FimH and Anti-Adhesive Therapeutics

Subjects: Infectious Diseases
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Chaperone-usher fimbrial adhesins are powerful weapons against the uropathogens that allow the establishment of urinary tract infections (UTIs). As the antibiotic therapeutic strategy has become less effective in the treatment of uropathogen-related UTIs, the anti-adhesive molecules active against fimbrial adhesins, key determinants of urovirulence, are attractive alternatives. The best-characterized bacterial adhesin is FimH, produced by uropathogenic Escherichia coli (UPEC). Hence, a number of high-affinity mono- and polyvalent mannose-based FimH antagonists, characterized by different bioavailabilities, have been reported. Given that antagonist affinities are firmly associated with the functional heterogeneities of different FimH variants, several FimH inhibitors have been developed using ligand-drug discovery strategies to generate high-affinity molecules for successful anti-adhesion therapy. As clinical trials have shown d-mannose's efficacy in UTIs prevention, it is supposed that mannosides could be a first-in-class strategy not only for UTIs, but also to combat other Gram-negative bacterial infections.

Keywords: FimH ; adhesins ; uropathogenic Escherichia coli ; uropathogenic Klebsiella pneumoniae ; uropathogenic Proteus mirabilis ; urinary tract infection ; antagonists ; mannose-binding lectin ; affinity

1. Introduction

Urinary tract infections (UTIs) caused by different microbial agents, including both Gram-positive and Gram-negative bacteria and fungi, are among the most prevalent infectious diseases affecting millions of people annually [1][2][3][4][5][6]. Most UTIs are community- and nosocomial-acquired infections, which can occur in any part of the urinary tract [4][6][7][8][9][10]. Among the Gram-negative bacteria, the uropathogenic *Escherichia coli* (UPEC) accounts for 50% of nosocomial- and up to 95% of community-acquired UTIs, followed by the uropathogenic *Klebsiella pneumoniae* (UPKP) and the uropathogenic *Proteus mirabilis* (UPPM) [1][6][11][12]. Although UTIs can occur in both male and female patients, the number of female patients is significantly higher than male patients due to the anatomical features of women. These features include the shortness of the urethra, the urethral meatus's proximity to the anus, and the more humid surrounding environment compared to the male anatomy [1][4][5][6][10]. Despite receiving appropriate antibiotic therapy, 20–30% of women who have already experienced an initial UTI develop a recurrent infection (rUTI) within 4–6 months. The high recurrence rates of UTIs, and the increasing antimicrobial resistance among uropathogens, impose a significant economic burden. As such, the inefficacy of antibiotic therapies determines the urgent need for the development of alternative strategies for UTIs [1][13][14]. In this regard, alternative non-antibiotic strategies that target uropathogenic adhesive factors are highly attractive. Indeed, adhesion to host cells represents a critical step in the early stages of infection, allowing bacteria to contact, colonize and, eventually, establish the infection. Thus, uropathogens have developed a plethora of adhesive structures that enable bacterial colonization of the urinary tract [5][6][8][13][15][16]. The three most common uropathogenic pathogens, UPEC, UPKP and UPPM, are armed by adhesive pili or fimbriae, mostly belonging to the Chaperone-usher (CU) pathway [5][12]. The axial and pivotal role of these important virulence factors in bacterial pathogenicity and virulence has sped up the development of anti-fimbrial therapeutic approaches [17]. Among these new approaches, the anti-adhesive strategy seems an effective and valid treatment procedure for UTIs. Detailed structural characterization of these adhesive organelles, as well as their receptors and ligands, may help us to find new antagonists to compete with adhesins. However, lab costs have reduced the speed of success in this regard. Fortunately, in recent years, computational biology and chemistry, bioinformatics, and different databases and software tools have hastened the achievements in this field.

2. FimH is a Highly Adapted Virulence Factor

Among the CU pili from uropathogenic members belonging to *Enterobacteriaceae*, one of the best-characterized is type 1 pili. These pili are expressed by 80% and 90% of UPKP and UPEC strains, respectively [5][18][19][20]. It has been reported that more than 95% of all *E. coli* isolates express type 1 fimbriae [21][22][23]. The type 1 pilus is 2 µm in length and 10 nm in width, and is highly represented in the bacterial surface (100–500 pili per cell) [5][18][19][20]. This pilus is defined as

