Original Article

Designing of Multi-Epitope Peptide Vaccine Based on Outer Membrane Proteins OmpF, OmpC, and PgtE of *Salmonella enterica* Typhi

Fereshteh Ezzati Ghadi[1*], Zahra Roudbari[2], Razieh Razavi[3]

1. Department of Biology, Faculty of Science, University of Jiroft, Jiroft, Iran
2. Department of Animal Science, Faculty of Agriculture, University of Jiroft. Jiroft, Iran
3. Department of Chemistry, Faculty of Science, University of Jiroft, Jiroft, Iran

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Corresponding Author's E-Mail:
f.ezzati@ujiroft.ac.ir

ABSTRACT

Consumption of contaminated water and foods by *Salmonella* Typhi cause the most common enteric disease known as Typhoid fever in both humans and animals. Despite the existence of various vaccines but infectious diseases remain a major cause of mortality worldwide. Nowadays, in-silico tools design a reliable and stable vaccine to combat such infections. The study aimed to design and evaluate a multi-epitope vaccine based on the outer-membrane proteins of *Salmonella* Typhi. B-cells and T-cells epitopes were predicted. Predicted epitopes were connected by AAY, KK, and GPGPG linkers. Heparin-Binding Hemagglutinin Adhesin (HBHA) has been attached to the N-terminal of the final vaccine as a potent immune adjuvant. Epitope's antigenicity, allergenicity, immunogenicity, and physicochemical characteristics were defined using in-silico tools. Molecular docking of vaccine-TLR4 was done. ΔG of vaccine-TLR4 is -3.91×10^{4} Kcal mol^{-1} with 1.93 RMSD. The results indicated protein was stable and non-allergen. In conclusion, the multi-epitope vaccine base on outer membrane proteins of the *Salmonella* Typhi bacterium might be considered to combat typhoid fever.

Keywords: In-Silico, Typhoid, Multi-epitope, Vaccine

1. Introduction

Salmonella enterica is a gram-negative bacterium that causes salmonellosis and belongs to the Enterobacteriaceae family. *S. enterica* is a flagellating bacillus that causes gastrointestinal disease in humans and animals (1). There are six subspecies of *S. enterica*, which are: *Enterica*, *Arizonae*, *Diarizonae*, *Salamae*, *Indica*, and *Houtenae* (2-4). There are over 2600 Salmonella enterica serovars, and the most important serovars of *S. enterica* are *Salmonella* Typhi and *Salmonella* Typhimurium (5). *Salmonella* Typhi causes typhoid fever with typical symptoms, such as persistent fever, chills, and abdominal pain, killing many people each year. *Salmonella* is transmitted through feces and spread through contaminated water and food (6). An estimated 11 to 21 million cases of typhoid fever and approximately 128000 to 161000 deaths worldwide each year (7). Antimicrobial resistance of pathogenic intestinal bacteria is one of the global health problems today. Multiresistance of *S.* Typhi to ampicillin, chloramphenicol, and cotrimoxazole has been reported (8-10).

In recent years, various approaches have been taken to develop vaccines against *Salmonella* Typhi. This vaccine contains injectable Ty21a and Vi, which are moderately effective (50-80% protection) (11). Despite the fact that vaccines against typhoid fever are commonly administered, it is important to consider the immune mechanisms for enteric infections (12).

In recent years, immunoinformatics has been well established and applied to vaccine information of various microorganisms (13). Data based on the genomes of microorganisms can help predict the epitopes that can interact with the host immune system. However, mapping epitopes in immunogenic proteins is critical for peptide vaccine development. In *S.* Typhi, the immune potential of outer membrane proteins (Omps) has been studied (14). They are good candidates for the development of multi-epitope vaccines against *Salmonella* Typhi. The studies revealed that the epitopes can bind to human leukocyte antigen alleles (HLA) and major histocompatibility complex (MHC) class I and class II proteins, which play a central role in the immune system and are recognized by cytotoxic $CD8^+$ T-cells and $CD4^+$ T-cells, respectively (15). $CD4^+$ and $CD8^+$ epitopes are used for vaccine models that target a variety of pathogens (16). Vaccines based on multiple epitopes are cheaper, safe, time-saving, stable, highly specific, and bind to multi-epitope alleles simultaneously compared with vaccines produced in a conventional manner (17). Therefore, the goal was to develop an effective multiepitope vaccine against the bacterium *S.* Typhi. The vaccine was developed from the antigens OmpC, OmpF, and PgtE. Finally the docking of the ligand to a receptor (TLR4) was analyzed. Stability energy and electron surface map analysis was performed for a refined vaccine with TLR4 receptor.

