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Homology Modeling and Topology Prediction of OMPL1 in *Leptospira*

Haniye Eskandari and Fateme Sefid
Department of Biology, Science and Art University, Yazd, Iran

Abstract: Leptospirosis is a widespread zoonosis caused by members of the genus *Leptospira*. These highly invasive spirochetal pathogens are capable of infecting a broad range of mammalian hosts through either direct contact with an infected animal or indirect contact with soil or water contaminated with urine from a chronically infected animal. In humans, acute leptospirosis accounts for roughly 10% of hospitalizations for acute febrile illness in tropical areas of the world.

Key words: *Leptospira,* OMPl1, topology and 3D structure, complement

INTRODUCTION

Leptospirosis is a widespread zoonosis caused by members of the genus *Leptospira*. These highly invasive spirochetal pathogens are capable of infecting a broad range of mammalian hosts through either direct contact with an infected animal or indirect contact with soil or water contaminated with urine from a chronically infected animal. In humans, acute leptospirosis accounts for roughly 10% of hospitalizations for acute febrile illness in tropical areas of the world (Everard *et al*., 1987). Leptospirosis is an important cause of morbidity in US military personnel, occurring in 2-8% of soldiers undergoing jungle training in Panama (Takafuji *et al*., 1984). Leptospirosis also causes significant abortion, stillbirth, infertility, decreased milk production and death in livestock (Thiermann, 1984). Control efforts have been hampered by the fact that virulent leptospires are persistently shed from the urinary tracts of wildlife and livestock and subsequently are able to survive in the environment. Currently available vaccines for prevention of leptospirosis produce only short-term immunity and do not provide cross-protection against many of the 170 different serovars of pathogenic *Leptospira* sp. Because of a growing appreciation of leptospiral diversity, there are now six pathogenic species and three nonpathogenic species within the genus *Leptospira* (Yasuda *et al*., 1987). Freeze fracture electron microscopy has shown that pathogenic *Leptospira* sp. Belong to a group of virulent spirochetes, including Treponema pallidum and Borrelia hernsii, that have a low density of Outer Membrane Proteins (OMPs) relative to that in enteric gram-negative bacteria (Haake *et al*., 1991; Isberg and Falkow, 1985; Baumann *et al*., 1984; Walker *et al*., 1991, 1989). For treponemes and leptospires there appears to be a correlation between low OMP density and virulence (Haake *et al*., 1991; Walker *et al*., 1989). The kinetics of complement activation and outer membrane particle aggregation indicate that *T. pallidum* OMPs are important targets of treponemicidal antibody (Blanco *et al*., 1990).

Because of outer membrane fragility and the fact that OMPs are present in small amounts, there have been no reports identifying spirochetal proteins that span the outer membrane. OMPs of gram-negative bacteria are of great interest because they are located at the cell surface, where bacterial pathogens interact with the host (Nikaido, 1988). OMPs may play a role in bacterial pathogenesis by acting as adhesins (Bessen and Gotschlich, 1986; Isberg and Falkow, 1985; Miller and Falkow, 1988; Sansonetti, 1991), targets of bactericidal antibody (Elkins and Sparling, 1990; Murphy and Bartos, 1988; Saukkonen *et al*., 1987) porins (Murphy and Bartos, 1988; Jeanteur *et al*., 1991; Li *et al*., 1991) and receptors for soluble molecules such as siderophores (Stoebner and Payne, 1988) and complement proteins (Hoffman *et al*., 1992). There is also evidence that OMPs can elicit protective antibodies against disease (Hansen *et al*., 1982; Saukkonen *et al*., 1987). In order to identify potential leptospiral OMP candidates we describe the topology and 3D structure of a novel antigen which was discovered by mining the bacterial genome and that is very effective in inducing bactericidal antibodies. This antigen is a very good candidate for inclusion in universal vaccines against *Leptospira*.

MATERIALS AND METHODS

Sequence availability and homology search: The OMPl1 reference sequence with accession No. NP-713318.2 and GI 294828247 acquired from NCBI at http://www.ncbi.nlm.nih.gov/protein was saved in FASTA format for further analyses. The sequence

Corresponding Author: Haniye Eskandari, Departeman of Biology, Science and Art University, Yazd, Iran

served as a query for protein BLAST at http://blast.ncbi.nlm.nih.gov/Blast.cgi against nonredundant protein database. Probable putative conserved domains of the query protein were also searched for, at the above address.

