J Med Microbiol Infect Dis, 2018, 6 (1): 1-7
DOI: 10.29252/JoMMID.6.1.1

Original Article

OprF and OprL Conjugate as Vaccine Candidates against *Pseudomonas aeruginosa*; an in Silico Study

Zahra Payandeh[1], Bahman Khalesi[2], Maysam Mard-Soltani[3], Fateme Sefid[4*]

[1]Department of Biotecnology, Faculty of Medicine, Zanjan University of Medical Science, Zanjan, Iran; [2]Department of Research and Production of Poultry Viral Vaccine, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extention Organization (AREEO), Karaj, Iran; [3]Department of Clinical Biochemistry, Faculty of Medical Sciences, Dezful University of Medical Sciences, Dezful, Iran; [4]Department of Biology, Shahed University, Tehran, Iran

Received Oct 09, 2017; Accepted July 30, 2018

Introduction: Vaccine studies against *Pseudomonas aeruginosa* have often focused on outer membrane proteins (OPRs) due to their potent stimulation of the immune response. Using major outer membrane proteins of cell walls (mOMPs) of *P. aeruginosa* and other Gram-negative bacteria actively stimulate the immune system without any toxic side effects. Moreover, these antigens show immunological cross-reactivity with mOMPs of other serotypes belonging to the same species. The main OPRs of *P. aeruginosa*, OprF, and OprL, have received much attention from biologists as the potential OPR-based vaccine candidates. **Methods:** Homology modeling of OprF and OprL was done based on the template structures obtained from the BLAST search. The quality of OprF and OprL molecules was assessed using GMQE and QMEAN4 quality assessment tools. The secondary structure of the proteins was predicted as well as the structural topology, subcellular localization, functional analyses, signal peptide and B cell epitopes of proteins. **Results:** The structures of OprF and OprL proteins were successfully modeled and assessed using 4RLC-A and 4G4V-A as template structures. The regions of the proteins with a high B cell epitope density were identified as candidates for vaccine design. These regions contain functional and exposed amino acids. In these regions, the majority of amino acids were hydrophilic, flexible and accessible. **Conclusion:** It should be noted that in silico approaches are appealing alternatives for empirical methods. These approaches could pave the way for precise vaccine design efforts with lower cost and time. *J Med Microbiol Infec Dis, 2018, 6 (1): 1-7.*

Keywords: *Pseudomonas aeruginosa*, Vaccine, OprF, OprL, Bioinformatics.

INTRODUCTION

The nature and acquired resistance of *P. aeruginosa* to a variety of new antibiotics have led researchers to look for new ways to treat and prevent infections caused by this bacterium. Since 1960, the immunological methods based on pathogenicity and virulence factors in addition to new antibiotics were considered as significant approaches to fight against this infection [1, 2]. The most crucial factor in reducing the mortality rate caused by this bacterium is early diagnosis followed by appropriate antibiotic treatment. The control of *Pseudomonas* infection frequently fails due to the indiscriminate use of drugs and increased drug resistance, especially against conventional antibiotics. Currently, the immunotherapy with harmless and effective vaccines to prevent complications caused by *P. aeruginosa* is very promising. Subcutaneous injection of an effective dose of the purified bacterial lipopolysaccharide as an active immunogen has been suggested by many researchers [2, 3].

The pathophysiological nature of LPS and its serotype-specific immunological activities has limited LPS applications for *Pseudomonas* infection control, whereas major outer membrane proteins of cell walls (mOMPs) of this bacteria and other Gram-negative bacteria have received much attention. The mOMPs are non-toxic and active stimulants of the immune system. Moreover, these molecules show immunological cross-reactivity with mOMPs of other serotypes belonging to the same species. Today, the protection of mOMPs of many pathogenic Gram-negative bacteria against the corresponding etiologic factors have been approved [4]. The OprF and OprL are lipoprotein constituent of secretory pumps in the outer membrane of *P. aeruginosa* [5]. The OprF and OprL proteins are respectively used to identify the genus *Pseudomonas* and *P. aeruginosa* species. These two genes encode the major lipoproteins of outer membrane involved in antibiotic resistance of bacteria [6].

The first step to achieve an effective vaccine against the target organism is to identify the optimal combinations of protective regions.

