Structural and Functional Study of the Receptor Binding Site for FimH Adhesin in Uropathogenic Strains of *Escherichia coli*

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We evaluated binding capacity of FimH-FocH hybrid adhesins during their interaction with model 1M and 3M substrates and epithelial cells. Introduction of the Glu89Lys point mutation into the *fimH* gene induced a new 1M-specific phenotype of adhesin. The role of a new pathoadaptive sign in the population of *E. coli* is discussed.

Key Words: *Escherichia coli; type I pili; FimH-FocH hybrid adhesin; 1M-specific phenotype*

FimH adhesin is a component of type I pili or fimbriae responsible for adhesion of *E. coli* to epithelial cells in the macroorganism. Bacterial cell carries 100-500 fimbriae that are regularly distributed on its surface. Type I pili are heteropolymers primarily consisting of FimA structural protein. Three additional subunits FimF, FimG, and FimH are also polymerized in the structure of fimbriae, but their contribution is about 1-3%. The adhesive subunit FimH is localized on the tip of fimbriae or incorporated over their whole length at various distances. Expression of pili is regulated by fim operon (DNA fragment of 9000 nucleotides) on *E. coli* chromosomes [2,4-7].

FimH recognizes and specifically binds to mannose receptors on the surface of target cells. Tropism of various FimH variants is similarly high for trimannose (3M) receptors, but different for monomannose (1M) receptors [12-15].

Recent studies revealed a population of uropathogenic *E. coli* carrying F1C fimbriae. They contain an equivalent of FimH adhesin named FocH [8]. FocH cannot recognize mannoside residues, but adheres to cells of the vascular endothelium and epitheliocytes of the urinary bladder via binding to glycosylceramide receptors. The amino acid sequence in FimH and FocH adhesins is identical (37%), which allows interchanging of their fragments with the formation of hybrid fimbriae. The specificity of receptors is determined by the structure of adhesin.

Here we studied binding capacity of hybrids during the interaction with 1M and 3M substrates and epithelial cells of various origins, assayed the variant of spontaneous mutant K12 strains with similar phenotypic characteristics, and evaluated localization of the FimH lectin domain determining its receptor specificity.

MATERIALS AND METHODS

Experiments were performed with recombinant strains obtained from phenotypically nude *E. coli* containing the pPKL114 plasmid. It carried *fim* operon including inactivated *fimH* gene and *AmpR* gene determining resistance to ampicillin (Fig. 1). The *fimH* gene from the studied natural strains of *E. coli* (uropathogenic strains MJ12 and J96, fecal strain F18, and laboratory strain K12) was amplified in the polymerase chain reaction, sequenced, and cloned in pACYC184-derived pGB2-24 vector carrying chloramphenicol resistance CmR gene under the control of the *bla* promoter. The set of hybrids included the N-terminal segment of FimH from strain K12 (amino acids 125-217) sequentially bound to the C-terminal segment of FocH [9].

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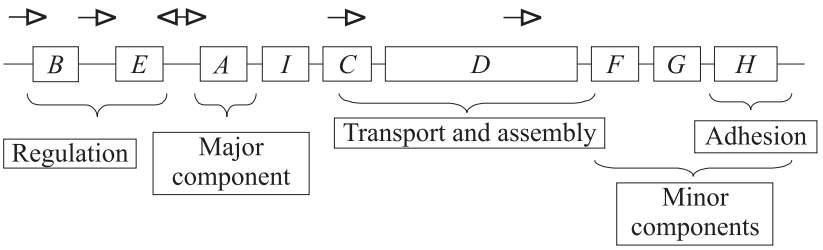

Fig. 1. Genetic structure of the *fim* operon.

Transcomplementation of plasmids carrying the fim cluster and inactivated *fimH* gene (similarly to the gene of adhesin alleles or chimeric *fimH-focH* gene) induces expression of fimbriae that are structurally undistinguished from fimbriae of wild-type FimH-K12.

