Original Article

In silico study of ligand binding site of toll-like receptor 5

Nastaran Sadat Savar [1,2], Saeid Bouzari[1]

[1]Department of Molecular Biology, Pasteur Institute of Iran, [2]Department of Biology, Science and Research Branch, Islamic Azad university, Tehran, Iran

Abstract

Background: Toll-like receptor-5 (TLR-5) is a member of TLRs family and responsible for bacterial flagellin recognition. The activation of TLR-5 with flagellin leads to initiation of signaling cascades, which in turn results in transcription of pro-inflammatory cytokines. Regarding the critical role of TLR-5 agonists and antagonists in activation of innate immune responses, an increasing number of studies have focused on their therapeutic applications in drug and vaccine design. In this study, to identify the most critical region and residues of TLR-5 for interaction with flagellin, different truncated forms of TLR-5 were designed and subjected to protein-protein interaction studies.

Materials and Methods: The interactions of the full native TLR-5 and its truncated forms with bacterial flagellin (FliC) were evaluated using *Hex* docking server and molecular interaction analysis was performed using Dimplot analysis.

Results: According to our *in silico* results, truncated form C (an amino acid sequence containing residues 174-401 of TLR-5) has the most suitable interaction with FliC and seven amino acids within this region were found to be crucial for the interaction with flagellin.

Conclusions: These results provide new insights in to potential drug target sites of TLR-5, which may guide future TLR-5 targeting studies.

Key Words: *In silico* study, ligand binding site, toll-like receptor-5

Address for correspondence:
Dr. Saeid Bouzari, Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran. E-mail: saeidbouzari@yahoo.com
Received: 21.12.2012, **Accepted:** 13.02.2013

INTRODUCTION

Innate immunity plays an essential role in host defense against microbial pathogens and initiation of signaling pathways and subsequent activation of adaptive immune responses. The toll-like receptors (TLRs) initiate the activation of innate immunity responses via recognition of pathogen associated molecular patterns (PAMPs) including microbial components such as proteins, lipids, lipopoly sacharides, lipoproteins, and nucleic acids. To date, over 13 TLRs have been identified in mammals.[1] TLRs 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] Recognition of PAMPs by TLRs, triggers the signaling pathways leading to the activation of anti-microbial responses. TLR signaling consists of two major pathways: MyD88-dependent, which results in NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation and subsequent production of inflammatory

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.125730

How to cite this article: Savar NS, Bouzari S. *In silico* study of ligand binding site of toll-like receptor 5. Adv Biomed Res 2014;3:41.

cytokines and MyD88-independent pathway, which doesn't require MyD88 for NF-κB activation and is associated with the stimulation of interferon (IFN) regulatory factors (IRFs) and production of type 1 IFNs.[3-5] Regarding the important role of these pathways in innate immune activation and initiation of inflammatory responses, TLR agonists and antagonists could be considered as effective immune regulators, suggesting that these reagents have potential therapeutic value in inflammatory diseases and cancers. TLR-5 is one of the TLRs, which is mainly expressed on antigen presenting cells (APCs) and recognizes bacterial fllagelin. Recognition of flagellin by TLR-5, leads to the activation of immunomodulatory responses in APCs.[6,7] Regarding the ability of flagellin to activate innate immune responses, an increasing number of studies have focused on its adjuvant properties.[8,9] According to these studies, TLR-5 ligand could be an effective vaccine adjuvant and it may also serve as a valuable adjuvant for cancer radiotherapy, the treatment of degenerative diseases and myocardial infarction.[10,11] Identification of the nature of flagellin/TLR-5 interaction plays a crucial role in understanding the mechanisms underlying the TLR-5 activation. Studies have shown that TLR-5 binding site on bacterial flagellin is localized in conserved domain D_1[12] but, in the absence of three dimensional structure of TLR-5, the complimentary portion of TLR-5, which interacts with flagellin, remains less well-defined. Previous theoretical and experimental studies have predicted different interaction sites (residues 386-407, 552-561, and 174-401) in TLR-5.[13-15] The recently solved 3D structure of TLR-5[16] can give us the opportunity to further investigate TLR-5 ligand binding sites. In this study, we designed truncated forms of TLR-5 based on its putative interaction sites with flagellin. Subsequently the interaction tendency of each truncated form to the ligand was investigated via protein-protein docking protocols. Finally, the best truncated form was selected and its critical residues involved in ligand binding were identified.