***Correspondence:** Fateme Sefid
Department of Biology, Shahed University, Tehran, Iran, 3319118651.
Email: sefid.fateme@yahoo.com
Tel: +98 (21) 51212200 **Fax:** +98 (21) 51212201

Polysaccharide capsules, as well as protein moieties, are among the protective antigens used in the formulation of multiple vaccines. However, the need for a culture of pathogenic bacteria and the molecular instability of their antigens, has hindered the introduction of effective vaccines. Today, development of vaccines have focused on identification and production of recombinant protein antigens [7]. Access to whole genome sequencing data of pathogenic bacteria and various bioinformatics tools have initiated a new era for scientists to identify the immunogenic antigens. The interaction between the exposed surfaces or secretory proteins with host cells is the crucial step in virulence and pathogenicity. Bioinformatics tools could be employed to achieve novel insights into these interactions and their uses in vaccine design studies.

One of the leading bioinformatics objectives in immunology is algorithm development for production of new vaccines. Identifying valid and reliable epitopes through bioinformatics computing reduces the costs of laboratory analysis of bacterial, viral and parasitic gene products [8]. In the present study, bioinformatics tools were used for further characterization of OprF and OprL proteins of *P.aeruginosa*, and the obtained results were used to design an effective vaccine against this bacterium. In this regard, the structures of these proteins were predicted and evaluated. The obtained structures were used for prediction of the regions with the higher B cell epitope density [9].

MATERIAL AND METHODS

Sequence availability and homology search. The OprF and OprL protein sequences with accession numbers of WP_004885687.1 and WP_003111417.1 were acquired from NCBI (http://www.ncbi.nlm.nih.gov/protein) and saved in FASTA format for further analyses. The sequences served as a query for protein BLAST search against a non-redundant protein database (http://blast.ncbi.nlm.nih.gov-/Blast.cgi). We also searched for the probable putative conserved domains of the query proteins.

Template search. The query protein sequences were used as an input for the PSI-BLAST search against protein data bank (PDB) at "http://blast.ncbi.nlm.nih.gov/Blast.cgi" to identify its homologous sequences with resolved structures.

Primary sequence analysis. Protparam [10] online software (http://expasy.org/tools/protparam.html) and InterPro program (https://www.ebi.ac.uk/interpro) were employed for estimation and determination of the properties such as molecular weight, theoretical pI, amino acid composition, the total number of negatively and positively charged residues, instability index, aliphatic index and molecular function.

Subcellular localization. The subcellular localization of the proteins was predicted by CELLO [11] and PSORTII (https://psort.hgc.jp/form2.html).

Topology and signal peptide prediction. SignalP 4.1 software [12] was used to predict the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms. The method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks. The SPOCTOPUS programme [13] and PRED-TMBB software (http://bioinformatics.-biol.uoa.gr/PRED-TMBB/input.jsp) were employed to determine the membrane protein topology and signal peptides.

Secondary structure prediction. The secondary structure of the protein was predicted by PSIPRED [14] software. The PSIPRED Protein Sequence Analysis Workbench aggregates several University College London (UCL) structure prediction methods into one location. Phyre2 software [15] at "http://www.sbg.bio.ic.ac.uk/phyre-2/html/page.cgi?id=index" employed to validate the PSIPRED predictions.

3D structure prediction, evaluation, and refinement. The SWISS-MODEL [16] Workspace at "http://swiss-model.expasy.org" is a web-based integrated service dedicated to protein structure homology modeling. 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 the target sequence and template structure(s), model building, and model quality evaluation. These steps can be repeated until a satisfying modeling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases. All the designed 3D models of the proteins were qualitatively estimated by GMQE and QMEAN4 scores. The obtained structures were refined using the 3Drefine program at "http://sysbio.rnet.missouri.-edu/3Drefine". The quality of refined models was estimated by the RaAMPAGE program at "http://mordred.bioc.cam.-ac.uk/~rapper/rampage.php".

Identification of functionally and structurally important residues. The Consurf [17] program at "http://consurf.tau.ac.il" was used for annotating functional residues of protein structure in the twilight zone.

Ligand binding site predictions. The Cofactor [18] is a structure-based method for biological function annotation of protein molecules. This software predicts the relevant amino acid involved in the ligand binding site.

