In Silico Modeling of Human Toll like Receptor 1

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ABSTRACT

Toll-like receptors (TLRs) are membrane-spanning receptors that play a key role in the innate immune system.. They play an important role in providing innate immunity. TLR1 is one such protein that belongs to this family .It recognizes pathogen-associated molecular pattern and is specific for gram-positive bacteria. TLR1 dimerizes with TLR2 and identifies a mycobacterial lipoprotein .This interaction and recognition is considered to be a key step in the invasion of Mycobacterium based infections like tuberculosis. The protein structure for TLR1 has not yet been predicted, either theoretically or experimentally. This gave us scope for building a model structure for Human Toll like Receptor 1 using multiple templates and MODELLER 9v7 .

Keywords

TLR, Homology modeling, Modeller, Mycobacterium, Tuberculosis

1. INTRODUCTION

1.1 TOLL-LIKE RECEPTORS

Toll receptors were first discovered in Drosophila [1].They provide innate immunity by producing antimicrobial peptides upon activation. Toll-like receptors (TLRs) found in different organisms share structural similarity. [2]. In mammals they represent a family of pattern recognition receptors (PRRs) [3]. This family includes a known group of 10 human transmembrane proteins that are essential for innate immunity [4].

1.2 Toll Like Receptor 1 (TLR1)

TLR 1 belongs toToll-like receptor family (TLR) .It is a pattern recognition receptor that forms a part of innate immune system.TLR1 identifies a unique molecular pattern that is usually associated with gram-positive bacteria. TLR1 is designated as CD281. TLR1 dimerizes with TLR2 and identifies a mycobacterial lipoprotein .This interaction and recognition is considered to be a key step in the invasion of Mycobacterium based infections like tuberculosis [5].The members of TLR family are known to elicit varied immune response with respect to the type of antigen involved. [6]

The toll like receptors form dimers with other members of the family.TLR1 forms a dimer with TLR2.They can form homodimers or heterodimers but exhibit specificity for ligands. Dimerisation plays a crucial role in TLR1 function since it cannot recognize an antigenunless it is dimerised with TLR2. Transfection studies with TLRs 1, 2, 6 and 10 reveal that TLR1 and TLR2 together mediate strong activation of NF-KB-driven luciferase activity in response to LAM [7].

2. METHODOLOGY

2.1. Homology modeling

The aim of comparative modeling or homology protein modeling is to build a 3D model for a protein of unknown structure (the target) based on one or more related protein structures (templates).

2.2 Query Sequence

The query is a 786 amino acid human Toll Like Receptor 1 (gi|146291086|sp|Q15399.3|TLR1_HUMAN) reported to NCBI on 1st May 2007 and the annotation for which was updated on 2nd March 2010. The query was submitted to the SignalP server [8] to recognize the signal peptide.

2.3 Template Sequences:

Multiple templates were used to model the protein TLR1. Template searching was performed using the HHPRED server [9]. This server uses the HHSEARCH program to predict templates which runs 8 PSI-BLAST iterations so that even the most remote homologues of the target protein can be given as results. The templates are 2Z7X B chain, 2ID5 B chain, 1FYV.Secondary Structure Prediction was performed using GOR IV [10].Alignment was performed using the ClustalW server [11]

2.4 Model Building:

The alignment file was used as an input for the modeling step and the "alignment-multiple.ali" was generated. This was used as input to the Modeller9v7 program. [12]. The atom files for all three templates namely, 2Z7X, 2ID5 and 1FYV were made and were selected as multiple templates with which an initial model was generated.

2.5 Loop Modeling and Model Optimization:

To find the residues which are in the disallowed regions, this structure was submitted to the RAMPAGE server [13]. Based on this result, the residue range for loop modeling was decided. Based on the RAMPAGE server results, the region 500-580 residues was used for loop modeling and is modified using the loop modeling program of Modeller. A final model was generated. Which was further analysed for Ramachandran Plot statistics.

2.6 Model Evaluation:

Model evaluation was done using Verify 3D [14] PDB sum [15] and RAMPAGE server. PDB sum uses version 3.6.2 of PROCHECKthe generates a Ramachanran plot for analysis.