2. Methods and Materials

2.1. Finding the protein retrieval

The sequence of OmpF (Uni-prot Accession no Q56113), OmpC (Uni-prot Accession no P0A264), and PgtE (Uni-prot Accession no P06185) from *S.* Typhi was retrieved from http://www.uniprot.org. The sequence obtained was stored in a text-based format known as FASTA.

2.2. Prediction of sequence-based $CD8^+$ epitopes

The IEDB Analysis Resource tool was used to predict MHC-I alleles (http://tools.iedb.org/mhci), and a threshold of 0.5 was used to identify nine mer-amino acids. This software is freely available on the public website. This web server predicts epitopes in proteins based on accessibility, flexibility, antigenicity, and hydrophobicity.

2.3. Prediction of sequence-based $CD4^+$ epitopes

IEDB (http://tools.iedb.org/mhcii/) was used to predict nine mer-linear $CD4^+$ alleles. For this tool, the criterion for selecting candidate epitopes is a p-value greater than 0.5.

2.4. B-cell epitope

B-cells are lymphocytes responsible for humoral immunity and produce antibodies. IEDB software

was used to predict and select appropriate linear and continuous B-cell epitopes in the vaccine structure. This web-based software allows prediction and analysis of B-cell epitopes using computational tools.

2.5. Allergenicity, Antigenicity, and immunogenicity

The VaxiJen server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) was used to predict the antigenic properties of epitopes. This server determines the antigenicity of the sequence based on the physicochemical properties of the proteins with a cut-off value of 0.5 and independent of the orientation of the protective antigens.

We used the AllerTop server (http://www.ddg-pharmfac.net/AllerTOP) to test the allergenicity of epitopes. The immunogenicity of OmpF, OmpC, and PgtE epitopes was checked at http://tools.iedb.org/immunogenicity. This web server predicts vaccine allergenicity based on key physical and chemical properties of the proteins.

2.6. Physicochemical activities

The ProtParam server (http://web.expasy.org/protparam) is used to study the physicochemical activities. It is used to report the amino acid composition, theoretical pI, molecular weight (MW), instability index, half-life, aliphatic index, and grand average hydropathy (GRAVY) of the construct.

2.7. Design and fusion of Epitopes

Epitopes were arranged as $CD4^+$, $CD8^+$, and B-cell of OmpF, OmpC, and PgtE, respectively (Fig. 1). Linker sequences of AAY were used in the case of MHC- I, KK for MHC- II, and GPGPG were incorporated into B-cell epitopes. Immunogenic proteins can increase the potency and efficacy of an epitope-based vaccine. For example, HBHA (AN: ZP_07011362.1) from *Mycobacterium tuberculosis* (N-terminal of the vaccine) was used as an adjuvant that can bind to TLR4 (18). The EAAAK linker with helical structure was placed on both sides of HBHA to minimize domain interaction (19).

2.8. Prediction of secondary structure

PSIPRED tool (http://bioinf.cs.ucl.ac.uk/psipred) was used to predict the secondary structure. Four conformational states (helices, sheets, turns, and coils) of candidate genes were analyzed.

2.9. Modeling and refinement of the tertiary structure

RAPTORX server (http://raptorx.uchicago.edu/StructPredV2/) was used to predict the 3D structure. PyMOL is a comprehensive molecular software used to visualize 3D designs. ProSA is a powerful server (http://prosa.services.came.sbg.ac.at/prosa.php) used to check for possible errors. The predicted 3D structural model was refined using the Galaxyrefine server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE). Ramachandran plot (http://swissmodel.expasy.org/workspace) predicts the structural stereochemical property in PROCHECK software (20).