MATERIALS AND METHODS

3D structure determination

The 3D structures of TLR-5 and *Salmonella typhimurium* flagellin (FliC) were determined from RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (PDB IDs 3A5X and 3J0A). Tertiary structure of TLR-5 was generated by electron microscopy single-particle image reconstruction at 26Å resolution.

Based on the suggested interaction sites of TLR-5, three truncated forms were designed. The truncation and geometry optimization of structures was carried out with YASARA (Yet Another Scientific Artificial Reality Application) (12.4.1) and Swiss-PDB Viewer 4.0.[17]

Protein-protein docking

Docking of ligand and receptor was performed using *Hex* docking server.[18] Total energy of interactions were calculated based on shape and electrostatics as correlation type and the final search was set to 25 (N = 25) and the angular search range of docking was limited by selecting an interface residue for flagellin (amino acid 411)[12] and the range angle of 45°. Other parameters were set to default values. The best truncated form of TLR-5, which showed the largest binding affinity to flagellin was subjected to further molecular interaction studies.

Molecular interaction studies

The molecular interaction plot between truncated form of TLR-5 and flagellin 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 interactions plotted include hydrogen bonds and non-bonded contacts.

RESULTS

Generation of truncated forms of TLR-5

In order to identify the most critical region of TLR-5 involved in interaction with flagellin, Three truncated forms of TLR-5 were designed based on suggested flagellin binding sites; truncated forms A (552-560), B (386-407), C (174-401). Structures were generated via truncation of 3D structure of TLR-5 (PDB: ID 3J0A) [Figure 1].

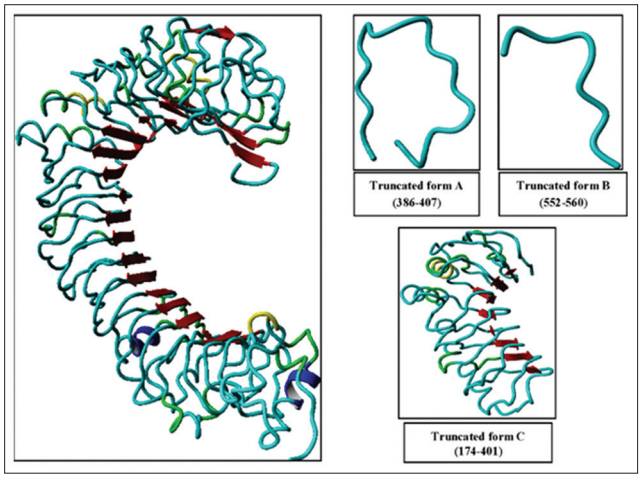

Figure 1: 3D structure of toll-like receptor-5 and its truncated forms A, B and C

Docking analysis

The interaction free energies are listed in Table 1. According to the Table, full-length TLR-5, containing all interacting epitopes shows the lowest free energy of interaction and has the best interaction tendency to the ligand. Truncated form C is the next best structure, which shows the best binding affinity compared to other truncated forms of TLR-5 and its total free energy is close to that of full-length TLR-5. The truncated forms A and B present the lowest affinity to flagellin.

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

Receptor (TLR-5)	Ligand (FliC)	E-total (kJ/mol)
Whole molecule	Whole molecule	−613.2
Truncated form A (552-560)	Whole molecule	−428.8
Truncated form B (386-407)	Whole molecule	−450.6
Truncated form C (174-401)	Whole molecule	−589.7

TLR-5: Toll-like receptor-5, FliC: Flagellin

Molecular interaction analysis

Hydrogen bonding and hydrophobic interaction between truncated form C and FliC are found by Dimplot analysis of the docked complex. Interaction plot indicates the formation of nine hydrogen bonds between nine residues of truncated form C and eight residues of FliC [Figure 2]. Amino acids (Arg 224, Lys 252, Arg 287, Arg 291, Arg 306, Asp 313, Phe 382, Glu 384, and Lys 385) of truncated form C are found to form hydrogen bonds with amino acids (Ser 423, Thr 420, Gln 409, Asp 140, Glu 405, Gln 417, Asp 395, and Lys 378) of FliC. The hydrogen bond lengths are shown to be shorter than 3.33Å. Additionally, 23 residues of truncated form C are involved in hydrophobic interaction with 23 amino acids of FliC.

