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Original Article

In silico Study of Toll-Like Receptor 4 Binding Site of FimH from Uropathogenic *Escherichia coli*

Mehri Habibi, Mohammad Reza Asadi Karam, *Saeid Bouzari

Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran

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Introduction: The innate immune system as the first line of defense against the pathogens recognizes Pathogen-Associated Molecular Patterns (PAMPs) by Toll-Like Receptors (TLRs). Interaction of bacterial PAMPs by TLRs results in activation of innate and acquired immunity. FimH adhesin, a minor component of type 1 fimbriae encoded by Uropathogenic *Escherichia coli* (UPEC) is a PAMP of TLR4 that stimulates the innate immunity against infections. The FimH involves N- terminal and C- terminal domains. Detailed information about the TLR4 interaction with FimH is lacking. **Methods:** In this study, we evaluated interaction between TLR4 and whole FimH and two domains of FimH using computational methods. Two truncated forms of FimH that included N- terminal and C- terminal truncated forms were selected from PDB. Molecular docking analysis of TLR4 against FimH was done using HEX docking tool. The molecular interaction plot between TLR4 and FimH was generated Dimplot in LIGPLOT software (v. 4.5.3). **Results:** Based on the total free energy, C- terminal truncated form had the best interaction tendency to the receptor. Dimplot analysis showed that there are 11 intermolecular hydrogen bonds in the TLR4 and C- terminal truncated form of FimH complex. **Conclusion:** The high affinity of C- terminal truncated form to TLR4 suggests that this portion of FimH has important effect on the adjuvant activity and innate immune response and could utilize as adjuvant for vaccine application against microbial infections and cancers. *J Med Microbiol Infec Dis, 2014, 2 (1): 35-39.*

Keywords: Toll-Like Receptors, FimH, Molecular Docking Analysis.

INTRODUCTION

The innate immune system recognizes conserved microbial components, known as Pathogen-Associated Molecular Patterns (PAMPs), via a limited number of Pattern-Recognition Receptors (PRRs), which constitutes the first line of defense against the pathogens [1]. Toll-Like Receptors (TLRs) as important PRRs are type I trans-membrane receptors, with three different domains; intracellular toll-interleukin 1 receptor domain, which plays an important role in signal transduction, trans membrane domain and an extracellular ligand recognition domain containing leucin-rich repeats [2]. Since this major discovery, thirteen TLRs have been identified in mammals. TLRs 1, 2, 4, 5, 6 and 11 are surface-exposed, whereas TLRs 3, 7, 8 and 9 are located within endosomes. The surface-expressed TLRs primarily recognize structural components of pathogens, while the endosomal TLRs are dedicated to recognizing nucleic acids [3].

Two independent pathways have been distinguished for TLR signaling: MyD88-dependent pathway can be activated upon engagement by all TLRs except for TLR3. MyD88 recruitment leads to activate NF-κB, activator protein 1 and IFN regulatory factor 3/7, leading to the secretion of cytokines and dendritic cells maturation. MyD88-independent pathway or TRIF dependent pathway, that is associated with the stimulation of interferon (IFN) regulatory factors (IRFs) and production of type 1 IFNs [4, 5].

Recognition of bacterial PAMPs by specific TLRs triggers a signaling pathway resulting in production of proinflammatory cytokines/chemokines and up-regulation of co-stimulatory molecules, thereby activation of not only innate immunity but also acquired immune responses [6, 7].

The studies showed that several PAMPs of bacterial origin including LPS, flagellin, peptidoglycan and bacterial DNA can activate the innate immune system via TLRs [8]. Regarding the involvement of TLRs in the immune response against pathogens and current knowledge of their ability to activate innate and direct adaptive responses make them an attractive adjuvant candidate for vaccine formulations [9].