mannose-sensitive, because it is able to interact with the mannosylated receptors expressed by epithelial cells, particularly urothelial cells [12][24][19][25][26]. This specific function relies on the expression of the adhesin FimH located at the tip of the type 1 pilus. Type 1 pili of *K. pneumoniae* and *E. coli* are highly homologous in uropathogenic strains. However, the slight sequence variations between the FimH from *E. coli* and *K. pneumoniae* result in huge differences in the ability to colonize the urinary tract, FimH from UPEC being much more efficient in adhering to the mannosylated structure; for these reasons, it was chosen as the model for the bacteria–urothelium interaction [27]. Proper functioning of urothelium depends on the precise assemblage of highly specialized glycoproteins called uroplakins (UPs), the end products and differentiation markers of urothelial cells. On the apical surface, four major UPs are expressed by the umbrella cells lining the bladder, forming hexagonal plaques characterized by six tetramers linked by two heterodimers, UPIa/II and Ib/IIIa. UPs can be synthesized in several glycoforms; UPIa contains high-mannose N-glycans, and UPIb and IIIa carry complex N-glycans, whereas mature UPII lacks sugar moieties [28][29]. FimH binds to high-mannosylated UPIa, thereby ensuring a stable bacterial adhesion to the tissue. It is noteworthy that FimH is also responsible for biofilm formation, proliferation, and invasion of and internalization into eukaryotic cells, mediating the formation of intracellular bacterial communities (IBCs) [8][26][30]. Moreover, FimH is also able to interact with the Tamm–Horsfall soluble proteins, which are secreted by kidney cells, within the urine to exert a protective role against FimH adhesion [25][31]. Finally, CD48, types I and IV collagens, fibronectins and laminins are other receptors that can be bound by the UPEC FimH [32]. Due to the multiplicity of ligands and functions, type 1 pili armed with FimH represent a pivotal virulence factor within UPEC [5][8][24][33][34].

The whole FimH adhesin is composed of 279 amino acids. The N-terminal domain (NTD) carries a lectin domain ($FimH_{LD}$) encompassing the carbohydrate-binding domain (CBD), while the C-terminal domain (CTD) bears a pilin domain ($FimH_{PD}$) (**Figure 1**) [24][26][35]. The interaction between these two domains, $FimH_{LD}$ and $FimH_{PD,}$ determines the conformational state of the FimH adhesin, thereby influencing the level of affinity of FimH with the related molecule/receptor/ligand [13][26]. The conformation of FimH is highly dynamic, and interdomain interactions can be influenced by different factors, including the shear stress. Normally, FimH is in the low affinity conformation, also known as T-state; however, it switches to the high affinity structure in the presence of shear forces (R-state). The mechanism by which FimH binds to the mannosylated uroplakins is known as the catch and bond mechanism, which enables bacteria to establish long-lived interactions with host cells [13][26].

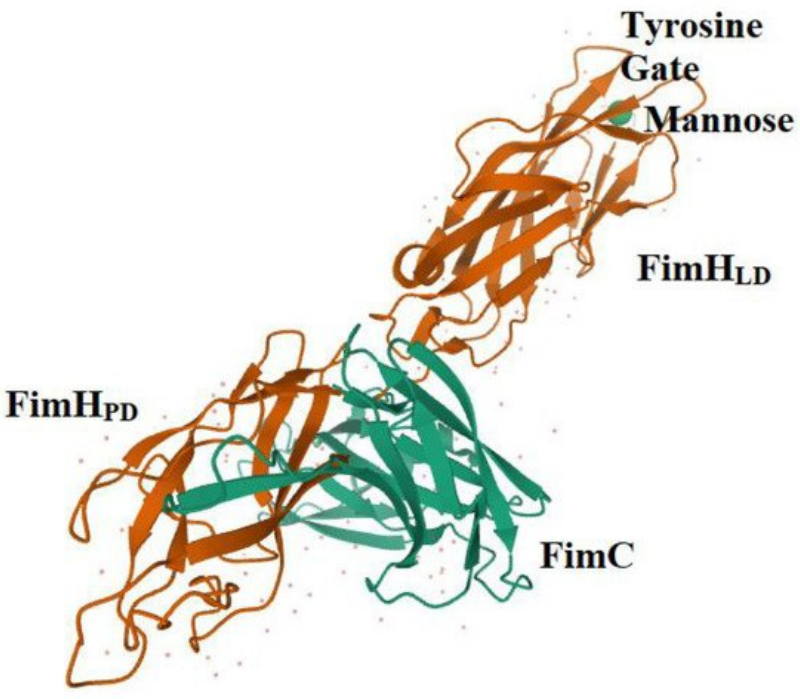

Figure 1. The structure of FimH co-crystallized with FimC. The $FimH_{PD}$, $FimH_{LD}$, Tyrosine Gate (Tyr137, Ile52 and Tyr48), molecule of mannose and FimC is shown (1KLF PDB file) [35]. FimC is a chaperone protein that does not belong to the mature fimbria.

