affect bacterial metabolism. They are particular for a certain type of procedure or bacterial species and do not disrupt the host-microbiota [22,54]. The stability of bacterial phenotype, lack of selection of modified *FimH* variants after long-term exposure to mannose, minimal support for bacterial growth and metabolism, and emphasis of the bladder cell tolerance to the safe use of mannose require paying more attention to mannose to prevent and treat UTIs caused by UPEC [52]. A balance should be found between the ability of *FimH* antagonists to cross the cell membrane and the degree of lipophilicity in oral absorption [55]. Therefore, designing drugs based on *FimH* inhibition, quantum chemistry, and thermodynamics of *FimH*-ligand interactions can be a solution for treatments [51]. In recent years, several monovalent and multivalent *FimH* antagonists are synthesized. The n-heptyl α-D-mannoside treated with β-cyclodextin is a highly effective heptavalent antagonist when introduced into the bladder of C3H/HeN mice infected with the UTI89 bacterial strain [56]. The interest is higher in optimizing monovalent antagonists than multivalent antagonists. Since the former has little penetration into the gastrointestinal tract, and oral dosing is not possible due to its high molecular weight and high polarization. The advantage of multivalent antagonists is the increase in avidity [9]. In addition to the use of these compounds to treat UTIs, recent efforts to treat Corn's disease show that *FimH* antagonists can reduce the population of adherent and invasive *E. coli* (AIEC) *in vivo* [54]. Pilicides or substituted 2-pyridones regulate the level of pili expression by inhibiting the pilus assembly process without affecting the biophysical properties of the pilus rod [57]. IBCs are probably the leading cause of recurrent or chronic UTIs and are resistant to antibiotic clearance. Therefore, vaccines are an excellent option to eradicate these intracellular reservoirs of UPEC strains. *FimH* is extremely immunogenic among *E. coli* antigenic proteins and provides high protection against UTIs in mice and primates [58]. Also, it is a good target for the UT β Is vaccine since it possesses conserved and surface-exposed epitopes that mimic the humoral immune system [59]. Immunization with *FimH* vaccines reduces the binding of UPEC strains to human bladder cells *in vitro* and a 99% reduction in bacterial colonization in a murine model *in vivo*. This approach may even prevent acute and recurrent UTIs [60]. A study in 2019 showed that the non-pathogenic and recombinant *Lactococcus lactis* containing *FimH* prevents the spread of biofilm of uropathogens by biofilm formation in the bladder of mice [59]. The production of antibodies that target epitopes at the binding site of ligand proteins is essential to develop therapeutic antibodies [50]. However, antibodies against *FimH* may cause high-affinity conformation of the adhesin and increased bacterial binding [24]. But, genetically engineered low-affinity variants including A10P, R60P, V67K, and V27C/L34C may be useful vaccines. These variants, due to creating strong low-affinity conformation, can also be used to study *FimH*-mediated bacterial adhesion [61]. Inhibitors act in three ways; the first orthosteric inhibitors compete with the ligand to bind to the receptor. The second allosteric inhibitors exert their effect by binding distinctly to the ligand-binding site. The third type has a non-competitive impact on the ligand-binding place and has a better effect than the first two types [50]. Also, a *FimH*-based expression system is developed that provides the expression of functional eukaryotic proteins. One of the available methods is the production of recombinant proteins in genetically modified bacteria for biotechnology, therapeutic, and analytical purposes. After the production of recombinant protein, the carrier protein is required to transfer the desired protein to the cell surface. One of the carrier proteins is *FimH*, which can carry more abundant proteins than other carrier proteins such as LamB, OmpA, and PhoE (Figure 2) [62]. Besides, *FimH* can be used as a vaccine adjuvant for human cancer immunotherapy due to its water solubility and strong stimulatory effects on the immune system. By the immune system induction and subsequently the promotion of TLR4 and dendritic cells and activation of T cells, *FimH* prevents tumor cell growth, including melanoma and carcinoma, in mice [63].

Concluding Remarks

The type 1 fimbrial adhesin is important for adhesion, biofilm formation, and invasion. The affinity of *FimH* variants to mannose targets can differ due to changes in the primary structure of this protein. Mutations in different bacterial conditions in the urinary tract by altering the binding strength of the isolates caused changes in the bacterial tropism. Mutations in the *FimH* gene were employed for *FimH* typing. *FimH* conformational flexibility of LBP shifting was observed between open and tight conformations, with comparatively low- and high-affinity to mannose. In this direction, designing drugs based on *FimH* inhibition is newly considered as possible alternatives to treat UPEC. The current review study discussed the promising methods of using *FimH* antagonists as an alternative antimicrobial treatment for UTIs.

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