TLR4 is one of the TLRs, which is mainly expressed by cells of the innate immune system, including dendritic cells and macrophages. It is also expressed by many non-immune cells including fibroblasts and epithelial cells [10]. A diversity of ligands interact with TLR4, including lipopolysaccharide (LPS), mannans of *Candida albicans*, glycoinositolphospholipids of Trypanosoma, viral envelope proteins (RSV and MMTV) and endogenous antigens including fibrinogen and heat shock proteins. Recognition of the ligands by TLR4 induces a signaling cascade that utilizes both the MyD88 and TRIF-dependent pathways, leading to NF-κB and IRF3/7 activation, respectively [11]. Recent researches showed FimH adhesin, a minor component of type 1 fimbriae encoded by Uropathogenic *Escherichia coli* (UPEC), is also a PAMP of TLR4 that has been

***Correspondence:** Saeid Bouzari
Department of Molecular Biology, Pasteur Institute of Iran, No. 69, Pasteur Ave, Tehran, Iran, 1316943551.
Email: saeidbouzari@yahoo.com

Tel: +9821 66953311; **fax:** +9821 66492619

shown to stimulate the innate immune system and elicits protective responses against bacterial and viral infections. These findings may have important implications to utilize FimH as a promising adjuvant against infections, inflammatory diseases and cancers [12, 13].

FimH can directly interact with TLR4 independently of cofactors CD14 and MD-2 [13]. FimH is produced as a precursor of 300 amino acids that is processed into a mature form of 279 amino acids. FimH is folded into two domains of the all-beta class connected by a short extended linker. The N- terminal domain, or lectin domain comprises residues 1H to 156H and contains the binding pocket for D-mannosyl residues, that involved in bacterial attachment to mucosal epithelial cells and the C- terminal domain, which is used to anchor the adhesin to the pilus, comprises residues 160H to 279H [14]. The studies showed that FimH adhesin is conserved among different UPEC strains [15]. Studies have indicated that blocking the mannose-binding site of FimH with D-mannose has no effect on the FimH-induced innate immune response activity. This suggested that FimH may bind to TLR4 independent of mannose to induce innate responses [13]. Although the recently reports provide useful information about the recognition and binding of FimH to TLR4, detailed information about the precise sequence and structural requirements of the TLR4 interaction with FimH is lacking. However, It remains unclear which domain of FimH is involved in binding to TLR4. Advances in the field of structural biology have provided tremendous opportunities for analysis of interaction between receptor and ligand [16]. Identification of the nature of FimH/TLR4 interaction plays a crucial role in understanding the mechanisms underlying the TLR4 activation.

In this study, we investigated interaction tendency between TLR4 and whole FimH as well as two domains of FimH using computational methods. Finally, critical residues involved in receptor-ligand interaction were identified.

MATERIALS AND METHODS

Data set. X-ray diffraction structure of human TLR4 (PDB code: 3FXI) was retrieved through Protein Data Bank (PDB). The 3D structures of full-length FimH and two truncated forms of FimH include N- terminal and C-terminal domains were obtained from RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (PDB IDs: 3JWN, 3ZPD and 1ZE3, respectively). The tools Pymol and Swiss-PDB Viewer 4.0 were used to visualize the modeled 3D structures [17].

Molecular docking analysis of TLR4 and FimH. Molecular docking analysis of TLR4 against FimH was accomplished using HEX docking tool to obtain the best native conformation. In case of HEX dock tool [18], the input parameters were the PDB coordinate files for TLR4 and FimH with default parameters. TLR4 was defined as the receptor, and FimH was used as the ligand. Total energy of interactions was calculated based on shape and electrostatics as correlation type and the final search was set to 25 (N = 25). Other parameters used for the docking process were set to the default values.

Molecular interaction studies. The molecular interaction plot between TLR4 and FimH were generated using Dimplot in LIGPLOT software (v. 4.5.3) [19]. Default criteria were used for determining hydrogen bonds and hydrophobic interactions. The Dimplot program produces a plot of the interactions across a dimer or a domain-domain interface and the plotted interactions include hydrogen bonds and non-bonded contacts. Protein structure illustrations were generated with the Swiss-PDB Viewer 4.0.

RESULTS

Determination of truncated forms of FimH. Two truncated forms of FimH, including N- terminal (21-158) and C-terminal (159-279) domains that have 3D structure in PDB site were obtained from RCSB-PDB to determine which domain involved in interaction with TLR4. The 3D structure of the domains obtained from PDB is shown in Figure 1.

Docking analysis. Docking of the FimH and two truncated forms of FimH with TLR4 was performed by Hex docking server. Interaction free energies and docking conformations are shown in Table 1. Considering the total free energy, C-terminal truncated form had the best interaction tendency to the receptor (-861.6 kJ/mol). Full-length FimH was the next best structure. Finally, N- terminal truncated form showed the lowest affinity to the receptor. Figure 2 shows the 3D structure from docking of C- terminal truncated form with TLR4.