The CBD within $FimH_{LD}$ is responsible for the binding to the mannosylated molecules, in that the amino acid's composition allows the formation of the negatively charged mannose-binding pocket (MBP), explaining why amino acids encompassing the MBP are extremely conserved among UPEC strains [34][36]. The MBP is surrounded by hydrophobic amino acids, comprising Ile13, Tyr48, Ile52, Tyr137 and Phe142 [34][36][37]. The dynamic conformation of amino acid

residues Tyr 48 and 137 constitutes the structure of the tyrosine gate (**Figure 2**) [34][36][37], which covers the hydrophobic groove of the mannose-binding site (MBS) [36][37].

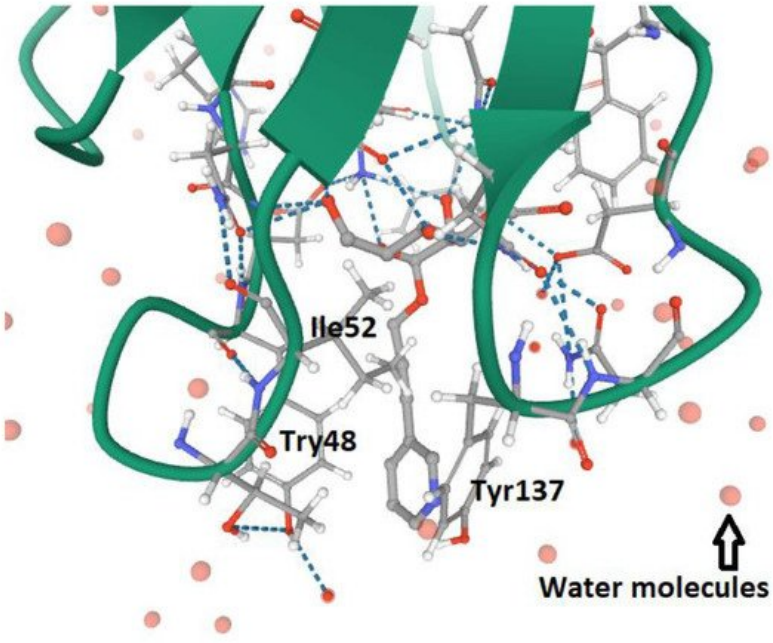

Figure 2. The structure of the tyrosine gate of $FimH_{LD}$ MBP in UPEC (Tyr137, Ile52 and Tyr48). The ligand is α-D-mannoside O-linked to a propynyl pyridine (4AV4 PDB file) [37].

The $FimH_{LD}$ MBS consists of a hydrophobic region (including Phe142, Phe1 and Ile13), a stretch of seven polar amino acids (Asn46, Asp47, Asp54, Gln133, Asn135, Asn138 and Asp140), the tyrosine gate (Tyr137, Ile52, Tyr48) and the Thr51 amino acid residue. The affinity between ligands and the MBP of FimH can be increased by Van der Waals bonds within the hydrophobic groove [24][36]. The side chains of the Tyr molecules at positions 48 and 137 are dynamic rotamers that can define three different tyrosine gate configurations: the full open, full close and partly open gate [36][37]. Any mutation in the tyrosine gate amino acids may lead to the loss of affinity with mannosylated molecules by FimH (**Figure 3**) [38]. Moreover, any mutation within the amino acids of the MBP of FimH, including Phe1, Asn46, Asp47, Asp54, Gln133, Asn135, Asp140 and Phe142, results in the abrogation of the adhesin function [24][35][39].