Single-scale amino acid properties assay and B cell epitope prediction. The IEDB database [19] parameters such as hydrophilicity, flexibility, accessibility, turns and the antigenic propensity of polypeptide have been correlated with the location of B cell epitopes. This has led to a search for empirical rules that allows prediction of the position of B cell epitopes from specific features of the protein sequence. The ElliPro [20] at "http://tools.immun-eepitope.org/tools/ElliPro/tutorial.jsp" predicts linear and discontinuous antibody epitopes.

RESULTS

Sequence availability and homology search. The OprF sequence with 344 residues and OprL sequence with 168 residues were obtained from NCBI and saved in FASTA format. The protein sequence serving as a query for

BLAST analysis returned a set of sequences as the highest similar sequence. The BLAST search revealed numerous hits to the OprF and OprL subunit sequence. All hits belonged to the genus *Pseudomonas*. The putative conserved domains were detected within OprF and OprL sequences. Most of the sequences belong to ompA_C-like (Peptidoglycan binding domains similar to the C-terminal domain of outer-membrane protein OmpA). The OmpA-like domains (named after the C-terminal domain of OmpA protein) have shown to be non-covalently associated with peptidoglycan, a network of glycan chains composed of disaccharides, which are cross-linked via short peptide bridges.

Template search. The PSI-BLAST against protein data bank (PDB) results displayed several hits as homologous structures. The first hit possessing the highest score was selected as a template for homology modeling (Table 1).

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 the grand average of hydropathicity index that indicates the solubility of the proteins (positive GRAVY (hydrophobic), negative GRAVY (hydrophilic)) are summarized in Table 2.

Table 1. The template structures for homology modeling of OprF and OprL structures.

PDB BLAST	Accession	Max score	Query coverage	Max ident
OprF	4RLC-A	148	44%	50%
OprL	4G4V-A	134	59%	63%

The Table presents the PDB ID of each template along with the BLAST search score, the sequence coverage of the template over the OprF and OprL sequences and the identity of sequences between OprF and OprL and the templates

Table 2. Physical and chemical parameters of OprF and OprL

	Residue number	Molecular weight	Theoretical pI	Instability index	Aliphatic index	GRAVY
OprF	344	36549.1	4.69	30.77(stable)	69.74	-0.448
OprL	168	17925.0	5.95	25.79	73.27	-0.432

Subcellular localization. The subcellular localization of the OprF and OprL was predicted to be the outer membrane. The predicted location was associated with the highest reliability index (4.848 and 4.016 respectively).

Topology and signal peptide prediction. The OprF and OprL signal peptide cleavage site predicted by SPOCTOPUS and SignalP programs are shown in Figure 1. The first 24 amino acids of OprF and 21 amino acids of OprL were predicted to compose the signal peptides. The topology of the transmembrane proteins was predicted to be single span transmembrane by SPOCTOPUS and PRED-TMBB programs.

Secondary structure prediction. The secondary structure of the proteins was predicted by PSIPRED program. The coil, helix, and strands are the main secondary structure components constituting the secondary structure of the proteins. The results from Phyre2 confirmed the results of the PSIPRED program. The secondary structure could be used to validate the tertiary structures. The attribution of the secondary structure components in the proteins is listed in Table 3.

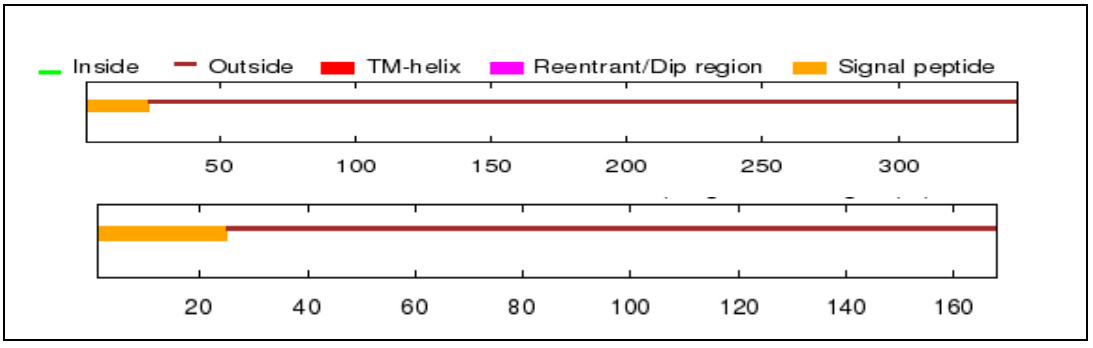


Fig. 1. Topology prediction for OprF (up) and OprL (down) by the SPOCTOPUS programs
The yellow line represents the signal peptide, and the red line represents the sequences located outside of the cell (extracellular).