2.10. Docking of vaccine (ligand) to TLR4 (receptor)

Ligand-receptor docking is an in-silico method (ClusPro) used to determine the best match and estimate the binding dependence between ligand and receptor. For docking, water molecules and additional binding molecules of the ligand (unsuitable epitopes) were separated from the receptor, polar hydrogen bonds were added to the receptor, and finally the energy of the system was optimized.

2.11. Energy and surface map of a vaccine-TLR4 complex

To confirm the stability of the model structure, the ligand-TLR4 construct and the surface map of the vaccine, a computational analysis was performed using Amber10: EHT/all-atom force field with rigid water molecules at room temperature.

3. Results

In the current study, a total of 18 epitopes with nine mer lengths were obtained from three genes and subjected to vaccine modeling. The immunogenicity score of all MHC- I was retrieved from the IEDB server. The results showed that the range of immunogenicity score of MHC-I epitopes was between -0.008 and -0.2 (Table. 1). To see the MHC-II epitopes, the IEDB tools were used and 9 epitopes of 15 mer length were found. To detect linear B-cell epitopes, the sequences were subjected to the IEDB antigen sequence properties, and 22 epitopes from 9 to 34 mer in length were obtained (Table. 2).

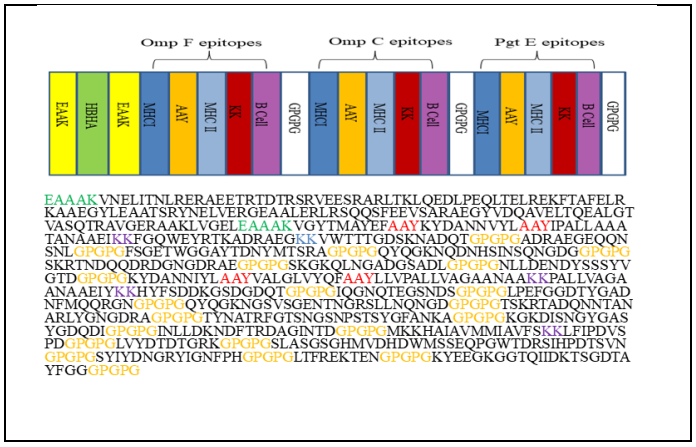

Figure 1. Designing and fusing of epitopes

Table 1. MHC- I (CD8$^+$ T-cell) and MHC- II (helper T lymphocyte or CD4$^+$)

MHC- I					
Gene	**Alleles**	**Start**	**End**	**Sequence (peptide)**	**Score**
OmpF	H2-Db	17	25	AATANAAEI	0.834497
	H2-Dd	195	203	VGYTMAYEF	0.116599
	H2-Kb	320	328	ATYYFNKNM	0.791775
	H2-Kd	239	247	KYDANNVYL	0.779589
	H2-Kk	260	268	VENTVTDTV	0.856953
	H2-Ld	132	140	APYFSGETW	0.340812
OmpC	H2-Db	150	158	ATYRNTDFF	0.794161
	H2-Dd	272	280	SNPSTSYGF	0.184829
	H2-Kb	334	342	ATYYFNKNM	0.791775
	H2-Kd	245	253	KYDANNIYL	0.740712
	H2-Kk	179	187	GENTNGRSL	0.681186
	H2-Ld	370	378	VALGLVYQF	0.236613
PgtE	H2-DB	168	176	YIYDNGRYI	0.504859
	H2-Dd	184	192	RGIGYSQRF	0.153985
	H2-Kb	257	265	SNAKIFAEF	0.550266
	H2-Kd	253	261	YYITSNAKI	0.949666
	H2-Kk	271	279	EEGKGGTQI	0.882369
	H2-Ld	30	38	SPDSVTTSL	0.74587
MHC- II					
Gene	**Alleles**	**Start**	**End**	**Sequence**	**Percentile**
OmpF	H2-IAd	1	15	MMKRKILAAVIPALL	1.03
	H2-IAb	11	25	IPALLAAATANAAEI	0.09
	H2-IEd	76	90	FGQWEYRTKADRAEG	2.30
OmpC	H2-IAd	8	22	LLVPALLVAGAANAA	11.00
	H2-IAb	11	25	PALLVAGAANAAEIY	0.69
	H2-IEd	332	346	VGATYYFNKNMSTYV	10.15
PgtE	H2-IAd	1	15	MKKHAIAVMMIAVFS	2.85
	H2-IAb	248	262	IDAGYYITSNAKIFA	4.10