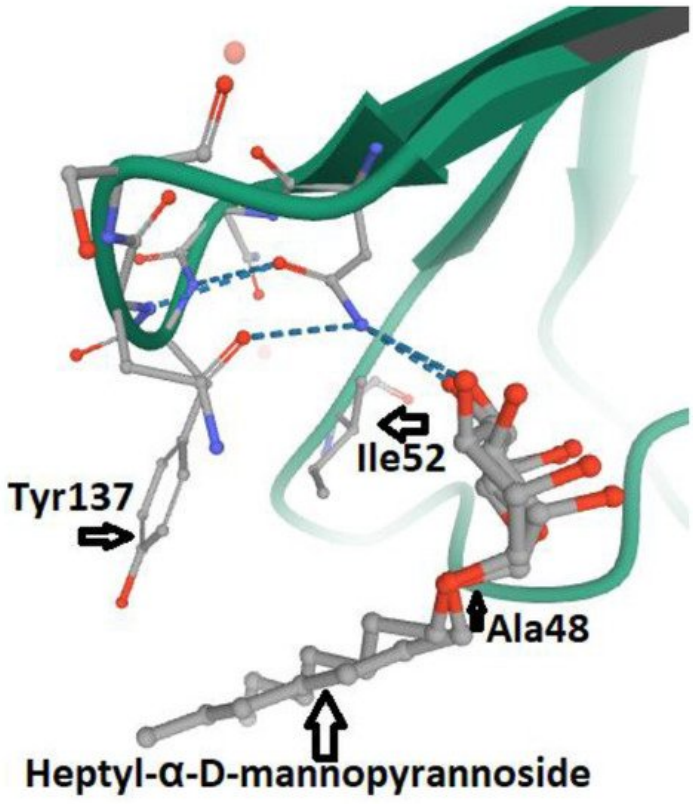

Figure 3. The Tyr48Ala mutation within the relaxed Tyrosine Gate of MBP in $FimH_{LD}$ from UPEC. The linkage of Heptyl-α-D-mannopyrannoside with mutated Tyrosine Gate is shown. The stacking pattern between Tyr137, Ile52, Ala48 and

Heptyl-α-D-mannopyrannoside is shown (4CA4 PDB file) [38].

3. FimH and Glycomimetics

Since FimH emerged as the most appropriate target for the development of anti-adhesive therapeutic strategies, several studies began, decades ago, to analyze the effects of FimH antagonists. Duguid and Gillis were the first authors to report mannose as an anti-adhesive substance in *E. coli* bacteria in 1957 [24][40]. Then, in 1977, the anti-adhesive activity of mannose was described in detail by Ofek, Mirelman and Sharon for *E. coli* [24][41], and later on for other uropathogens [42][43][44]. Up until now, different categories of soluble mono- and polyvalent FimH inhibitors (α-d-mannosides, and their chemically modified derivatives and glycodendrimers, respectively) have been selected, synthesized and analyzed [17][26]. Due to the huge number of available molecules, researchers in this field established standardized protocols and techniques to test FimH inhibitory activity. The relative inhibitory potency (RIP) index describes the extent of the affinity of the various FimH antagonists, compared to known synthetic mannoside derivatives such as the Methyl α-d-mannoside (MeMan), which is considered as a high-affinity molecule with regards to FimH [19][45]. The application of this method ensures the comparability of the results obtained by applying different experimental procedures. High RIP values result in the strong affinity of the analyzed molecules. For example, it has been shown that a monovalent antagonist with a high RIP value possessed the same affinity toward a variety of UPEC strains, while a polyvalent antagonist showing high RIP displayed a strain-dependent affinity. This result points out that more studies should be performed in assessing the therapeutic efficacy of polyvalent molecules [46]. However, despite their high and broad activity, monovalent glycosides, such as natural d-mannose, have no stable structures in vivo, and are rapidly hydrolyzed within the mouth, gastrointestinal tract and other organs and tissues, or they are quickly excreted from the body [47][48]. So, the application of antagonist therapeutic strategies, such as glycosidic drugs, represents a big challenge. The advances in understanding carbohydrate–protein interactions led to the development of a new class of small-molecule drugs for the treatment of several human diseases, known as glycomimetics. These molecules mimic the bioactive function of pure carbohydrates without presenting their drawbacks, such as low activity and stability [49]. Thus, specific chemical modifications represented a good approach to enhancing the bioavailability and metabolic stability of glycoside molecules [47]. Today, several glycomimetics, based on α-d-mannose derivatives, have been selected that show a strong affinity with FimH [34]. Glycosides are chemically linked to the aglycone (non-carbohydrate) portion in order to improve the glycomimetics' affinity to FimH, as well as their stability and bioavailability. Alkyl-, aryl- biaryl-, biphenyl-, butyl- dioxocyclobutenylaminophenyl-, indolylphenyl-, methyl-, phenyl-, triazolyl-, thiazolylamine- and umbelliferyl- are the most common aglycone groups combined with monovalent α-d-mannosides [24][26][34][36][50][51]. Vice versa, the polyvalent d-mannosides include cyclodextrin-based heptyl mannosides (CD-based HMs), divalent mannosides, glycoclusters, glycodendrimers, neoglycoproteins and trivalent mannosides, which contain more than one mannose subunit [24][34][36][52]. Indeed, lectins may carry several carbohydrate-binding sites (CBS), and this property enables them to significantly increase their affinity towards sugar residues. Exploiting this feature, multivalent glycomimetics are bound by FimH with high affinity, and this multivalent effect is known as molecule avidity [53][54]. These types of modifications result in an inhibition effect on FimH a million times greater than that exerted by the d-mannose sugar [33][55][56].