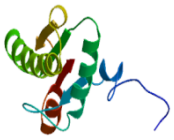
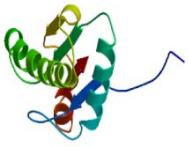
Table3. Attribution of secondary structure components in the OprF and OprL

	alpha helix	beta strand	random coil
OprF	19%	35%	46%
OprL	42%	8%	50%

3D structure prediction, evaluations, and modification. Building a homology model comprises four main steps: identification of structural template (s), alignment of the target sequence and template structure (s), model building, and model quality evaluation. These steps can be repeated until a satisfying modeling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases. Swiss model software recruited for homology modeling introduced 2 models. All the models were selected for further analyses. The 3D models estimated qualitatively by two programs revealed that there was a consensus on a single model. The results are shown in Table 4. Ramachandran plot evaluations indicated that the predicted models have more than 90% of their residues in the favored region.

Identification of functionally and structurally important residues. The Consurf annotated functional residues on the 3D structure of OprF and OprL in twilight zone (Figure 2).

Table 4. Estimated quality scores of the predicted 3D models for OprF and OprL before and after refinement.

Model	Figure	Seq Identity	Seq Similarity	Coverage	GMQE	QMEAN4	Refined QMEAN4
OprF 1		53.33%	0.46	0.44	0.29	-3.60	-2.77
OprF 2		50.99%	0.45	0.44	0.26	-4.23	3.14
OprL 1		42.86%	0.41	0.75	0.58	-3.39	2.56
OprL 2		50.40%	0.43	0.74	0.57	-1.97	-1.12