H2-IEd	225	239	DEHYMRKLTFREKTE	2.77

The results showed (Table. 3) a total of nine interferon-γ- inducing epitopes (15 mer). A total of five interferon-γ- inducing epitopes were predicted with positive scores and four interferon-γ- generating epitopes were predicted with negative scores

Antigenicity of the construct was indicated by ANTIGENpro for both $CD4^+$ and $CD8^+$ epitopes (Tables. 4 and 5).

The psipred server at http://bioinf.cs.ucl.ac.uk/psipred is used to display secondary structure. According to the results in Fig. 2 (a). Of the 749 amino acids analyzed, 246 (32.84%) were involved in α-helix formation, 94 (12.55%) in β-strand formation, and 410 (54.73%) were engaged in coil formation.

ProSAweb was used to predict the quality and errors of the 3D structure of the engineered vaccine. The Z-score of the best refined model was -5.38 (Fig.2 (b, c, and d)).

The Galaxy web server displayed the five models with GDT-HA, MolProbity, RMSD, and Ramachandran plot. Model 3 proved to be the best based on its parameters, including GDT-HA (0.939), MolProbity (2.111), and RMSD (0.468). The clash

Table 2. B-cell

Gene	Start	End	Sequence (peptide)	Length
OmpF	44	57	VWTTTGDSKNADQT	14
	85	97	ADRAEGEQQNSNL	13
	135	153	FSGETWGGAYTDNYMTSRA	19
	176	194	QYQGKNQDNHSINSQNGDG	19
	215	232	SKRTNDQQDRDGNGDRAE	18
	254	276	TRNMSIVENTVTDTVEMANKTQN	23
	298	312	SKGKQLNGADGSADL	15
	337	352	NLLDENDYSSSYVGTD	16
OmpC	42	55	HYFSDDKGSDGDQT	14
	81	92	IQGNQTEGSNDS	12
	128	147	LPEFGGDTYGADNFMQQRGN	20
	169	193	QYQGKNGSVSGENTNGRSLLNQNGD	25
	215	237	TSKRTADQNNTANARLYGNGDRA	23
	260	284	TYNATRFGTSNGSNPSTSYGFANKA	25
	309	326	KGKDISNGYGASYGDQDI	18
	350	367	INLLDKNDFTRDAGINTD	18
PgtE	24	32	LFIPDVSPD	9
	50	59	LVYDTDTGRK	10
	94	127	SLASGSGHMVDHDWMSSEQPGWTDRSIHPDTSVN	34
	167	181	SYIYDNGRYIGNFPH	15
	233	241	LTFREKTEN	9
	269	292	KYEEGKGGTQIIDKTSGDTAYFGG	24

Table 3. γ- Interferon

Gene	Start	End	Sequence	IFN-γ	Score
OmpF	1	15	MMKRKILAAVIPALL	Positive	0.37242047
	11	25	IPALLAAATANAAEI	Positive	0.062773151
	76	90	FGQWEYRTKADRAEG	Positive	0.21147028
OmpC	8	22	LLVPALLVAGAANAA	Positive	0.15535542
	11	25	PALLVAGAANAAEIY	NEGATIVE	-0.042811389
	332	346	VGATYYFNKNMSTYV	NEGATIVE	-0.56728272
PgtE	1	15	MKKHAIAVMMIAVFS	Positive	0.66910732
	248	262	IDAGYYITSNAKIFA	NEGATIVE	-0.22423073
	225	239	DEHYMRKLTFREKTE	NEGATIVE	-0.32178762

score of model 3 was 11.5 with a poor Rotamers score of 0.6 and a Rama favored score of 90.5. The Ramachandran diagram of the refined structure of the multi-epitope vaccine shows 39% helix, 9% beta and 51% coil of residues (Fig. 2 (e)).