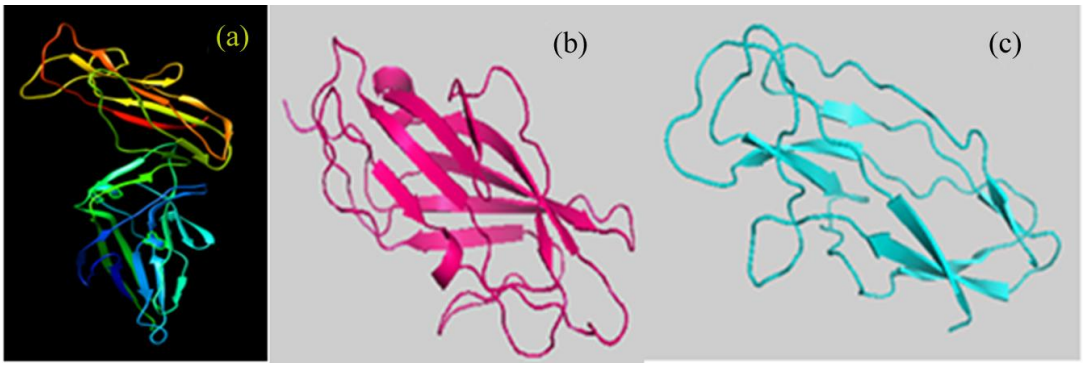


Fig. 1. Three-dimensional structures of FimH and truncated forms of FimH. (a) full-length FimH, (b) N- terminal truncated form of FimH, and (c) C- terminal truncated form of FimH.

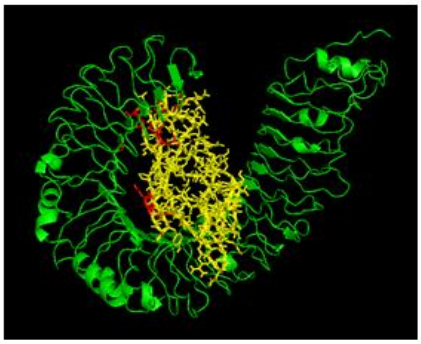


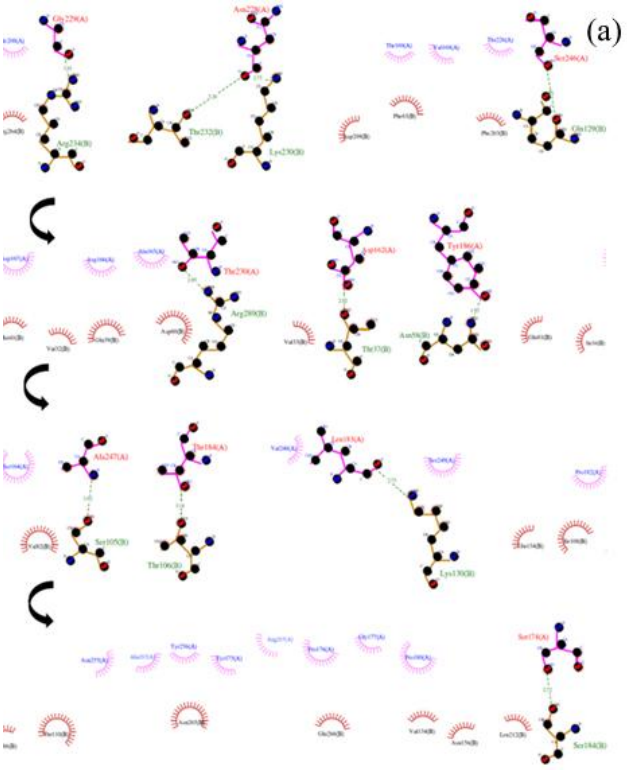
Fig. 2. 3D structure from docking of C- terminal truncated form of FimH with TLR4. C- terminal form of FimH (yellow), and TLR4 structure (green). Red labels show the amino acids of C-terminal truncated domain of FimH that interact with TLR4.

Molecular interaction analysis. The importance of hydrogen and hydrophobic bonds in the binding affinity of a ligand receptor has been described extensively. In the first, Hydrogen bonding and hydrophobic interaction between C-terminal truncated form and TLR4 are found by Dimplot analysis of the docked complex.