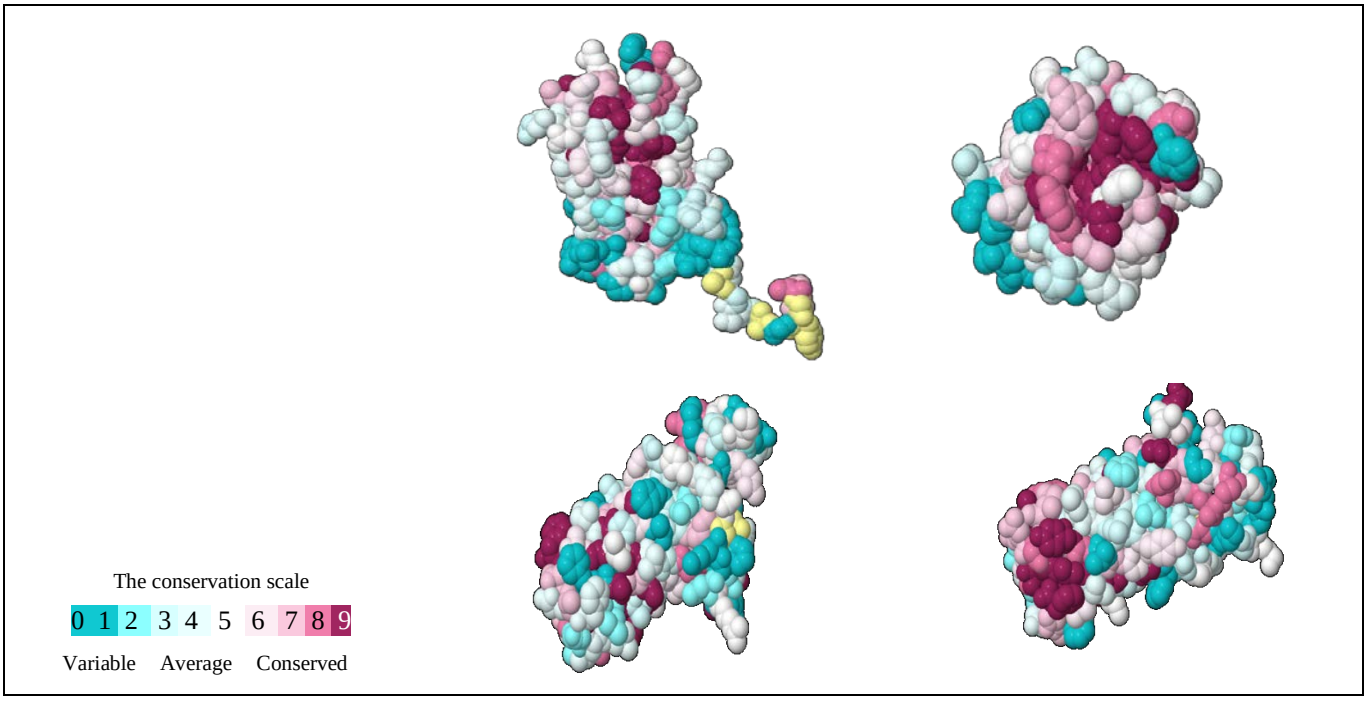


Fig. 2. Functionally and structurally essential residues in OprF (up) and OprL (down)

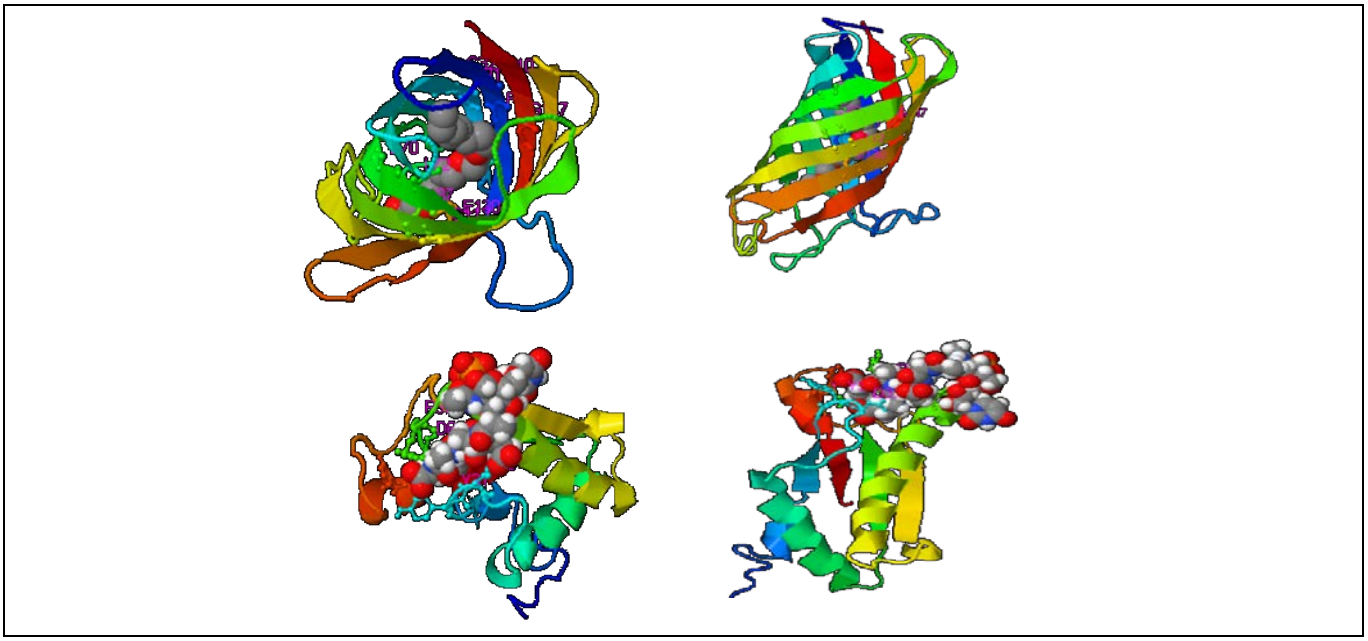


Fig. 3. Cofactor ligand binding site prediction in OprF (up) and OprL (down)

Ligand binding site predictions. The OprF ligand binding sites determined by COFACTOR software indicated the involvement of conserved residues 31, 32, 70, 72, 87 and 112 in the binding site with the highest $Cscore^{LB}$ (the confidence score of predicted binding site). The OprL ligand binding sites determined using COFACTOR software, indicate the involvement of conserved residues 31, 32, 65, 67, 72 and 118 in binding site with the highest $C\text{-}score^{LB}$ (Figure 3).