Template search: The query protein sequence was used as an input data for the PSI-BLAST against rotein Data Bank (PDB) at http://blast.ncbi.nlm.nih.gov/Blast.cgi to identify its homologous structures.

Primary sequence analysis: Protparam online software at http://expasy.org/tools/protparam.html was employed for estimation and determination of properties such as molecular weight, theoretical pI, amino acid composition, total number of negatively and positively charged residues, instability index and aliphatic index.

Subcellular localization: Subcellular localization of protein was predicted by CELLO at http://cello.life.nctu.edu.tw/

Homology modeling: In the process of modeling, default restraint settings were applied and a rigorous relaxation protocol involved 2000 simulated annealing relaxation cycles (4.4 ps stepwise warming from 0-1000 K, followed by 19.2 PS stepwise cooling back down to300 K, all done through Charm force field and charges). The loop regions geometry was corrected using MODELER/Refine Loop command. The SWISS-MODEL workspace at http://swissmodel.expasy.org/ is a web-based integrated service dedicated to protein structure homology modelling. Secondary structure of the protein was predicted by SWISS-MODEL too. It assists and guides the user in building protein homology models at different levels of complexity.

Building a homology model comprises four main steps: identification of structural template(s), alignment of target sequence and template structure(s), model building and model quality evaluation. These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases.

Models evaluations: All 3D models of the proteins built, were qualititatively estimated by GMQE and QMEAN4 scores.

Topology prediction: Prediction of the hydrophobic transmembrane regions in a protein sequence forming probable β-barrel could help determination of the 3D protein structure. Full-length protein served as input in topology prediction. PRED-TMBB at http://biophysics.biol.uoa.gr/PRED-TMBB/is a sever that predicts transmembrane β-strands in protein sequences of Gram-negative bacteria. The web-server could find the topology of the loops in addition to localize the transmembrane strands.

RESULTS AND DISCUSSION

Sequence availability and homology search: The OMPl1 protein sequence with 320 residues obtained from NCBI and saved in FASTA format. Protein sequence serving as query for BLAST produced a set of sequences as the highest similar sequence.

BLAST search revealed numerous hits to the OMPl1 sequence. All hits were of *Leptospira.* Putative conserved domains were detected within this sequence. Most of the sequences belong to OMPl Porin superfamily. *Leptospira* porin protein OmpL1; OmpL1 is a member of the Outer Membrane (OM) proteins in the mammalian pathogen *Leptospira*. Specifically, it is a porin. Putative conserved domains have been detected within the sequence are shown in Fig. 1.

Template search: PSI-BLAST against Protein Data Bank (PDB) result displayed several hits as homologous structures. The first hit possessing the highest score was selected as a template for homology modelling. This top hit for OMPl1 sequence blast results was a protein with PDB code 3J7Y-Q (32% identity, 23% query coverage, 36.6 max score and 36.6 total score, chain Q, structure of the large ribosomal subunit from human mitochondria).

Our BLAST results showed that OMPl1 exists in all pathogenic strains of leptospira. This protein antibodies cross-react with a range of leptospira isolates for high similarity reason.

In this regard, OMPl1 sequence served as a query for BLAST search against Protein Data Base (PDB) to find the best template for 3D structure prediction. In addition to E-value, query coverage and Max. identity are also involved in max. score definition. Lower E-value and higher query coverage and max. identity are appropriate criteria for the selection. Thus, a hit with the highest total score could be the most reliable template. The use of some sequence alignment methods to identify a relationship between the target sequence and one or more possible templates is the first step in structure prediction. Based on BLAST search and alignments generations, the predicted 3D model of the OMPl1 could be applied to all OMPl1 proteins in leptospira.