ProtParam sever was used to check the physiochemical activities of the constructed model. The results showed that the isoelectric point (pI) of all vaccine is 5.00. The constructed vaccine consists of 749 amino acids with a molecular weight of 77981.61. The total number of residues with positive and negative charges was 93 and 72, respectively. The half-life of the vaccine is estimated to be one h in mammalian reticulocytes (*in vitro*), 30 min in yeast (*in vivo*), and > 10 h in *E. Coli* (*in vivo*). The hydrophobicity of the protein was estimated by a grand average of hydropathicity (GRAVY). GRAVY score of the construct was -0.821. The predicted scaled solubility was 0.582. The instability index of the protein was 16.19. In addition, the aliphatic index was 52.51.

Multi-epitope vaccine and TRL4 docking were performed using ClusPro server. Of the 10 models obtained, the model with the minimum energy value of -965.5 was considered. The best docking of a ligand-receptor is shown in Fig. 3.

To estimate the structural stability of the docked vaccine constructTLR4, some statistical features were

Table 4. MHC- I antigenicity, allergenicity, and immunogenicity

Gene	Sequence (peptide)	Immunogenicity Score	Antigenicity Score	Probable	Allergenicity
OmpF	AATANAAEI	0.18032	0.8979	ANTIGEN	PROBABLE ALLERGEN
	VGYTMAYEF	0.13127	1.0554	ANTIGEN	PROBABLE NON-ALLERGEN
	ATYYFNKNM	-0.08159	0.1067	NON-ANTIGEN	PROBABLE NON-ALLERGEN
	KYDANNVYL	0.07316	0.5367	ANTIGEN	PROBABLE ALLERGEN
	VENTVTDTV	0.13242	0.6525	ANTIGEN	PROBABLE ALLERGEN
	APYFSGETW	0.25568	0.1224	NON-ANTIGEN	PROBABLE ALLERGEN
	ATYRNTDFF	0.17454	-0.2619	NON-ANTIGEN	PROBABLE ALLERGEN
OmpC	SNPSTSYGF	-0.30552	0.3842	NON-ANTIGEN	PROBABLE NON-ALLERGEN
	ATYYFNKNM	-0.08159	0.1067	NON-ANTIGEN	PROBABLE NON-ALLERGEN
	KYDANNIYL	0.15064	0.6091	ANTIGEN	PROBABLE NON-ALLERGEN
	GENTNGRSL	0.10624	2.9062	ANTIGEN	PROBABLE NON-ALLERGEN
	VALGLVYQF	-0.00144	0.8219	ANTIGEN	PROBABLE NON-ALLERGEN
	YIYDNGRYI	0.09454	-1.5659	NON-ANTIGEN	PROBABLE NON-ALLERGEN
PgtE	RGIGYSQRF	-0.18915	0.0030	NON-ANTIGEN	PROBABLE NON-ALLERGEN
	SNAKIFAEF	0.11432	-0.1130	NON-ANTIGEN	PROBABLE ALLERGEN
	YYITSNAKI	-0.01681	0.6889	ANTIGEN	PROBABLE NON-ALLERGEN
	EEGKGGTQI	-0.10834	2.7029	ANTIGEN	PROBABLE ALLERGEN
	SPDSVTTSL	-0.18663	0.9775	ANTIGEN	PROBABLE ALLERGEN

analyzed based on the 100-ns trajectory of the molecular dynamics simulation and shown in Fig. 4 (a) and (b). The RMSD of all atoms in the ligand-receptor complex gradually increases with timeand persists until 20 ns. The RMSD curve of all atoms means that the ligand-receptor complex remained mostly stable, and the RMSD value converged within ~0 .1 nm. Moreover, the ΔG of the stable structure is -3.91×10^4 Kcal mol^{-1} with 1.93 RMSD. Van der Waals forces are a general term for the attraction of