Single-scale amino acid properties assay and prediction of B cell epitopes. The IEDB programs predicted several properties such as hydrophilicity, accessibility, antigenicity, flexibility, and beta-turn secondary structure in the protein sequence. The propensity scale methods assigned a propensity value to each amino acid which measures the tendency of an amino acid to be part of a B-cell epitope (as compared to the background). To reduce fluctuations, the score for each target amino acid residue in a query sequence was computed as the average of the propensity values of the amino acids in a sliding window centered at the target residue. Hydrophilicity, accessibility, antigenicity, flexibility and secondary structure properties have a fundamental role in B cell epitope prediction. A reliable result cannot be achieved regarding B cell epitope prediction by relying only on just one of these properties. Therefore, to confirm the results of B cell epitope predictions the single-scale amino acid properties of the proteins were predicted. B cell epitope prediction results indicated that four linear along with 11 discontinuous B cell epitopes exist in OprF protein and five linear along with 8 discontinuous B cell epitopes in OprL protein (Table 5, 6). The epitopes were mainly predicted to be situated in the C terminus region.

DISCUSSION

P. aeruginosa, an opportunistic gram-negative bacillus, is one of the most critical hospital pathogens [21]. The mortality resulted from infections caused by this bacteria is very common due to its inherent resistance to most common antibiotics. This bacteria causes urinary tract infections, respiratory system infections, inflammation and dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in immunosuppressed patients with severe burns, cancer, and AIDS (acquired immune deficiency syndrome). Virulence factors of this bacteria including toxins, enzymes, flagella, lipopolysaccharide, pili, and secretory proteins create severe and fatal infections [1, 2].

Table 5. OprF and OprL linear Epitopes Predicted by ElliPro

Protein	No.	Start	End	Peptide	Number of residues	Score
OprF	1	209	344	CPDTPANVTVDADGCPAVAEVVRVELDVKFDFDKSVVKPSSYGDIK NLADFMQQYPQTSTTVEGHTDSVGPDAYNQKLSERRANAVKQVLV NQYGVGASRVNSVGYGESRPVADNATESGRAVNRRVEAEVEAQAK	136	0.792
OprF	2	1	7	MKLKNTL	7	0.592
OprF	3	156	165	QYNIDQGNTE	10	0.583
OprF	4	117	132	QSIGQDARGGRDGSTF	16	0.555
OprL	1	138	168	LELVSYGKERPVATGHDEQSWAQNRRVELKK	31	0.837
OprL	2	93	111	GSGQRVVLEGHTDERGTRE	19	0.682
OprL	3	75	85	DLKPEAMRALD	11	0.665
OprL	4	53	64	GSLSDEAALRAI	12	0.625

Table 6. OprF and OprL discontinuous Epitopes Predicted by ElliPro

Protein	No.	Residues	Number of residues	Score
OprF	1	_:R334, _:V335, _:E336, _:A337, _:E338, _:V339, _:E340, _:A341, _:Q342, _:A343, _:K344	11	0.983
OprF	2	_:R318, _:P319, _:V320, _:A321, _:D322, _:N323, _:A324, _:T325, _:E326, _:S327, _:G328, _:R329, _:A330, _:V331, _:N332	15	0.942
OprF	3	_:Y302, _:G303, _:V304, _:G305, _:A306, _:S307, _:R308, _:V309, _:N310, _:S311, _:V312, _:G313, _:Y314, _:G315, _:E316, _:S317	16	0.862
OprF	4	_:M260, _:Q261, _:Q262, _:Y263, _:P264, _:Q265, _:T266, _:S267, _:T268, _:T269, _:V270, _:E271, _:G272, _:H273, _:T274, _:D275, _:S276, _:V277, _:G278, _:P279, _:D280, _:A281, _:Y282, _:N283, _:Q284	25	0.846
OprL	1	_:R163, _:V164, _:E165, _:L166	4	0.772
OprL	2	_:W158, _:A159, _:Q160, _:N161	4	0.759
OprL	3	_:E155, _:Q156, _:S157	3	0.75
OprL	4	_:Q137, _:E139, _:L140, _:V141, _:S142, _:Y143, _:G144, _:K145, _:E146, _:R147, _:P148, _:V149, _:A150, _:T151, _:G152, _:H153, _:D154	17	0.684