As shown in Figure 3(a), there are 11 intermolecular hydrogen bonds in the human TLR4 and C- terminal truncated form of FimH complex. Interaction plot indicated that 10 residues of C- terminal truncated form and 11 residues of TLR4 interact together by H bond. Amino acids (Asp 162, Ser 178, Leu 183, Thr 184, Tyr 186, Asn 228, Gly 229, Thr 230, Ser 246, and Ala 247) of C- terminal truncated form are found to form hydrogen bonds with amino acids (Thr 37, Asn 58, Ser 105, Thr 106, Gln 129, Lys 130, Ser 184, Lys 230, Thr 232, Arg 234, and Arg 289) of TLR4. The hydrogen bonds length is shown to be shorter than 3.2 A. Furthermore, 31 residues of C- terminal truncated form were involved in hydrophobic interaction with 34 amino acids of TLR4. Then, the molecular interaction of full-length and N- truncated form of FimH was analyzed (Figure 3(b) and 3(c)). Briefly, full-length FimH-TLR4 complex showed five hydrogen bonds. The involved amino acids responsible for formation of H bond were in N- terminal end of full-length FimH. Also, N-terminal truncated form of FimH showed three hydrogen bonds that one residue (Ser139) of the N- truncated form was responsible for H bond.

Table 1. *Hex* docking results based on interaction free energy (E-total)

Receptor (TLR4)	Ligand (FimH)	E-total (kJ/mol)
Whole molecule	Whole molecule	−625.6
Whole molecule	N- truncated form	−615.8
Whole molecule	C- truncated form	−861.6



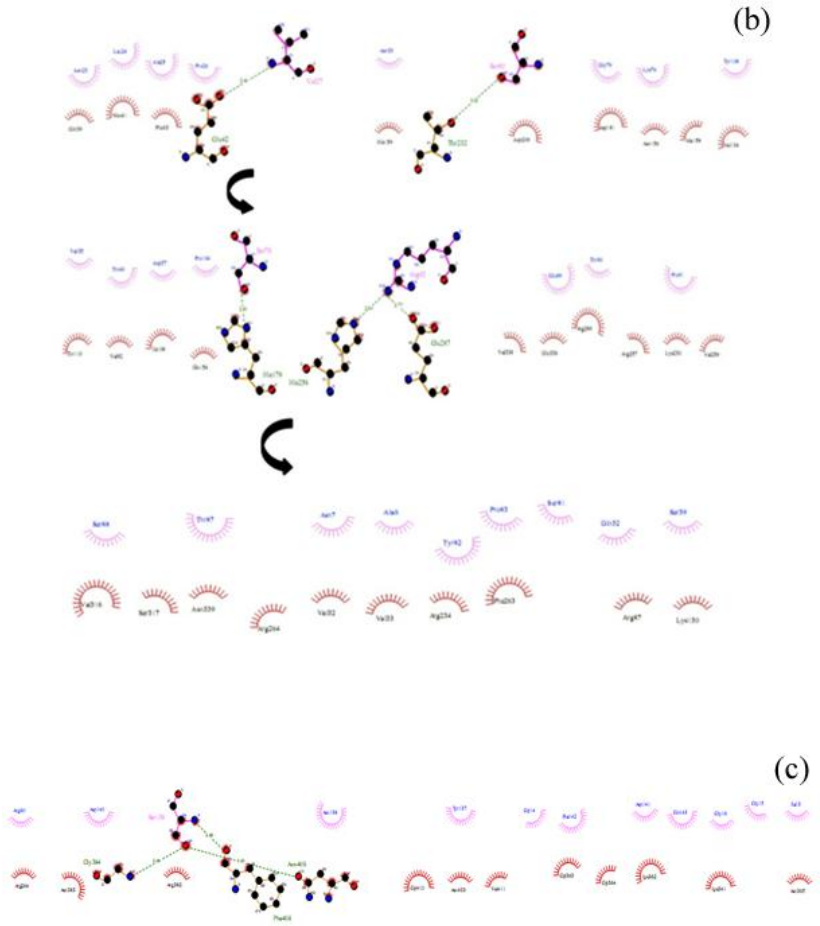


Fig. 3. Hydrophobic interactions and hydrogen bonding between: (a) C- terminal truncated domain, (b) Full-length and (c) N- terminal truncated domain of FimH and TLR4. Hydrogen bonds are shown by dashed lines (green) between C- terminal truncated form of FimH (red) and TLR4 (green) residues and hydrophobic interactions are shown by spoked arcs between residues.