Table 5. MHC- II antigenicity, allergenicity, and immunogenicity

Gene	Sequence	Antigenicity Score	Probable	Allergenicity
OmpF	MMKRKILAAVIPALL	0.2413	NON-ANTIGEN	PROBABLE NON-ALLERGEN
	IPALLAAATANAAEI	0.5863	ANTIGEN	PROBABLE NON-ALLERGEN
	FGQWEYRTKADRAEG	0.9059	ANTIGEN	PROBABLE NON-ALLERGEN
	LLVPALLVAGAANAA	0.8210	ANTIGEN	PROBABLE NON-ALLERGEN
OmpC	PALLVAGAANAAEIY	0.5361	ANTIGEN	PROBABLE ALLERGEN
	VGATYYFNKNMSTYV	0.3450	NON-ANTIGEN	PROBABLE NON-ALLERGEN
PgtE	MKKHAIAVMMIAVFS	0.5106	ANTIGEN	PROBABLE NON-ALLERGEN
	IDAGYYITSNAKIFA	-0.1221	NON-ANTIGEN	PROBABLE NON-ALLERGEN
	DEHYMRKLTFREKTE	0.4331	NON-ANTIGEN	PROBABLE NON-ALLERGEN

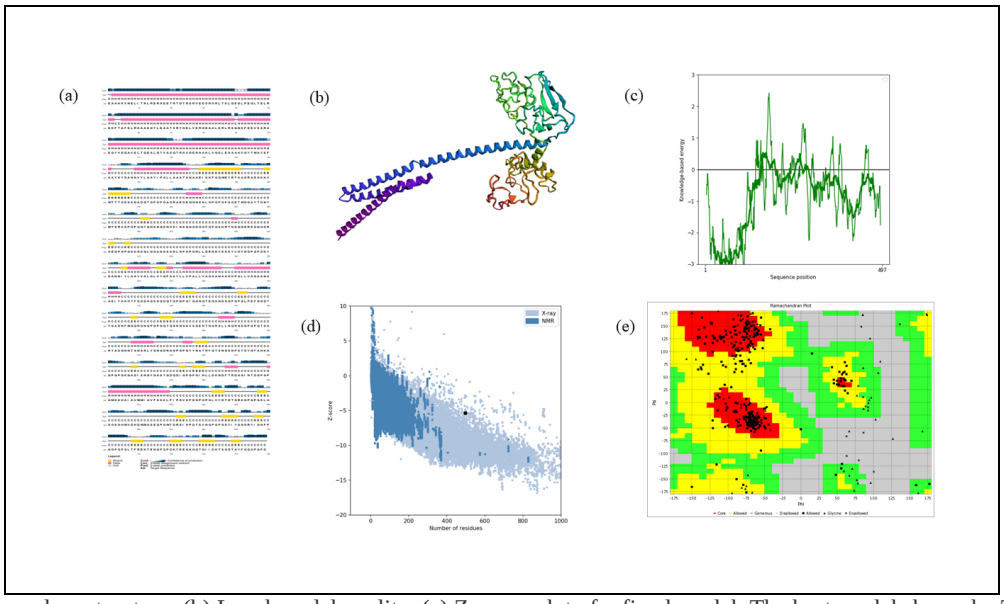

Figure 2. (a) Secondary structure, (b) Local model quality, (c) Z-score plot of refined model. The best model showed a Z-score of -5.38 (large black spot). Z-scores of all model protein chains (in PDB format) indicated by NMR spectroscopy (dark blue)