Adhesive properties of recombinant and hybrid strains were quantitatively assayed in the growth test with model substrates RNase B (3M substrate, Sigma) and yeast mannan (1M substrate, Sigma). The substrates dissolved in 0.2 M $NaHCO_3$ (~100 µl, 10-20 µg/ml) were added to wells of a flat-bottom microtitration plate (Nunc) and incubated at 37°C for 45 min. Unbound substrate was removed by washout (150 µl phosphate buffered saline, PBS, pH 7.2). Nonspecific binding was blocked with 0.1% bovine serum albumin (BSA) in PBS. The bacterial suspension in 0.2% BSA-PBS (100 µl) was added to wells and incubated at 37°C for 40 min. Incubation with the inhibitor α-methylmonomannoside (α-mM, 2% solution in 0.1% BSA-PBS) was performed to confirm specificity of binding mediated by type I pili. The degree of inhibition was 90%. Unbound cells were washed 6 times with PBS. The nutrient medium (brain heart infusion, BHI) was added to wells. Plates were incubated in a rotor at 37°C for 2.5-3.0 h. Optical density in each well was estimated on an automatic microplate reader (Molecular Devices, Inc.; Menlo Park Calif.).

Adhesion of *E. coli* to epithelial cells was studied in model systems with buccal epithelial cells (BEC) and monolayer of urinary bladder cells (UBC) from the J82 culture (American Type Culture Collection, Rockville, Md.). Agglutination of yeasts and guinea pig erythrocytes was studied by routine methods [1, 2,16].

Electron microscopy was performed after treatment of bacteria with 0.5% phosphotungstic acid using an S-3000H Standard SEM microscope (Hitachi) [3].

RESULTS

Study of adhesive activity of strains during the interaction with model substrates showed that FimH of *E. coli* K-12 (FimH-K12) displays strong 3M specificity and moderate 1M specificity (1M/3M ratio 0.25-0.35). FimH of *E. coli* fecal strain F-18 (FimH-F18) was characterized by typical 3M-binding specificity (1M/3M ratio 0.05-0.15). FimH of *E. coli* uropathogenic strain MJ2 (FimH-MJ2) had another 1M/3M-specific phenotype (1M/3M ratio 0.7-0.8, Fig. 2).

The strains expressing hybrid adhesins $FimH^{1-217}$-$FocH^{228-290}$ and $FimH^{1-201}$-$FocH^{213-290}$ bound to RNase B and 1M-BSA. Similar results were obtained in experiments with wild-type FimH-K12. However, the strains expressing $FimH^{1-125}$-$FocH^{134-290}$ and $FimH^{1-139}$-$FocH^{151-290}$ chimeras did not bind RNase B and 1M-BSA. Bacterial strains expressing FimH-FocH chimeras with incorporated 1-184, 1-170, and 1-158 regions of FimH were characterized by pronounced binding to 3M substrate and RNase B (similarly to the wild-type strain). As differentiated from the wild-type strain, they gained high affinity for 1M-BSA (1M/3M ratio 0.9-1.0, Fig. 2). These hybrids were of particular interest. Further detailed investigations were performed with one of the hybrids.

We studied whether the $FimH^{1-184}$-$FocH^{196-290}$ hybrid strain can recognize 3M and 1M substrates by the multivalent (3M) and monovalent specific mechanisms (1M), respectively. 1M-Specific binding differs

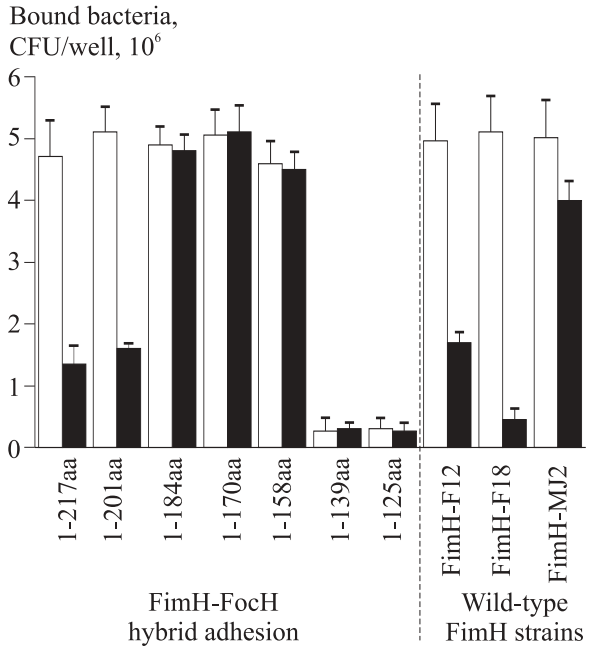

Fig. 2. RNase B and 1M-BSA binding of isogenic *E. coli* strains expressing FimH-FocH hybrids and natural variants. Light bars: RNase B. Dark bars: 1M-BSA.