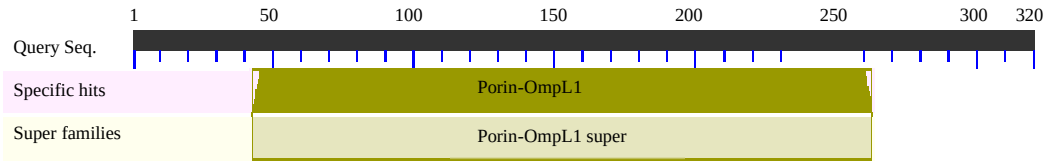

Fig. 1: Putative conserved domains have been detected

Primary sequence analysis: The protein sequence served as input for the computation of various physical and chemical parameters. The computed parameters included the molecular weight, theoretical pI, instability index, aliphatic index and grand average of hydropathicity (indicates the solubility of the proteins; positive GRAVY (hydrophobic), negative GRAVY (hydrophilic)) listed:

- Number of amino acids: 320
- Molecular weight: 33491.1
- Theoretical pI: 8.91

Amino acid composition:

- Ala (A) (37) = 11.6 (%)
- Arg (R) (12) = 3.8 (%)
- Asn (N) (17) = 5.3 (%)
- Asp (D) (13) = 4.1 (%)
- Cys (C) (2) = 0.6 (%)
- Gln (Q) (7) = 2.2 (%)
- Glu (E) (9) = 2.8 (%)
- Gly (G) (42) = 13.1 (%)
- His (H) (4) = 1.2 (%)
- Ile (I) (24) = 7.5 (%)
- Leu (L) (23) = 7.2 (%)
- Lys (K) (14) = 4.4 (%)
- Met (M) (6) = 1.9 (%)
- Phe (F) (12) = 3.8 (%)
- Pro (P) (13) = 4.1 (%)
- Ser (S) (20) = 6.2 (%)
- Thr (T) (26) = 8.1 (%)
- Trp (W) (3) = 0.9 (%)
- Tyr (Y) (14) = 4.4 (%)
- Val (V) (22) = 6.9 (%)

Total number of negatively charged residues (Asp+Glu): 22 are Total number of positively charged residues (Arg+Lys): 26.

Atomic composition:

- Carbon (C) = 1499
- Hydrogen (H) = 2355
- Nitrogen (N) = 405
- Oxygen (O) = 449
- Sulfur (S) = 8

Formula: $C_{1499}H_{2355}N_{405}O_{449}S_8$ total number of atoms: 4716

Extinction coefficients: Extinction coefficients are in units of M-1 cm^{-1}, at 280 nm measured in water. Ext. coefficient 37485. Abs 0.1% (= 1 gl^{-1}) 1.119, assuming all pairs of Cys residues form cystines. Ext. coefficient 37360. Abs 0.1% (= 1 gl^{-1}) 1.116, assuming all Cys residues are reduced.

Estimated half-life: The N-terminal of the sequence considered is M (Met). The estimated half-life is; 30 h (mammalian reticulocytes *in vitro*). About >20 h (yeast in vivo). About >10 h (*Escherichia coli in vivo*).

Instability index: The instability index (II) is computed to be 27.60. This classifies the protein as stable.

- Aliphatic index: 88.78
- Grand average of hydropathicity (GRAVY): 0.092

Subcellular localization: OMPl1 Subcellular localization predicted by CELLO was Extracellular with the highest reliability (2.788). CELLO results are shown below:

CELLO Prediction:

- Extracellular = 2.788*
- Outer membrane = 1.553
- Periplasmic = 0.464
- Inner membrane = 0.117
- Cytoplasmic = 0.078

3D structure prediction with homology medeling: The use of some sequence alignment methods to identify a relationship between the target sequence and one or more possible templates is the first step in structure prediction. Based on BLAST search and alignments generations, the predicted 3D model of the OMPl1 could be applied to all OMPl1 proteins. Accuracy of prediction depends on the degree of sequence similarity. If a structure template with sequence identity of >50% is found for a query protein, homology modeling could be chosen as the best in silico method with an accuracy equal to low-resolution X-ray predictions. When template and query sequences share

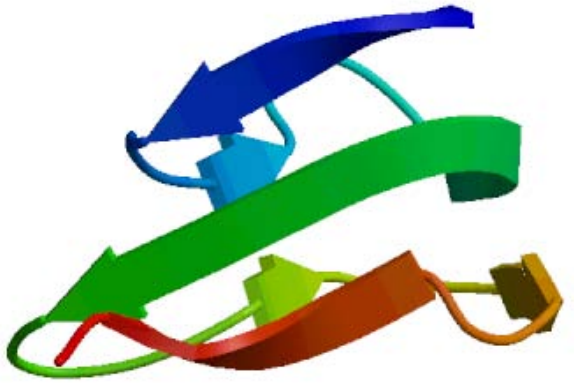

Fig. 2: Swiss model 3D structure prediction

30-50% identity, >80% of the C-atoms can be expected to be within 3.5°A of their true positions. Significant errors would occur in prediction when the sequences share <30% sequence identity. Since identity between the query and its template sequence was 35% (>30%) in our study, we assumed that homology modeling could be more powerful than threading.