Hydrogen bonds are shown by dashed lines (green) between truncated form of TLR-5 (green) and flagellin (pink) residues and hydrophobic interactions are shown by spoked arcs between residues.

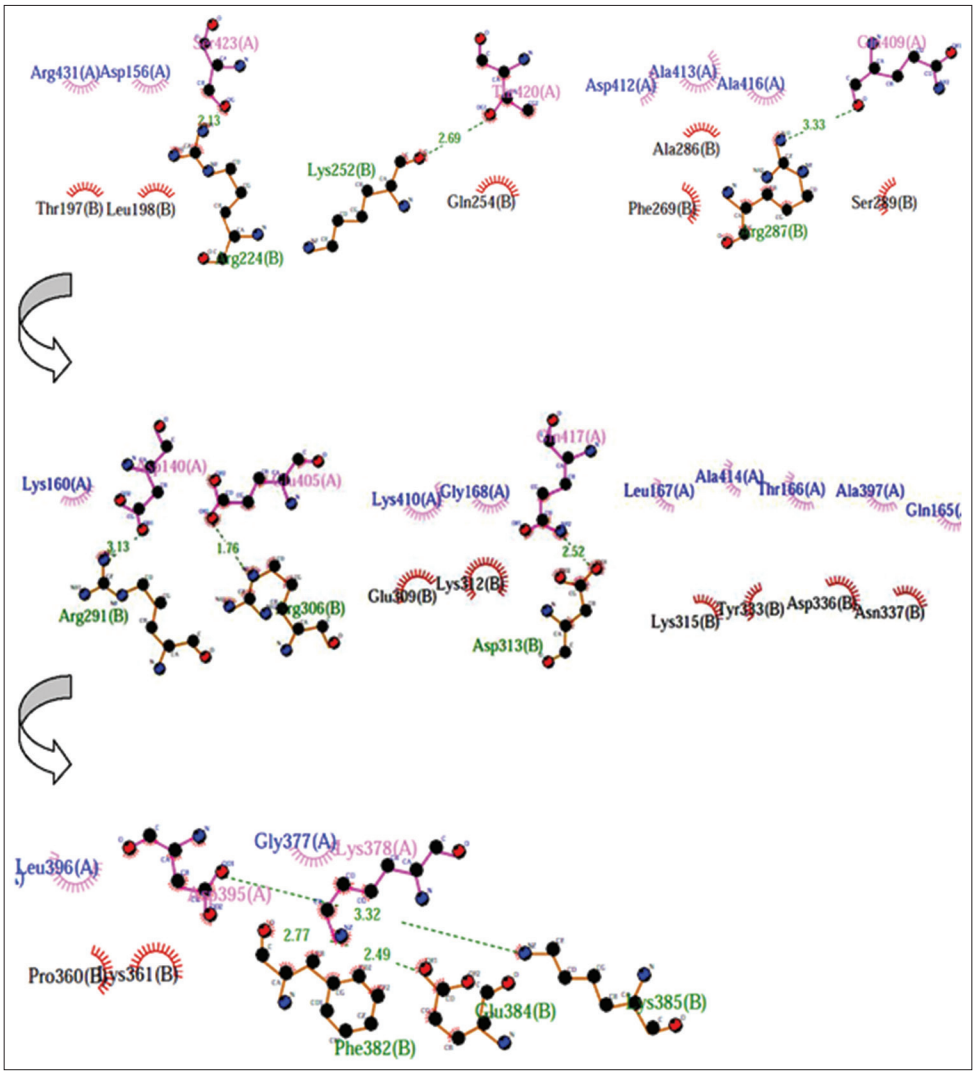

Figure 2: Hydrophobic interaction and hydrogen bonding pattern between truncated form C and FliC