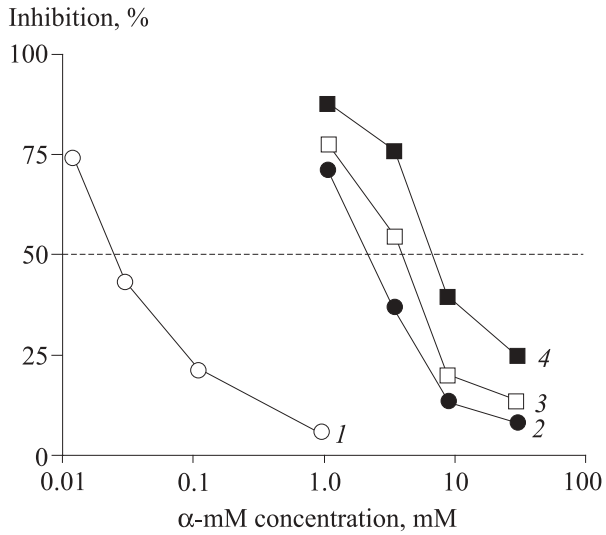

Fig. 3. Inhibition of 1M-BSA binding to isogenic *E. coli* strains expressing FimH-FocH hybrids and natural FimH variants by α-mM.

from 3M- binding by higher sensitivity to soluble inhibitors (1M, D-mannose, and derivatives). The D-mannose inhibition of interaction between FimH variants and 1M substrates reflects their ability to bind 1M-specific receptors.

FimH$^{1-184}$-FocH$^{196-290}$-mediated binding to 1M-BSA is more sensitive to the inhibition with α-mM (Fig. 3). The concentration of α-mM causing 50% inhibition (IC_{50}) was 50-200 times lower than that estimated in experiments with 1M-BSA binding induced by FimH-MJ2 and FimH-K12, respectively. It was impossible to evaluate accurately the degree of α-mM-induced inhibition of 1M-BSA binding for FimH-F18 due to low affinity of 1M-BSA. These results indicate that the FimH$^{1-184}$-FocH$^{185-290}$ hybrid strain has particular 1M-specific binding properties.

In additional experiments with these strains we studied α-mM-induced inhibition of FimH-mediated binding to RNase B (3M model substrate). Binding of strains expressing natural FimH variants was relatively resistant to inhibition with α-mM. These results are consistent with the ability of FimH variants to recognize 3M-specific receptors via the multivalent 3M binding mechanism.

However, binding of FimH$^{1-184}$-FocH$^{196-290}$ to RNase B was as sensitive to the inhibition as binding to the 1M substrate. As distinct from natural FimH adhesins, FimH$^{1-184}$-FocH$^{185-290}$ adhesin does not interact with 3M receptors by the multivalent 3M mechanism. It cannot be excluded that binding of this variant to RNase B proceeds via the monovalent 1M interaction with individual mannose residues that enter the composition of 3M oligomannose residues in RNase B.

Specificity of hybrid FimH-FocH variants was studied in 4 series of experiments with binding to eukaryotic cells [10-16]. Aggregation of yeasts and binding to BEC are characteristic of 3M and 1M/3M FimH variants. However, only strains expressing 1M/3M FimH variants formed rosettes with guinea pig erythrocytes and adhered to UBC.

Bacteria carrying FimH$^{1-184}$-FocH$^{196-290}$ adhesin were capable of binding in 1M-specific wells (Table 1). These results are consistent with strong 1M-binding activity of hybrid adhesin. However, only weak positive result was obtained in the 3M-specific test with this strain. Therefore, FimH$^{1-184}$-FocH$^{196-290}$ adhesin did not have 3M-receptor binding properties. Probably, bacterial binding to purified 3M receptors (RNase B) is associated with strong mono-binding activity of hybrid adhesin. It should be emphasized that 3M receptors on the surface of yeasts and BEC do not contribute to aggregation and activity of cells in the binding test, respectively.