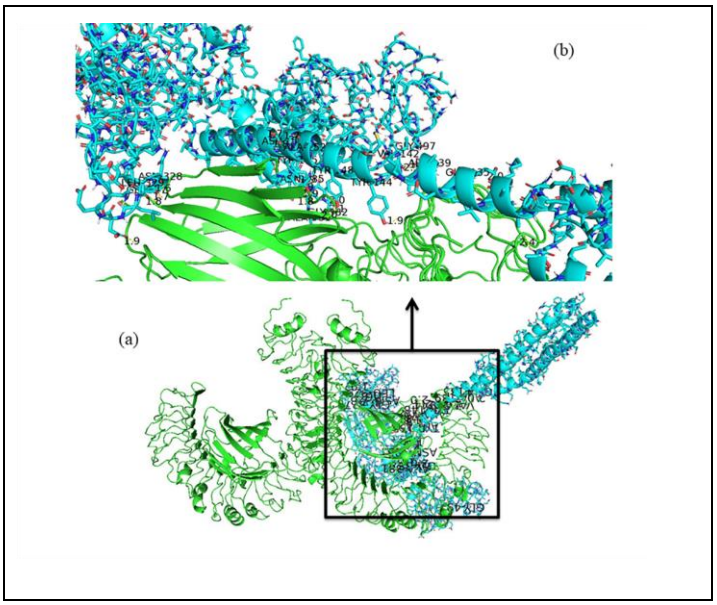

Figure 3. Docked complex of ligand-receptor by the ClusPro server. (a): ligand with blue color and receptor (TLR4) with green color, (b): interacting amino acid residues of ligand and receptor, which are labeled in black. Results revealed a probable

intermolecular forces between molecules. Fig. 5 (a) shows the Van der Waals surface of the vaccine construct-TLR4 complex. The Van der Waals forces depend on the distance between the atoms or molecules on the surface; the gray color shows that in this complex, the intermolecular forces are weak, resulting from the interactions between uncharged atoms/molecules. Fig. 5 (b) shows the electrostatic surface of the construct-TLR4 complex. The red site conducts the opposite electrostatic force, and the blue color shows the flattering posture of the molecule.

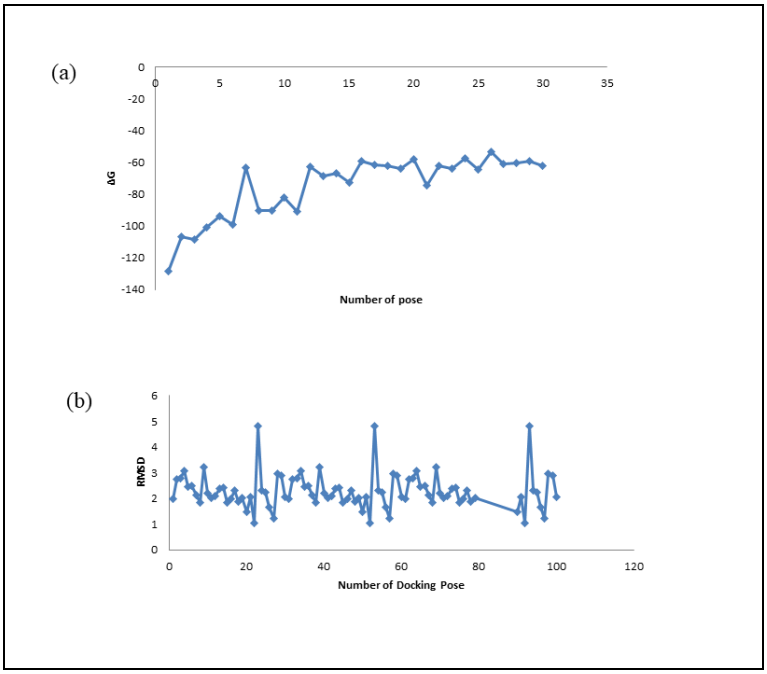

Figure 4. (a) Difference of ΔG of docking pose of vaccine with TRL4, and (b) RMSD structural stability analysis of vaccine with TRL4