Swiss modeler recruited for homology modeling introduced one model (Fig. 2). Its model was selected for further scrutinizes and validation analyses.

Models evaluations: QMEAN is a composite scoring function for the estimation of the global and local model quality. QMEAN consisting of four structural descriptors. The local geometry is analyzed by a torsion angle potential over three consecutive amino acids. Two pairwise distance-dependent potentials are used to assess all-atom and C-beta interactions. A solvation potential describes the burial status of the residues. The pseudo energies returned from the four structural descriptors and the final QMEAN4 score get directly related to what we would expect from high resolution X-ray structures of similar size using a Z-score scheme (Fig. 3 and 4).

The score of a model in also shown in relation to a set of high-resolution PDB structures (Z-score). The plot relates the obtained global QMEAN4 value to scores calculated from a set of high-resolution X-ray structures. Local estimates of the model quality based on the QMEAN scoring function are shown as per-reside plot. Each residue is assigned a reliability score between 0 and 1, describing the expected similarity to the native structure. Higher numbers indicate higher reliability of the residues. GMQE (Global Model Quality Estimation) is a quality estimation which combines properties from the target-template alignment. The resulting GMQE score is expressed as a number between zero and one, reflecting the expected accuracy of a model built with that alignment and template. Higher numbers indicate higher reliability. Once a model is built, the GMQE gets updated for this specific case by also taking into account the QMEAN4

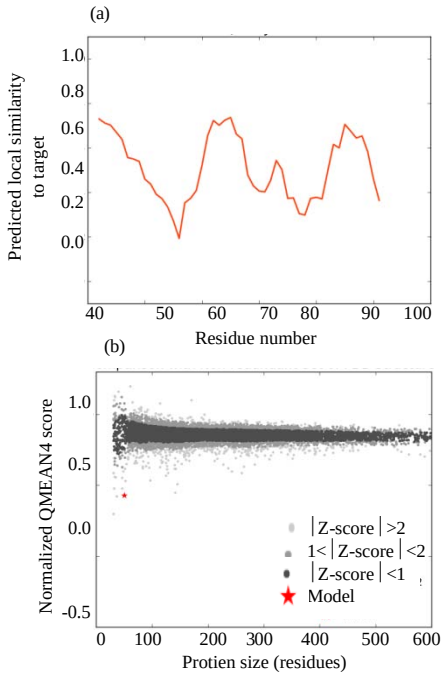

Fig. 3: 3D structure validations

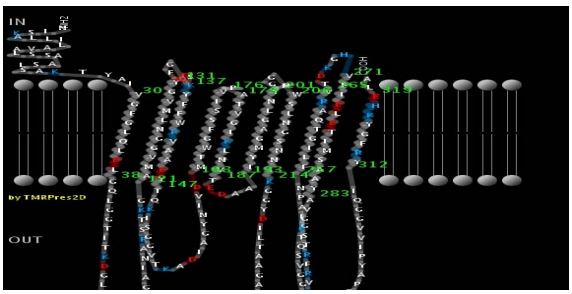

Fig. 4: A 2D topology model of OMPl1

score of the obtained model in order to increase reliability of the quality estimation. 3D structure validations are shown in Fig. 3.

Topology prediction: A 2D topology model of protein was built based on predicted inside, transmembrane and outside regions of the protein (Fig. 4).

CONCLUSION

The kinetics of complement activation and outer membrane particle aggregation indicate that *T. pallidum* OMPs are important targets of treponemicidal antibody. In order to identify potential leptospiral OMP candidates, we describe the topology and 3D structure of a novel antigen which was discovered by mining the bacterial genome and that is very effective in inducing bactericidal antibodies. This antigen is a very good candidate for inclusion in universal vaccines against *Leptospira*.

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