4. FimH Antagonists, Biochemical Characteristics and Bioavailability

According to the type of interaction with the d-mannosylated molecules, FimH adhesin changes its conformational structure, leading to different binding affinities. As outlined above, the low affinity (T-state) conformational structure of FimH, in which the LD and PD domains are in strict contact, occurs in the absence of shear stress. Vice versa, the high affinity conformation (R-state), in which $FimH_{LD}$ and Fim_{PD} are separated, represents the shear stress-induced allosteric regulation of its mannose-binding affinity, resulting in the strong attachment of $FimH_{LD}$ to the host urothelial cell receptors [26]. Thus, the balance between R- and T-states regulates the capability of the bacteria to colonize the urothelial niche or to spread the infection. At the molecular level, it is known that the interactions between α-d-mannose molecules (and related derivatives) and MBP in $FimH_{LD}$ occur in the presence of water, because water molecules support the hydrogen bonds between the hydroxyl groups of α-d-mannose molecules and the amino acid residues within MBP. Moreover, the presence of water drives the proper binding of α-anomer molecules to the MBP of FimH, increasing the affinity of the α-anomeric configuration of mannose and its derivatives with MBP [24][35][57][58]. Biochemical analyses of the interaction between $FimH_{LD}$ and α-d-mannose revealed that mannosides with an apolar (hydrophobic) substituent are able to mimic the interactions of high-mannose glycans with the MBD of FimH [56]. For this reason, n-hexyl- and n-heptyl-modified mannosides (i.e., MeMan) have a significant high affinity towards FimH [24][59]. This hydrophobic portion of aglycone interacts with the tyrosine gate through aromatic stacking (non-covalent interaction between aromatic rings) and Van der Waals bonds [51][59][60][61]. Moreover, it was shown that glycomimetics with inhibition constants in the range of 1–20 nM can be obtained by combining the α-anomeric configurations of d-mannose [58][62]. Hence, Wellens et al. generated a set

of α-d-mannosides carrying alkyl and aryl hydrophobic moieties. The determination of the crystal structure of $FimH_{LD}$ with the eight synthesized inhibitors, together with the analyses of their thermodynamic parameters, demonstrated that the presence of alkyl and aryl groups in the aglycone can induce the increased dynamics in the tyrosine gate responsible for the proper orientation of the interacting mannosides. This dynamic behavior of the tyrosine gate could contribute to FimH's ability to deal with less compatible high-mannose structures, while still making bacterial adhesion plausible [37]. Moreover, aromatic aglycone compounds mediate several interactions within the tyrosine gate in its hydrophobic space, increasing the affinity of the antagonist to the MBP of $FimH_{LD}$ [54][58]. An increase in the length of alkyl chains results in the higher affinity of the molecule with the $FimH_{LD}$ and, in particular, with the tyrosine gate area, showing that the affinity of the alkyl group with FimH adhesin is 100-fold greater than that exhibited by mannose [24][63][64].

It has been shown that O- and C-linked α-d-mannosides with hydrophobic and aryl substituents are potent *E. coli* FimH antagonists, having an affinity in the same range as that of nanomolar [65]. Indeed, the conformation and lipophilicity of aglycone moieties, their position with respect to the core sugar structure and the type of chemical group determine the RIP of antagonist molecules [34][54]. Para-substituted biphenyl derivatives were shown to be particularly appealing, owing to their numerous favorable binding interactions within the tyrosine gate. Thus, the structural and functional analyses of a series of O-, C-, and S-linked mannoside derivatives, incorporating the 1,1′-biphenyl pharmacophore and diverse aglycone atoms, demonstrated the suitability of these antagonists, establishing the possibility of further exploring these chemically modified mannosides [65][66]. Furthermore, it was shown that the biphenyl group linked to mannosides can be efficiently absorbed if orally administered [26][67]. Indeed, these mannosides show increased metabolic stability, bioavailability and intestinal permeability in in vivo pharmacokinetic studies, thereby recommending them for preclinical evaluation [68]. In addition, the reabsorption of biphenyl groups by renal tubuli results in stable and regular excretion into urine, leading to their high availability in the site of infection [26][67][69]. It was also demonstrated that 3′-chloro-4′-(α-d-mannopyranosyloxy) biphenyl-4-carbonitriler (**Figure 4**), synthesized using the bioisostere approach, is a highly effective FimH antagonist, also presenting optimal pharmacokinetic characteristics, such as proper solubility, low toxicity, intestinal permeability and renal excretion in mouse models [18]. Moreover, its oral application reduced the bacterial load in the bladder by almost 1000-fold 3 h after infection, highlighting its therapeutic potential [18].