DISCUSSION

TLRs are a class of innate immunity receptors, which play a key role in recognition of PAMPs. Binding of PAMPs to extracellular domain of TLRs initiates the signaling pathways resulting in innate immune responses.[1] 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.[20] TLR-5 recognizes bacterial flagellin, a major structural protein of flagellar filament.[15] The therapeutic applications of bacterial flagellin as TLR-5 natural agonist have been demonstrated.[10,11] Nevertheless, the mechanisms underlying the flagellin/TLR-5 interactions are not completely understood. The computational analysis of protein-protein interactions could provide insights in to the binding of flagellin to TLR-5 and TLR-5 activation. To date different interaction sites (residues 386-407, 552-561, and 174-401) were suggested in TLR5 structure.[13-15] In the present study, to identify the most critical region and residues of TLR-5 for the interaction with flagellin, different truncated forms of TLR-5 were designed and investigated via protein-protein interaction studies. Our docking results indicated that truncated form C, containing the central 228 amino acids (174-401) of extracellular domain of TLR-5, shows the best interaction tendency to the ligand while truncated structure A and B present low-affinity toward flagellin [Table 1]. This finding is consistent with previous reports indicating that residues 552-560 (truncated form A) and 386-407 (truncated form B) could not serve as flagellin binding sites.[15] According to the total free energy, the affinity of truncated form C to flagellin is close to that of TLR-5 wild type [Table 1], suggesting that the deletion of N and C terminal portions of TLR-5 doesn't have an important effect on the interaction of truncated form C in comparison to the full-length TLR-5. This finding is accordant with the previous studies, indicating that the central portion of TLR-5 is responsible for flagellin recognition.[15] In order to find TLR-5 binding sites, truncated form C/FliC complex was selected as the best docked complex for further molecular interaction studies. Dimplot analysis indicated the involvement of 18 amino acids in formation of nine hydrogen bonds. The hydrogen bond lengths are shown to be between 1.67-3.33 Å and the smallest hydrogen bond is found between Arg 306 of TLR-5 and Glu 405 of FliC [Figure 2]. Although, the short distance between these amino acids indicates the formation of a strong hydrogen bond, the obstruction of the region containing residues 292-366 by a glycan at the position of 342 of TLR-5,[16] may interfere with the formation of hydrogen bond. This can also include the interaction between Asp 313 and Gln 417. Other seven amino acids are found to be crucial in flagellin TLR-5 interaction. Amino acids Arg 224, Lys 252, and Arg 287 from truncated form C made hydrogen bonds with residues Ser 423, Thr 420, and Gln 409 of FliC all these three amino acids are located in conserved portion of FliC, which is known to form the TLR-5 recognition site.[12] Residues Phe 382, Glu 384, and Lys 385 donate the hydrogen bonds to amino acids Lys 378 and Asp 395 of FliC. Although, residues 395 and 378 are not located in conserved recognition site of flagellin, the participation of three adjacent residues in formation of hydrogen bonds could indicate their potential role in interaction with FliC. According to our results, all these seven amino acid residues are located on the lateral side, near the convex surface of TLR-5 [Figure 3]. In contrast, structural studies of TLRs have shown that, in most cases ligand binding occurs on the ascending lateral surface (dimmer interface) of the receptor, which completely lacks N-linked glycans.[21-24] However, the formation of asymmetric dimmers of TLR-5 in the absence of flagellin, suggests that TLR-5 may recognize two flagellin molecule cooperatively,[16] which propose the different activation mechanism for TLR-5. Additionally, the formation of molecular contacts on the interface side of subdomains may make this surface inaccessible to the large ligands such as flagellin.

This *in silico* study provides the structural analysis of TLR-5/agonist interaction and revealed the important residues of TLR-5 involved in interaction with FliC. These results provide new insights in to potential drug target sites of TLR-5, which may guide future TLR-5 targeting studies.

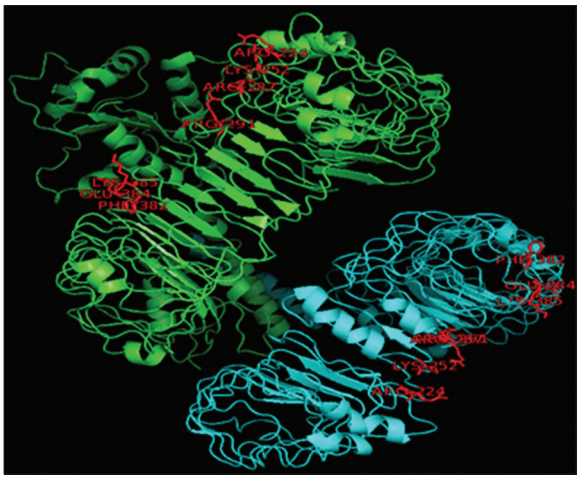

Figure 3: 3D structure of toll-like receptor-5 hetero dimmer. Red labeled amino acids are involved in flagellin binding