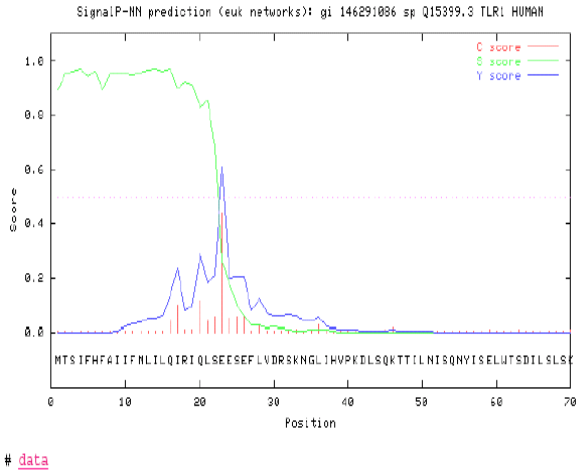
2.7 Energy Minimization

The above generated model was then further subjected to energy minimization using SPDBV. [16] The final protein structure was in the best of its conformation.

3. RESULTS and DISCUSSION

3.1 Signal P Results

Based on the result from the SignalP server, the first 22 residues of the protein were removed as signal peptide. (Fig 1)



data

Fig 1 : Signal P Results

3.2 HH Search Results

The sequence of the Human Toll Like Receptor 1 was retrieved from the NCBI database. Multiple templates were used to model the protein. The templates that were identified by HH Search are 2Z7X_B: The B Chain of Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. 2ID5_B: The B Chain of Crystal Structure of the Lingo-1 Ectodomain and 1FYV: Crystal Structure of the TIR domain of the Human TLR1

3.3 GOR IV Results

Results obtained from GOR IV indicate that 26.70% of the residues are in alpha helical conformation, 23.56 % are in extended beta strand form and majority of the residues ie 49.74% are in the form of coils **(Fig 2)**

```
        10        20        30        40        50        60        70
        |         |         |         |         |         |         |
EESEFLVDRSKNGLIHVPKDLSQKTTILNISQNYISELWTSDILSLSKLRILIISHNRIQYLDISVFKFN
ccccceeecccccccceecccccccceeeeecccccccchhhhhhhhhhhhhhhccchhhhccchhhhhcc
QELEYLDLSHNKLVKISCHPTVNLKHLDLSFNAFDALPICKEFGNMSQLKFLGLSTTHLEKSSVLPIAHL
hhhhhhhhhccceeeecccccccccccchhhhccccccccccccccchhhhhhhcccccccccchhhhhh
NISKVLLVLGETYGEKEDPEGLQDFNTESLHIVFPTNKEFHFILDVSVKTVANLELSNIKCVLEDNKCSY
hhhhhhhhhccccccccccccccccccceeeecccccceeeeecchhhhhhcccccceeeecccccccc
FLSILAKLQTNPKLSNLTLNNIETTWNSFIRILQLVWHTTVWYFSISNVKLQGQLDFRDFDYSGTSLKAL
hhhhhhhhcccccccccceeeceeccceeeeeeeeeeecceeeeeeeecccccccccccccccccchhhh
SIHQVVSDVFGFPQSYIYEIFSNMNIKNFTVSGTRMVHMLCPSKISPFLHLDFSNNLLTDTVFENCGHLT
hhcceecccccccceeeeeecccccceeeecceeeeeeeecccccceeeecccccceecceeeeccccch
ELETLILQMNQLKELSKIAEMTTQMKSLQQLDISQNSVSYDEKKGDCSWTKSLLSLNMSSNILTDTIFRC
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhcccceeecccccchhhhhcccccccceecceeccc
LPPRIKVLDLHSNKIKSIPKQVVKLEALQELNVAFNSLTDLPGCGSFSSLSVLIIDHNSVSHPSADFFQS
ccccceeeeecccccccccchhhhhhhhhhhhhhhhcccccccccccccceeeeecccccccccchhhh
CQKMRSIKAGDNPFQCTCELGEFVKNIDQVSSEVLEGWPDSYKCDYPESYRGTLLKDFHMSELSCNITLL
hhhheeecccccceeeeecccccccccccceeeeecccccccccccccceeeeecccccccccceee
IVTIVATMLVLAVTVTSLCSYLDLPWYLRMVCQWTQTRRRARNIPLEELQRNLQFHAFISYSGHDSFWVK
eeeeeeeeeeeeeeeccecccccceeeeecccccccccccccchhhhhhhhhceeeeeecccchhhh
NELLPNLEKEGMQICLHERNFVPGKSIVENIITCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEG
hhhccchhhhhceeeeeeecccccceeeeeeeeecccccceeeecccecccccceeeeecccceeccc
SNSLILILLEPIPQYSIPSSYHKLKSLMARRTYLEWPKEKSKRGLFWANLRAAINIKLTEQAKK
ccchhhhhhccccccccccchhhhhhhhhhhhhhhcccccccchhhhhhhhhhhhhhhhceec
```