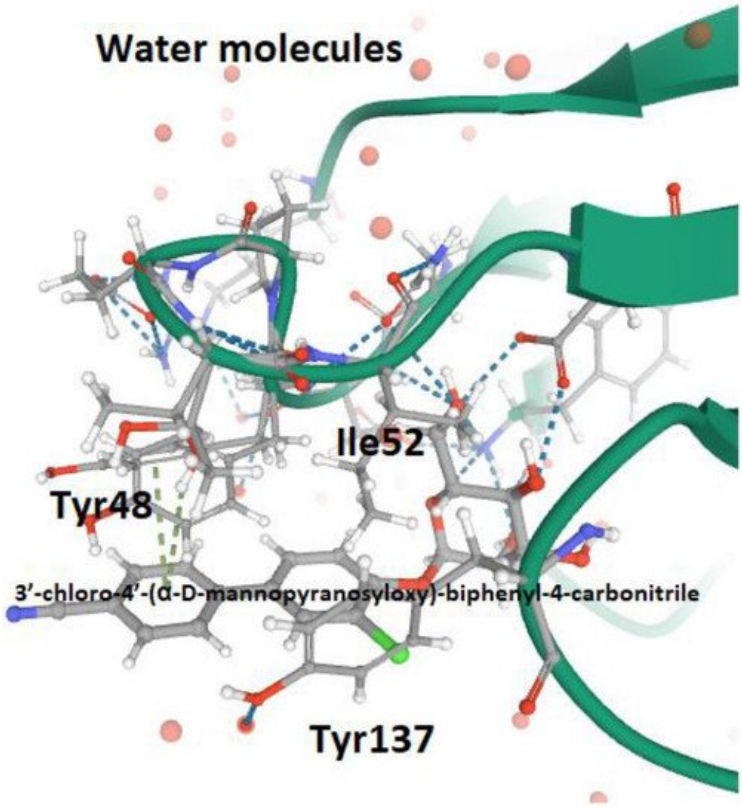

Figure 4. The successful linkage between Tyrosine Gate (Fim_{LD} MBP) and the bioisostere of 3′-chloro-4′-(α-d-mannopyranosyloxy)-biphenyl-4-carbonitrile (4CST PDB file) [18].

The polyvalent adhesin inhibitors (carbohydrate dendrimers) were designed to better mimic the interaction of FimH with high-mannose eukaryotic receptors [34]. The affinity, avidity and selectivity of mannosylated glycodendrimers are strengthened throughout by the presence of several mannose residues in the molecule; the so-called "cluster effect" [54][59][70]. Despite their higher affinity with the MBP of FimH, mannosylated glycodendrimers are large-size polar molecules, and these chemical properties reduce their absorption in the gastrointestinal tract, affecting their oral usage [34].

Apart from chemically synthesized mannose-based molecules, natural compounds, including cranberry and its derivatives, such as myricetin, cranberry extract standardized in proanthocyanidins (PACs) and PAC-derived polyphenol metabolites, have anti-adhesive effects on UPEC [25]. The mechanism by which these compounds exert their anti-adhesive activity is not totally understood yet. The complex molecular composition of these natural extracts can influence the establishment of the infection at different levels, acting on both bacteria and human physiology. Several investigations showed that PACs efficiently block the P fimbriae [71][72][73]. Vice versa, it was indicated that PAC-metabolites could be responsible for anti-adhesive effects on FimH [25]. Moreover, it was also suggested that cranberry induces the expression/secretion of the Tamm–Horsfall proteins by the kidney, thereby leading to its accumulation in the bladder. Thus, the interaction between UPEC FimH and the mannosylated Tamm–Horsfall glycoproteins causes bacterial release within the urine flux [74]. As such, cranberry-based supplements represent a source of natural compounds that are biochemically active against UPEC, which deserves further investigation.

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