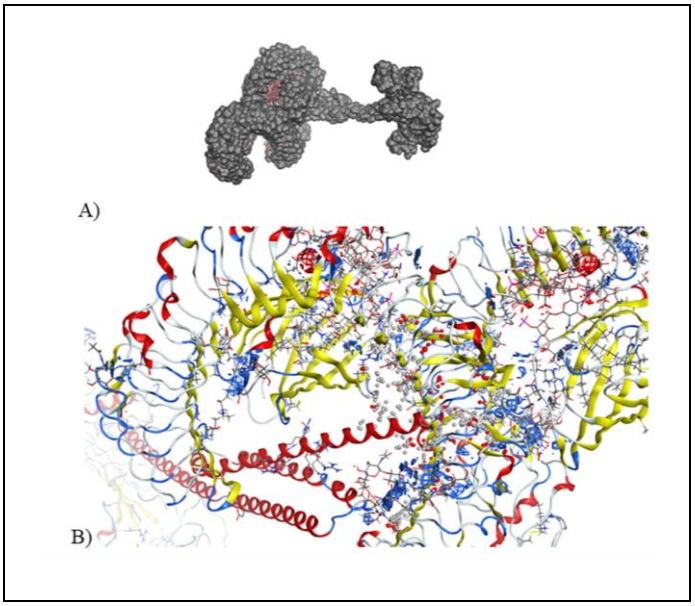

Figure 5. Van der Waals, (a) and electrostatic (b) maps for a vaccine with TRL4

4. Discussion

Classical strategies are used for the development of most human vaccines (21). Because of its time-consuming and costly approach, reverse vaccinology, which includes genomic information, could predict antigenic proteins that elicit an immune response (22). Multi-epitope vaccines are planned for various bacteria and parasites (23). In the present study, the predicted epitopes were joined by different linkers (AAY, KK, and GPGPG) as cleavage sites. These linkers were used to increase protein stability (24). In the present study, the EAAAK linkers were used before and after the adjuvant. The EAAAK linkers are defined as rigid linkers that can separate and reduce the interaction between vaccine domains and increase the thermal stability of chimeric proteins (25). The dilysine linker (KK) has been used in multi-epitopes as a suitable linker for cathepsin B, enhancing T-cell antigen presentation and increasing immunogenicity (26). AAY linkers are efficiently cleaved in cells, reducing cross-linking, to improve multi-epitope vaccines (27,28). GPGPG linkers have been used for B-cell cross-linking (29). In addition, the GPGPG linker can elicit a helper T lymphocyte (HTL) response and abrogate the immunogenicity of the compound and restore immunogenicity (30).

The antigenic proteins of *Salmonella* Typhi were extracted from the NCBI protein database based on the FASTA format of their genes. Cytotoxic Tlymphocytes are $CD8^+$ T-cell responses to cells with intracellular viral, bacterial, or protozoal infections. In the current study, lower percentile ranks were chosen because of the highest immunogenic properties of the epitopes (Table. 1). Epitopes identified by their corresponding receptors (B-cells) play an important role in vaccine modeling. Thus, 22 epitopes ranging from 9 to 34 mer in length were obtained. Antigenicity is the ability of an antigen to bind with specific antibodies and elicit an immunological response that includes allergenicity and immunogenicity. ANTIGENpro indicated a high probability of antigenicity for both $CD4^+$ and $CD8^+$ epitopes. Allergenic vaccines can induce sensitization and allergic reactions associated with the IgE antibody response, so the multi-epitope should be free of allergenicity.

The secondary structure of the construct showed the formation of α-helix, β-strands, and coil. In addition, the 3D structure of the vaccine was displayed using Raptrox and refined using GalaxyRefine server. The 3D structure of the engineered vaccine was within the range of native proteins with similar sizes. The Galaxy web server showed that model 3 was the best based on its parameters, including GDT-HA, MolProbity, and RMSD. The clash score of the best model showed a poor rotamer score.

In the current study, the isoelectric point of the vaccine indicates the basic behavior of the protein. ProtParam sever indicates the basic behavior of the protein with positive and negative charges. GRAVY score of the construct indicates that the molecule is hydrophilic. The instability index of the protein indicated a stable molecule.

The results showed that the RMSD of all atoms in the ligand-receptor complex increased with time. The RMSD diagram showed that the ligand-receptor complex remained mostly stable. Van der Waals forces are weak and arise from the interactions between uncharged atoms/molecules.

Conclusion: *Salmonella* Typhi causes typhoid fever and death of many people every year. The resistance of *S*. Typhi to many antibiotics, suggesting that it is extremely dangerous to living organisms. Since a polyepitope vaccine based on immunogenic outer membrane proteins of *S.* Typhi, was developed in the present study, the results of in-silico analyses indicate that the constructed vaccine can be used to control typhoid fever and needs further investigation.

Authors' Contribution

Fereshteh Ezzati Ghadi: Supervision, Writing, Revision, Investigation,

Zahra Roudbari: Writing, Methodology, Revision,

Razieh Razavi: Writing, Methodology, Visualization

Ethics

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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