REFERENCES

1. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. Nat Immunol 2010;11:373-84.
2. Akira S, Takeda K. toll-like receptor signalling. Nat Rev Immunol 2004;4:499-511.
3. Honda K, Yanai H, Mizutani T, Negishi H, Shimada N, Suzuki N, *et al*. Role of a transductional-transcriptional processor complex involving MyD88 and IRF-7 in toll-like receptor signaling. Proc Natl Acad Sci U S A 2004;101:15416-21.
4. Kawai T, Sato S, Ishii KJ, Coban C, Hemmi H, Yamamoto M, *et al*. Interferon-alpha induction through toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat Immunol 2004;5:1061-8.
5. Pandey S, Agrawal DK. Immunobiology of toll-like receptors: Emerging trends. Immunol Cell Biol 2006;84:333-41.
6. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, *et al*. The innate immune response to bacterial flagellin is mediated by toll-like receptor 5. Nature 2001;410:1099-103.
7. Means TK, Hayashi F, Smith KD, Aderem A, Luster AD. The toll-like receptor 5 stimulus bacterial flagellin induces maturation and chemokine production in human dendritic cells. J Immunol 2003;170:5165-75.
8. Huleatt JW, Nakaar V, Desai P, Huang Y, Hewitt D, Jacobs A, *et al*. Potent immunogenicity and efficacy of a universal influenza vaccine candidate comprising a recombinant fusion protein linking influenza M2e to the TLR5 ligand flagellin. Vaccine 2008;26:201-14.
9. Vassilieva EV, Wang BZ, Vzorov AN, Wang L, Wang YC, Bozja J, *et al*. Enhanced mucosal immune responses to HIV virus-like particles containing a membrane-anchored adjuvant. MBio 2011;2:e00328-10.
10. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, *et al*. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science 2008;320:226-30.
11. O'Neill LA, Bryant CE, Doyle SL. Therapeutic targeting of toll-like receptors for infectious and inflammatory diseases and cancer. Pharmacol Rev 2009;61:177-97.
12. Smith KD, Andersen-Nissen E, Hayashi F, Strobe K, Bergman MA, Barrett SL, *et al*. Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. Nat Immunol 2003;4:1247-53.
13. Jacchieri SG, Torquato R, Brentani RR. Structural study of binding of flagellin by toll-like receptor 5. J Bacteriol 2003;185:4243-7.
14. Mizel SB, West AP, Hantgan RR. Identification of a sequence in human toll-like receptor 5 required for the binding of gram-negative flagellin. J Biol Chem 2003;278:23624-9.
15. Andersen-Nissen E, Smith KD, Bonneau R, Strong RK, Aderem A. A conserved surface on toll-like receptor 5 recognizes bacterial flagellin. J Exp Med 2007;204:393-403.
16. Zhou K, Kanai R, Lee P, Wang HW, Modis Y. Toll-like receptor 5 forms asymmetric dimers in the absence of flagellin. J Struct Biol 2012;177:402-9.
17. Guex N, Peitsch MC. Swiss-Model and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis 1997;18:2714-23.
18. Ritchie DW, Venkatraman V. Ultra-fast FFT protein docking on graphics processors. Bioinformatics 2010;26:2398-405.
19. Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. Protein Eng 1995;8:127-34.
20. Krishnan J, Lee G, Choi S. Drugs targeting toll-like receptors. Arch Pharm Res 2009;32:1485-502.
21. Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, *et al*. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell 2007;130:1071-82.
22. Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, *et al*. Recognition of lipopeptide patterns by toll-like receptor 2-toll-like receptor 6 heterodimer. Immunity 2009;31:873-84.
23. Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, *et al*. Structural basis of toll-like receptor 3 signaling with double-stranded RNA. Science 2008;320:379-81.
24. Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature 2009;458:1191-5.

Source of Support: Nil, **Conflict of Interest:** None declared.