DISCUSSION

The innate immune system plays a crucial role in the early defense against microbial infections. TLRs recognize pathogen-associated molecular patterns (PAMPs) and are thought to be the key sensors of invading microbes in the innate immune system [1]. Recognition of bacterial or non-bacterial PAMPS ligands by specific TLRs leads to the activation of transcription factors, such as NF-κB, and members of the interferon (IFN)-regulatory factor (IRF) family [20]. Since the discovery of TLRs, studies have focused on the activity of TLRs agonists and antagonists for development of new generation of drugs and vaccines [21].

TLR4 is the main receptor for LPS from Gram negative bacteria. The recognition of LPS by TLR4 requires the presence of two other molecules, CD14 and MD-2 [22]. In searching for such microbial PAMPs, very few studies recently discovered that FimH, as Uropathogenic *E. coli* type 1 fimbrial adhesin is a TLR4 ligand that induces potent innate responses in a MyD88 and TRIF dependent manner. They reported that FimH was able to binds directly to TLR4. More importantly, cells unresponsive to LPS were responsive to FimH and the presence or absence of MD-2 and CD14 had no effect on FimH activity [12, 13]. Although FimH is known to be the ligand for TLR4 and is able to induce innate immunity response, but ligand-receptor interaction between FimH and TLR4 hasn't yet been reported in any research work. Moreover, it is unclear which domain of FimH recognizes and binds to TLR4.

An increased number of protein structures in the Protein Data Bank (PDB) have also provided novel opportunities for scientists to visualize interactions between molecules in three dimensions [16]. The computational analysis of protein-protein interactions could provide insights into the binding of FimH to TLR4 and TLR4 activation. In the present study, to identify the most critical regions and residues of FimH for the interaction with TLR4, two truncated forms of FimH exist in RCSB-PDB were selected and investigated via protein-protein interaction studies. Our docking results indicated that based on the total free energy, C- terminal truncated form, containing the amino acids 159-279 of FimH, showed the best interaction tendency to the TLR4, while full-length FimH and N-terminal truncated form presented low-affinity toward TLR4 (Table 1). This finding is consistent with a previous study, suggested that N- terminal binding domain of FimH had no effect on the FimH-induced innate antiviral activity. According to their study, blocking the mannose-binding portion of FimH with D-mannose had no effect on the

FimH-induced innate antiviral activity [13]. The high affinity of C- terminal truncated form to TLR4 suggests that the deletion of N- terminal portions of FimH has no effect on the adjuvant activity. [13, 23].

The docking analysis not only identified the best interaction tendency between receptor and ligand but also provided useful information about the detailed interaction between TLR4 and the FimH ligand. In this study, docking studies between truncated forms of FimH and two truncated forms of FimH with TLR4 have been carried out by HEX docking server (Figure 2). The best docking model and suitable binding conformation was selected according to the lowest free energy and the basis of hydrogen bond interactions between the ligand and receptor, respectively (Table 1). The lowest energy poses indicate the highest binding affinity as high energy produces the unstable conformations. It's well known that hydrogen bond plays an important role for the structure and function of biological molecules, especially for interaction in a complex. The importance of hydrophobic interactions has been already reported [16]. In our study, C- terminal truncated form showed lowest free energy (Table 1), which signifies that this form has highest ligand protein interaction with TLR4. Eleven intermolecular hydrogen bonds have been observed between C- truncated form and TLR4 (Figure 3(a)). While, full-length and N- terminal truncated forms only indicated five and three hydrogen bonds with TLR4, respectively (Figure 3(b) and 3(c)). These results show that docking is probably the best known of methods used to identify the fit between a ligand and a receptor.

In conclusion, this *in silico* study provides useful information about the structural analysis of TLR4/agonist interaction and reveals the important residues of FimH involved in interaction with TLR4. These results suggests that C- terminal region of FimH exhibits considerable potential in evoking broadly innate immunity and can utilize as a promising adjuvant with vaccine for application against microbial infections and cancers.

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CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

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