Lipopolysaccharide (LPS) based vaccines, PseudogenTM, a heptavalent preparation, and PEV-01 a 16-valent vaccine, showed to be the most potent vaccines tested against *P. aeruginosa* infections [22, 23]. However, due to the toxicity associated with their lipid, LPS vaccines failed to be approved for clinical use [24]. Despite many efforts to develop an effective vaccine based on less toxic vaccine moieties and even their success to induce humoral responses [23, 25], an FDA approved *P. aeruginosa* vaccine for clinical use is not available yet. Given the failure of the previous efforts to provide an efficient anti-*P. aeruginosa* vaccine, attentions were driven to OPRs due to their high conservancy and their ability to induce a cross-protective immunity among all 17 known *P. aeruginosa* serotypes, their ability to be produced by recombinant DNA technology free of contaminating LPS, their applicability to be designed as naked DNA immunization agents and their potential to be transfected into particular vectors like non-pathogenic Salmonella strains to induce a mucosal immune response [7, 26, 27]. These facts have convinced us to perform an in silico analyses to provide immunological insights about two main OMPs of the *P. aeruginosa.*

Our structural results indicate the high quality of predicted structures. These structures could be employed for accurate prediction of other structure-based features of the OprF and OprL proteins like conformational B cell epitopes. The positions of the predicted epitopes within the OprF and OprL sequences reveals that the C terminus regions of these proteins contain higher numbers of both linear and conformational B cell epitopes, while the N terminus region contains only one epitope. The results of the single-scale amino acid properties confirmed the results obtained from ElliPro software. This indicates that the B cell epitope predictions were reliable to a great extent. The regions of the OprF and OprL protein with the highest density of epitopes could be suitable candidates to be included in the future vaccine design studies. Higher protection could be anticipated using these regions as individual vaccines or as combined antigens. There are reports of combined OMPs vaccines comprising OprF and OprI, Vaccination with recombinant OprF-OprI increased protection to a 975-fold LD50. Even at a challenge dose of 5×10^3 CFU, highly significant protection could be acquired [28, 29]. We believe that fusion vaccine of OprF and OprL could provide promising results due to the high-density B cell epitopes within their sequences. Further studies are required for the rational design of such fusion vaccines.

Recently, in silico approaches for solving biological issues have gained much attention due to the arduous and time-consuming nature of empirical approaches. Using bioinformatics tools would provide novel insight into the unsolved problems. These tools allowed us to disclose several structural, functional and immunological properties of OprF and OprL, the two members of *P. aeruginosa* mOMPs. This information would pave the way for immunologist in their efforts to design more effective vaccines against this bacterium.

ACKNOWLEDGEMENT

The authors thank Yazd University of Medical Sciences and Zanjan University of Medical Sciences for their support to conduct this work.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Hancock R. Intrinsic antibiotic resistance of Pseudomonas aeruginosa. J Antimicrob Chemother. 1986; 18 (6): 653-6.

2. Von Specht B, Knapp B, Muth G, Bröker M, Hungerer K, Diehl K, et al. Protection of immunocompromised mice against lethal infection with Pseudomonas aeruginosa by active or passive immunization with recombinant P. aeruginosa outer membrane protein F and outer membrane protein I fusion proteins. Infect Immun. 1995; 63 (5): 1855-62.

3. Jaffe RI, Lane JD, Bates CW. Real-time identification of Pseudomonas aeruginosa direct from clinical samples using a rapid extraction method and polymerase chain reaction (PCR). J Clin Lab Anal. 2001; 15 (3): 131-7.

4. Hancock R, Carey AM. Outer membrane of Pseudomonas aeruginosa: heat-2-mercaptoethanol-modifiable proteins. J Bacteriol. 1979; 140 (3): 902-10.

5. Nikaido H. Outer membrane barrier as a mechanism of antimicrobial resistance. Antimicrob Agents Chemother. 1989; 33 (11): 1831-36.

6. Nicas TI, Hancock R. Outer membrane protein H1 of Pseudomonas aeruginosa: involvement in adaptive and mutational resistance to ethylenediaminetetraacetate, polymyxin B, and gentamicin. J Bacteriol. 1980; 143 (2): 872-8.

7. Priebe GP, Goldberg JB. Vaccines for Pseudomonas aeruginosa: a long and winding road. Expert Rev Vaccines. 2014; 13 (4): 507-19.