Hybrids containing amino acids 1-184 of FimH adhesin can interact with receptors on the target cells via monovalent binding. However, they do not have multivalent binding properties. Probably, these adhesins with the 1M-binding phenotype constitute a new

TABLE 1. Binding of *E. coli* Strains Expressing FimH$^{1-184}$-FocH$^{196-290}$ or FimH$^{1-201}$-FocH$^{213-290}$ Hybrid and Natural FimH Variants to Eukaryotic Cells (*M*±*m*)

Types of fimbrial adhesins	Monomannose-specific tests		Oligomannose-specific tests	
	binding to UBC J82 (number of bacteria per cell)	binding to erythrocytes (titer)	binding to BEC (number of bound cell in well)	agglutination of yeasts (titer)
FimH$^{1-184}$-FocH$^{196-290}$	65±12	1:16	12±7	1:1
FimH$^{1-201}$-FocH$^{213-290}$	19±4	1:4	174±14	1:16
FimH-K12	15±3	1:4	195±12	1:16
FimH-F18	4±2	1:2	180±11	1:24
FimH-MJ2	52±7	1:16	205±24	1:16
FimH-MS260	53±14	1:12	47±13	1:2

TABLE 2. Binding of *E. coli* Strains Expressing FimH-MS260 and FimH-K12 to Eukaryotic Cells (*M*±*m*)

FimH variant	1M-BSA/ RNase B	Inhibitory activity of α-mM (IC_{50}, mM)		1M-specific tests		3M-specific tests	
		1M-BSA binding	RNase B binding	J82 cell binding, number of bacteria per cell	erythrocyte binding, titer	BEC, cells per well	yeast agglutination, titer
FimH-MS260	0.94	0.025	0.04	53±14	1:12	47±13	1:2
FimH-K12	0.35	8.0	20.0	12±2	1:4	185±15	1:16

class of adhesins differing from natural 3M- and 1M/3M-specific FimH variants of pathogenic commensals and uropathogenic strains of *E. coli*.

The discovery of a new monovalent 1M-FimH binding phenotype determined the search for similar functional variants among random mutants FimH-K12 [11]. One of 100 variants had similar phenotype in the interaction with model 1M and 3M substrates. The nucleotide sequence of the *fimH* gene in clone MS260 differed from that in wild-type FimH-K12 in 1 amino acid. Glutamine in the 89-position was substituted for lysine. Morphological characteristics of clone MS260 estimated by electron microscopy showed that the length, shape, and number of fimbriae on the surface of MS260 and FimH-K12 were similar. The study with anti-FimH antisera did not reveal interstrain differences in the degree of FimH expression on the surface of *E. coli*. Thus, Glu89Lys substitution inducing the 1M phenotype did not affect morphological characteristics of fimbriae or expression of FimH on the surface of bacteria.

The study of adhesive activity of this variant during the interaction with epithelial cells showed that strain MS260 more significantly bound to 1M-BSA than to RNase B. It should be emphasized that binding to both substrates was highly sensitive to inhibition with α-mM (Table 2). When compared to wild-type FimH-K12, strain MS260 was highly active in the 1M-specific test with UBC and formed rosettes with guinea pig erythrocytes. However, strain MS260 had low activity in 3M-specific tests of cell binding during aggregation of yeasts and interaction with BEC.

Our results show that mutation in the N-terminal region of FimH produces functional changes the binding profile observed after substitution of the amino acid in the 158- and 185-279-position in the C-terminal region of FimH for the corresponding part of FocH adhesin. Both types of structural changes induce a monovalent 1M phenotype of adhesin in type I pili.

Our study revealed a new phenotypic variant of adhesin characterized by monovalent 1M-binding and not capable of interacting with 3M-specific substrates. This phenotype was obtained after substitution of amino acid residues in the 185-279-position within the pilar domain of FimH adhesin for the corresponding segment of fimbrial FocH adhesin in type F1C pili. Moreover, the 1M-specific phenotype appeared after substitution of 1 amino acid (Glu89Lys) in the lectin domain of FimH. Monovalent adhesin was not revealed in natural strains of *E. coli* and is suitable for study of the ligand-receptor interaction.

Changes that enhance 1M-binding occur in various regions of the FimH molecule distant from previously identified carbon-binding pocket [5]. It remains unclear why amino acid substitutions promoting monovalent 1M-binding of FimH lectin do not modulate 3M-biding activity. Probably, changes stimulating 1M-binding affect the conformation of receptor-binding subsites within the FimH site, modify one of the structurally distant receptor-binding sites, or violate the tertiary or quaternary structure of FimH proteins without affecting the binding site.

Localization of the Glu89Lys mutation confirms the assumption that structural substitutions or mutations can affect the quaternary structure of FimH, which is important for functioning of FimH as a whole unit. Interestingly, the natural mutation responsible for the dual 1M/3M-specific phenotype of *E. coli* and located in the same region of the FimH lectin domain does not induce changes in morphological characteristics of fimbriae and number of surface expressed FimH.

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