Fig 2: GOR IV Results

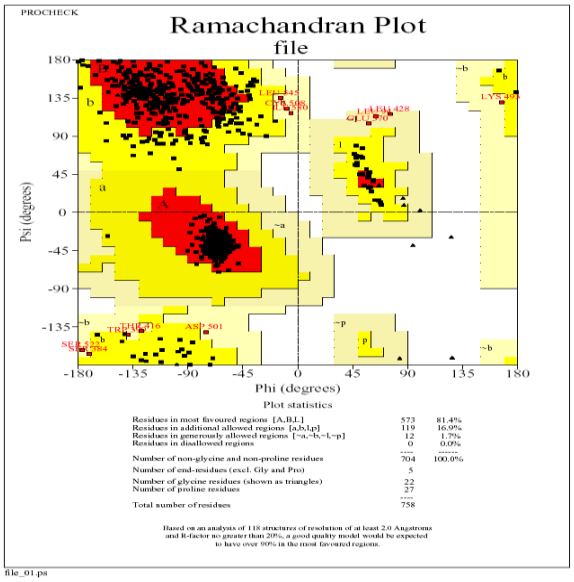
```
Sequence length :   764

GOR4 :
   Alpha helix      (Hh) :   204 is  26.70%
   3(10) helix      (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :   180 is  23.56%
   Beta turn        (Tt) :     0 is   0.00%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :   380 is  49.74%
   Ambigous states  (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```

3.4 Model Building:

Based on the structures of the template proteins, the protein structure of the query was built using Modeller 9v7. These were used to generate a model which had very low energy. The generated structure was then submitted to the PDB sum for Ramachandran Plot analysis which showed that 0.6% of the total number of the residues were in disallowed regions.

Based on this result, a loop between 500-580 residues was subjected to loop modeling by loop.py program in Modeller. This was again analysed by using PDB sum server and Ramachandran Plot analysis (Fig 3) indicated that there were no amino acids in the disallowed regions. Hence this model was considered as the best model generated for Human Toll Like Receptor 1.This protein had a very low energy and had no residues in the disallowed regions. (Fig 4)



file_01.ps

Fig 3 Ramachandran Plot of final model of TLR -1

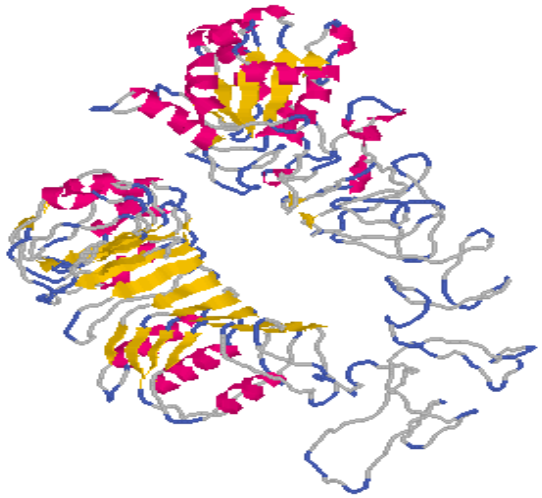


Fig 4 Final Model of TLR-1

4. CONCLUSION

A homology model for Human Toll Like Receptor 1 was built using multiple templates. The structure of the modeled TLR1 has 81.4 % residues in most favorable regions, 16.9 % in allowed regions, 1.7 % in generously allowed region and 0% in disallowed regions.

Based on this structure, further studies can be carried out as to find the mechanism of recognition of the mycobacterial lipopolysaccharides which could reveal important information in tuberculosis treatment.

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6. References

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