8. Soria-Guerra RE, Nieto-Gomez R, Govea-Alonso DO, Rosales-Mendoza S. An overview of bioinformatics tools for epitope prediction: Implications on vaccine development. J Biomed Inform. 2015; 53: 405-14.

9. Gasteiger E, Hoogland C, Gattiker A, Duvaud Se, Wilkins MR, Appel RD, et al. Protein identification and analysis tools on the ExPASy server: Springer; 2005.

10. Gardy JL, Brinkman FS. Methods for predicting bacterial protein subcellular localization. Nat Rev Microbiol. 2006; 4 (1): 741-51.

11. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. J Mol Biol. 2004; 340 (4): 783-95.

12. Viklund H, Bernsel A, Skwark M, Elofsson A. SPOCTOPUS: a combined predictor of signal peptides and membrane protein topology. Bioinformatics. 2008; 24 (24): 2928-9.

13. McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. Bioinformatics. 2000; 16 (4): 404-5.

14. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc. 2015; 10 (6): 845-58.

15. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. electrophoresis. 1997; 18 (15): 2714-23.

16. Glaser F, Pupko T, Paz I, Bell RE, Bechor-Shental D, Martz E, et al. ConSurf: identification of functional regions in proteins by surface-mapping of phylogenetic information. Bioinformatics. 2003; 19 (1): 163-4.

17. Roy A, Yang J, Zhang Y. COFACTOR: an accurate comparative algorithm for structure-based protein function annotation. Nucleic Acids Res. 2012: gks372.

18. Zhang Q, Wang P, Kim Y, Haste-Andersen P, Beaver J, Bourne PE, et al. Immune epitope database analysis resource (IEDB-AR). Nucleic Acids Res. 2008; 36 (suppl 2): W513-W8.

19. Ponomarenko J, Bui H-H, Li W, Fusseder N, Bourne PE, Sette A, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinf. 2008; 9 (1): 1.

20. Srinivasan A, Wolfenden LL, Song X, Mackie K, Hartsell TL, Jones HD, et al. An outbreak of Pseudomonas aeruginosa infections associated with flexible bronchoscopes. N Engl J Med. 2003; 348 (3): 221-7.

21. Cryz Jr S, Sadoff J, Fürer E. Octavalent Pseudomonas aeruginosa O-polysaccharide-toxin A conjugate vaccine. Microb Pathog. 1989; 6 (1): 75-80.

22. Cryz S, Sadoff J, Ohman D, Fürer E. Characterization of the human immune response to a Pseudomonas aeruginosa O-polysaccharide—toxin A conjugate vaccine. J Lab Clin Med. 1988; 111 (6): 701-7.

23. Cryz S, Fürer E, Cross A, Wegmann A, Germanier R, Sadoff J. Safety and immunogenicity of a Pseudomonas aeruginosa O-polysaccharide toxin A conjugate vaccine in humans. J Clin Invest. 1987; 80 (1): 51-6.

24. Pier G. Rationale for development of immunotherapies that target mucoid Pseudomonas aeruginosa infection in cystic fibrosis patients. Behring Inst Mitt. 1997 (98): 350-60.

25. Price BM, Legutki JB, Galloway DR, von Specht B-U, Gilleland LB, Gilleland Jr HE, et al. Enhancement of the protective efficacy of an oprF DNA vaccine against Pseudomonas aeruginosa. FEMS Immunol Med Microbiol. 2002; 33 (2): 89-99.

26. Baumann U, Mansouri E, Von Specht B-U. Recombinant OprF–OprI as a vaccine against Pseudomonas aeruginosa infections. Vaccine. 2004; 22 (7): 840-7.

27. Von Specht B, Knapp B, Muth G, Bröker M, Hungerer K-D, Diehl K-D, et al. Protection of immunocompromised mice against lethal infection with Pseudomonas aeruginosa by active or passive immunization with recombinant P. aeruginosa outer membrane protein F and outer membrane protein I fusion proteins. Infect Immun. 1995; 63 (5): 1855-62.

28. Knapp B, Hundt E, Lenz U, Hungerer K-D, Gabelsberger J, Domdey H, et al. A recombinant hybrid outer membrane protein for vaccination against Pseudomonas aeruginosa. Vaccine. 1999; 17 (13-14): 1663-6.